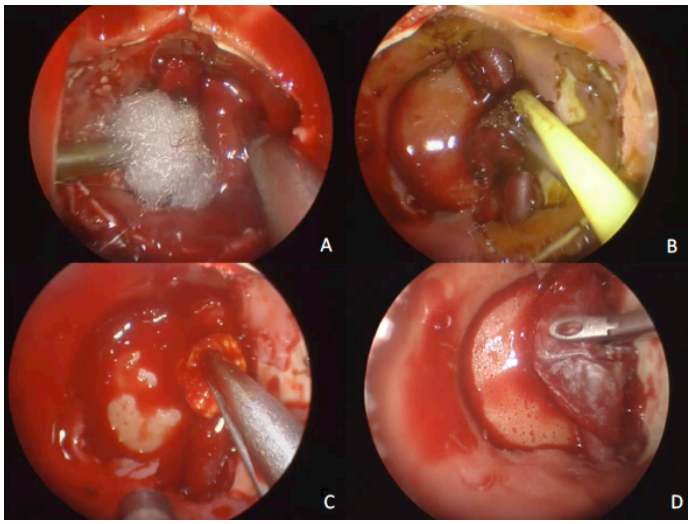
Haemostasis in Endoscopic Skull Base Surgery

Thesis submission for
Doctor of Philosophy

Alistair Jukes
MBBS(Hons) B.LibStud



The University of Adelaide

Discipline of Surgery

Department of Otolaryngology, Head and Neck Surgery
Department of Neurosurgery

Table of Contents

Abstract

The endoscopic approach to the skull base has revolutionised surgery in this region. Neurosurgery involves working around anatomical structures that are uniquely sensitive to damage and manipulation and patients may be left with the potentially devastating consequences of violating these structures. The endoscope allows the surgeon to visualise and reach areas that were previously only accessible with large amounts of destructive dissection. Tumours are able to be removed and aneurysms clipped without the need for large craniotomies and bony drilling.

There are, however, drawbacks. The midline endoscopic route takes the surgeon between the carotid arteries. It potentially violates the anterior communicating artery complex and the basilar artery region anterior to the brainstem. These are important arteries that supply critical structures. Damage to these, or diminution of blood flow through them, results in profound neurological dysfunction or death.

The rate of damage to the carotid artery with these approaches ranges from 1.1-9% depending on the specific approach and pathology. The carotid artery in this region does not generally lend itself to suturing, clipping or direct closure methods. Currently, the gold standard for repair is the application of crushed muscle patch to stop the bleeding and seal the vessel. The drawbacks to this are that it takes time to harvest and control the bleed (generally requiring 2 surgeons), and that there is a risk of pseudoaneurysm formation post recovery.

This thesis describes novel techniques that may replace the muscle patch in order that a single surgeon may have this technique available to them immediately.

Aims:

To demonstrate the use of fibrin/thrombin/gelatin patches, fibrin/thrombin glues, beta-chitosan patches and self-assembling peptides on a sheep model of carotid artery haemorrhage and quantify the rate of pseudoaneurysm formation.

To show the percentage of platelets activated by crushed and uncrushed muscle, chitosan, and fibrin and thrombin patches and gels using flow cytometry to further delineate the mechanism of action of crushed muscle as a haemostatic agent.

To quantify the stress response in surgeons training on this sheep vascular haemorrhage model de novo, to quantify its effect on surgeons' teamwork and communication skills, and determine the effect and value of training on modulation of this stress response.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint
-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Acknowledgements

This work was made possible by the Roy and Marjory Edwards Scholarship from the Royal Australasian College of Surgeons and funding from the Neurosurgical Research Foundation in South Australia.

Many people have made the work in this thesis possible. I would like to thank them all for their contributions and the kindness they have shown me.

To Professor PJ Wormald, for his supervision, inspiration, mentorship, friendship, and tireless demonstration of nuanced surgical techniques, decision making and departmental management.

To Associate Professor Alkis Psaltis for his guidance through this entire project, for his encouragement, mentorship, friendship and continuous teaching both in theatre and outside.

To Associate Professor Sarah Vreugde for her guidance through the world of laboratory science.

To my colleagues and friends who have helped me with these projects and studies – Dr Jae Murphy, Dr Sathish Paramasivan, Dr Dijana Miljkovic, Dr Rowan Valentine, Dr Mian Ooi, and Dr Vikram Padhye.

To the Neurosurgeons and Registrars who have taught me both the science and art of our profession, especially Dr Stephen Santoreneos, Dr Cindy Molloy, Dr Marguerite Harding, Dr Leigh Atkinson, Dr Rodney Allan, Dr Arthur Ellice-Flint, Dr Toby Loch-Wilkinson, Dr David Wilson and Dr Chris Huang.

To Dr Alyssa Parsons for my sight and for operating with me in the wee hours.

To Dr Annika Mascarenhas for her collaboration, friendship and assistance in everything.

To Dr Chantal Baldwin for her constant love and encouragement; and for being my sounding board in life.

For my father, Doug Jukes (1951-2009)

"But, he thought, I keep them with precision. Only I have no luck anymore. But who knows? Maybe today. Every day is a new day. It is better to be lucky. But I would rather be exact. Then when luck comes you are ready" – Ernest Hemingway. The Old Man and the Sea.

"You can't stay in your corner of the forest waiting for others to come to you. You have to go to them sometimes" – A.A. Milne

"But man is not made for defeat', he said. 'A man can be destroyed but not defeated" – Ernest Hemingway. The Old Man and the Sea.

Presentations

Components of this thesis were presented at

North American Skull Base Society Meeting 2017 – New Orleans

Australasian Society of Head and Neck Surgery 2017 – Adelaide

Neurosurgical Society of Australasia Annual Scientific Meeting 2017 – Adelaide

Australasian Military Medicine Conference 2017 – Brisbane

RP Jepson Prize 2017 – Royal Australasian College of Surgeons - Adelaide

Chapter 1 – Introduction

Skull Base Anatomy and Pathology

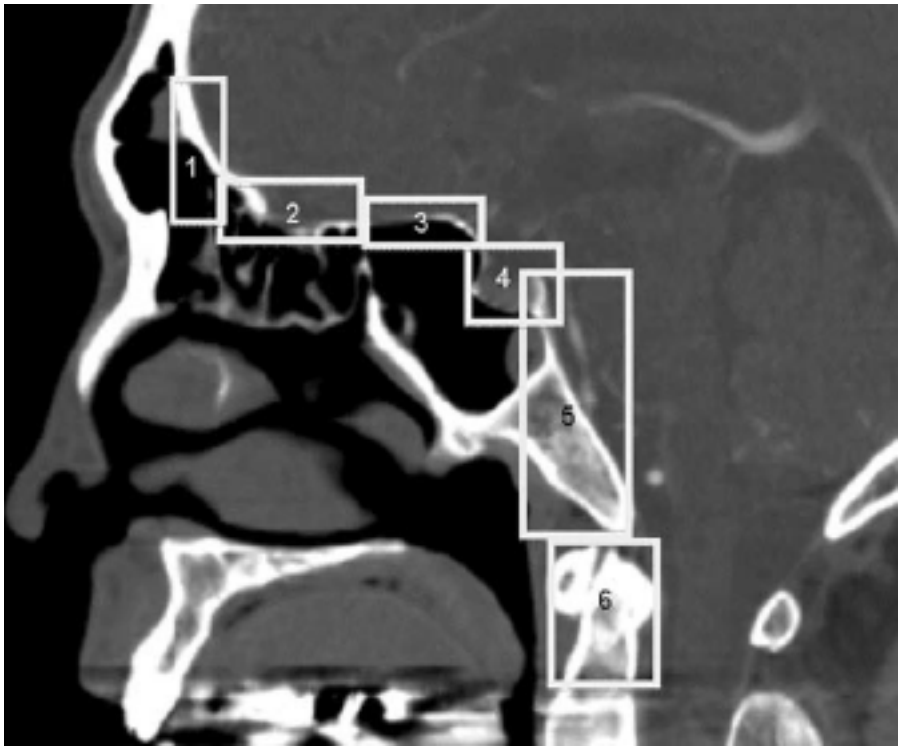
The skull base refers to the floor of the cranial cavity and forms a complex boundary between the brain and facial structures(1). It is conventionally described as comprising the ethmoid, sphenoid, occipital, frontal, and temporal bones(2). From a neurosurgical perspective, it is one of the most challenging anatomical areas to operate on. There are a number of crucial vascular and neural structures which pass through the skull base or lie upon its surface; all in close proximity to each other. Furthermore, there are a number of bony foramen and dural folds, which restrict both access and vision when operating in this area(3).

There are a huge variety of pathologies that occur within the skull base. These include tumours of the brain parenchyma and pituitary gland, tumours of nerves and nerve sheaths, tumours of dural origin and affixation, aneurysms of blood vessels, carotid-cavernous fistulae, traumatic fractures, & cerebrospinal fluid leaks – both spontaneous and traumatic(4-8). There are also multiple pathologies that occur within the pneumatised paranasal sinuses of the frontal, sphenoid, and ethmoid bones. The primary surgical challenge for the majority of these lesions is gaining access to the pathology without damaging surrounding structures(9).

Neurosurgeons and Otolaryngologists have long collaborated in designing approaches to this area however the concept of accessing the cranial cavity via the nose has existed for longer than either of these subspecialties. The midline trans-sphenoidal, trans-nasal

approach to the brain was described by the Egyptians over 2400 years ago. They used it to remove the cranial contents for embalming purposes(10-13). From the point of view of surgical practice, the trans-nasal approach was explored in the 1890s by Giordano on cadavers. In 1907, Schloffer described using this approach to successfully debulk a pituitary tumour(14). Results from transcranial approaches appeared superior however, and mainstream practice favoured transcranial approaches until Guiot demonstrated both efficacy and safety of the endoscope in the 1960s(5). His work described the first attempts to use an endoscope in this situation however, even he conceded that a microscope provided better visualisation.

The advent and refinement of the surgical endoscope by Harold Hopkins with his rod-lense system in the 1960s and ongoing development in camera and video technology led to the first neuro-endoscopy multi-specialty teams being formed in the 1990s(5, 15). Endoscopic surgery has subsequently become a valuable tool for accessing the anterior, middle, and posterior cranial fossae and even the odontoid process region(16-21). It can be a minimally invasive technique leaving few to no external scars and with potentially less requirement to mobilise intracranial structures and retract brain to access pathology(22). This does not mean that the approach is without morbidity, merely that the set of risks and potential morbidities have changed(22). The technique requires knowledge of where anatomical structures may be located in relation to fixed landmarks. The consequences of violating these structures can be neurologically devastating and potentially fatal for the patient.



Computerised tomography (CT) scan of the skull base (sagittal) demonstrating the endonasal endoscopic approaches possible. (1) Transfrontal, (2) Transcribriform, (3) Transplanum, (4) transphenoid, (5) transclival, (6) Trans-odontoid(23)

Challenges of Skull Base Surgery

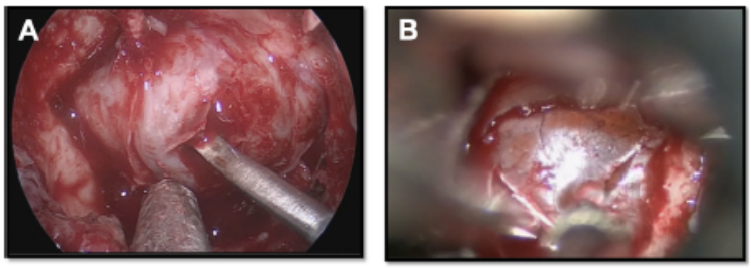
The bony contours of the skull base and the sensitive structures it contains pose a surgical challenge. The ideal approach would use the shortest and most direct route to the pathology, with the minimum of anatomical disruption. This, however, is rarely possible. Approaches must therefore be tailored to the patient's anatomy to provide access and visualisation but to avoid potentially irreversible damage to the sensitive neural and vascular structures that course through these areas. In situations where an anatomical corridor is not immediately accessible, the usual route is to remove bony prominences and divide dural folds that hinder access and visualisation(24). This follows the philosophy of most skull base surgeons who advocate removal of bone to prevent retraction injury on the brain or nerves that traverse this area. This can be a painstaking process as there are many

structures such as the internal carotid artery(ICA), greater and lesser superficial petrosal nerves, oculomotor, trochlear and abducens nerves that run in close proximity to, and in some cases through these bony areas. Given that bony structures are essentially incompressible and dural reflections can only be manipulated to a small degree, surgeons have developed strategies to increase their access and visualisation. Key amongst these is the draining of cerebrospinal fluid (CSF). There is between 75 and 150ml of CSF within the combined cranial cavity and spinal subarachnoid space. Drainage or diversion of CSF can significantly improve the ability to manipulate brain parenchyma in order to visualise pathology and aid dissection. This may be achieved through either the pre-operative placement of a lumbar drain with judicious release of CSF if the situation allows or the opening of arachnoid planes within the basal cisterns and subsequent gentle suction and drainage(25-27).

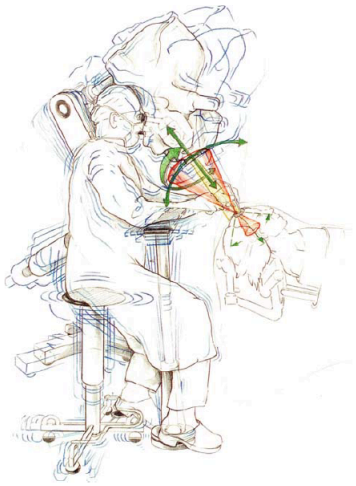
The skull base surgeon is also constrained by the ability to position the patient to adequately access these areas. Optimum positioning for visualisation may cause compression of venous channels with impedance of blood return, engorgement of deep and superficial veins and raised intracranial pressure (ICP)(28). This may also increase bleeding and hinder visualisation of the operative field. Surgery may occur near venous sinuses. If these are torn and air allowed to enter, an air embolism may result with potentially fatal results. The range of mobility of the patient's neck may pose a difficulty in positioning and allowing efficient and comfortable surgical corridors to be created. The surgeon's hands and line of site along the microscope's field of view should align in such a way as to allow for protracted operating times. If the surgeon has to hold him or herself in an uncomfortable or strained position, then muscle fatigue will occur and tremor may impede the surgery's

smooth progress. There is thus a compromise that must occur between optimum positioning for patient and surgeon.

The increasing use of endoscopic approaches to the skull base region has both ameliorated and exacerbated some of these problems. The direct approach, generally using loupes or microscope for magnification, has the advantage of providing the surgeon with a 3-dimensional field of view and, from a kinesiology perspective, allows the surgeon's hand-eye coordination to work in the way in which it is accustomed to. It does, however, have drawbacks. There must be a direct line of site down the line from eye, through microscope, and down to the operative field. This requires the use of retraction, bayonetted instruments and removal of bony and dural structures in this line(28).

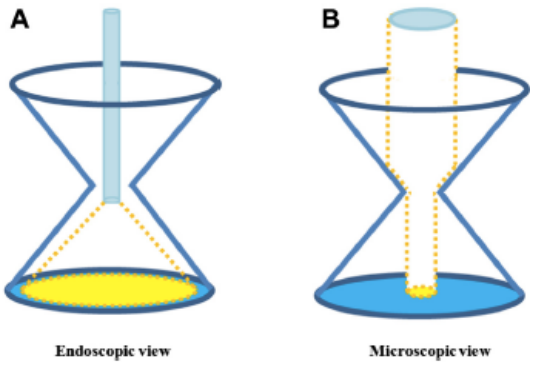


Intraoperative images – Endoscopy vs. Microscopy of approach to the pituitary. The endoscopic view is panoramic. The microscopic view is impeded by instruments(18)

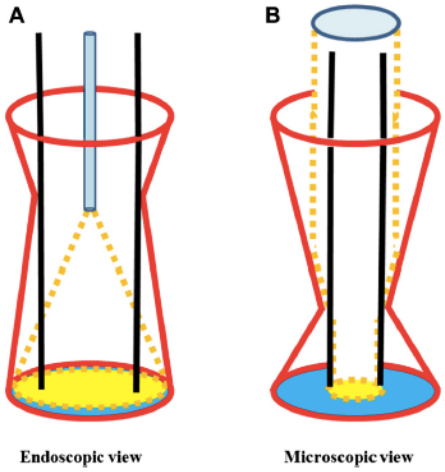


Surgeon using the microscope demonstrating the complex relationships between surgeon and bed heights, microscope angle and working instrument access(29)

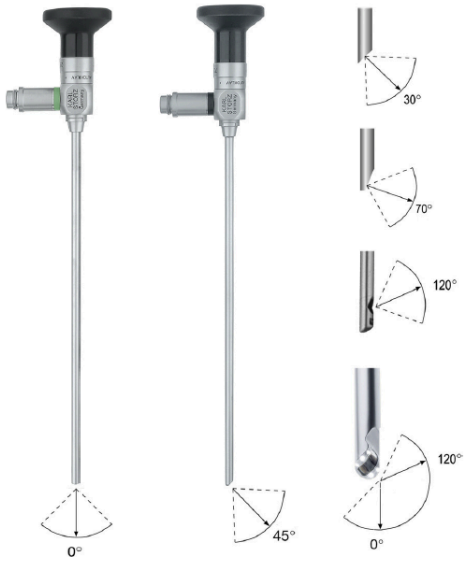
The endoscope has the drawback of (until the very recent addition of the 3D endoscope) providing a 2-dimensional picture to the surgeon however it does allow the surgeon to 'look around corners' with angled scopes, removing the need for a direct line of site. It also provides a source of illumination at the tip of the scope and, with the ever-improving quality of lenses and cameras, a high definition view of the operative area(30, 31). Endoscopic instruments may be introduced through or alongside the scope to manipulate and resect pathology(32). The patient may therefore be positioned in such a way as to provide a more comfortable and efficient stance for the surgeon. Endoscope and instrument rests may be utilised to stabilize vision(33). There is also less need for large craniotomy flaps or scars as the endoscope may be introduced through natural anatomical points of access – i.e. the nostrils. The endoscopic approach has traditionally been restricted to the midline through the sphenoid sinus with lateral vision being provided through the use of angled scopes. With appropriate removal of bony structures however, and careful dissection of overlying tissue, the endoscopic approach can provide access to lesions ranging from the floor of the anterior cranial fossa, planum sphenoidale, pituitary fossa, clivus and brainstem all the way down to the top of the spine(2, 19, 34-36).



Comparison of fields of illumination and vision – endoscopy(A) vs microscopy(B)(18)



Comparison of operative fields endoscopy(A) vs microscopy(B). The endoscopic field is wider and dissecting instruments do not interfere with the lines of sight(18)



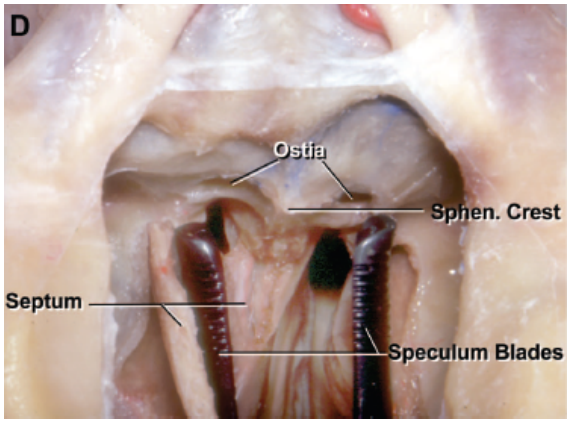
Storz endoscopes showing ranges of camera angles and vision achieved(37)

Both access to pathology and surgical results for midline structures have been comparable or even improved with endoscopic approaches compared to traditional open approaches(38-40). Evidence is also emerging that less invasive endoscopic approaches may be associated with improved quality of life outcomes including post-operative cognitive and physical functioning(22).

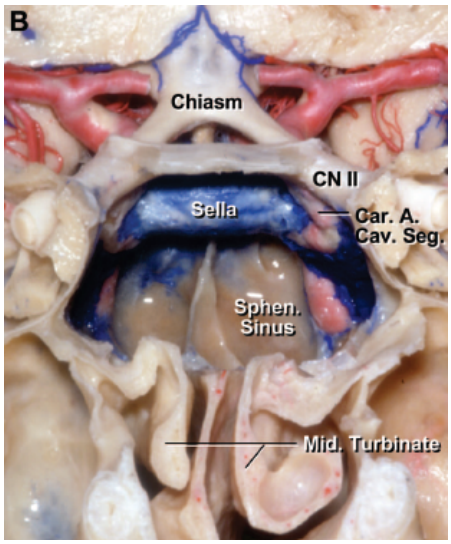
Neuro-navigation has also advanced. Pre-operative images may be loaded onto a navigation computer which triangulates the patient's skull, a fixed reference point on the operating table, and a camera affixed to the computer(41). Through the pre-operative registration of the patient's surface anatomy to either computerised tomography (CT) and/or magnetic resonance imaging (MRI) scans, surgeons can acquire a real-time view of where his or her instruments relative to the patient's anatomy. Although not a substitute for a sound understanding of skull base anatomy this can improve a surgeon's confidence during a case (3, 42, 43).

Anatomy as it pertains to Endoscopic approaches to the Skull Base and Carotid Artery Injury in Endoscopic Endonasal Surgery

Access to the skull base for most midline cases in the anterior and middle cranial fossae and pituitary region is achieved through the sphenoid sinus. The endoscope is introduced through the nostril and advanced to the back of the nasal cavity. If the surgeon has concerns regarding the possibility of CSF leak, they may raise a mucosal septal flap on an inferiorly-based vascular pedicle. The sphenoid ostia are then identified and enlarged before removing their entire anterior wall, intersinus septum and the vomer. The endoscope is further advanced to visualise the posterior wall of the sphenoid sinus and the important anatomical landmarks of the cavernous carotids, front face of the sella and medial and lateral optico-carotid recesses. The posterior wall is then thinned with a drill and the bone removed.

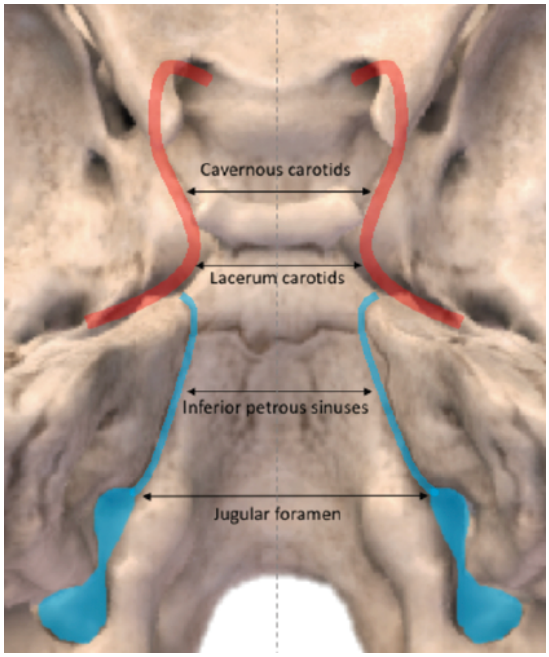


Superior view of approach to anterior wall of sphenoid sinus in cadaveric study (Rhoton)(44)

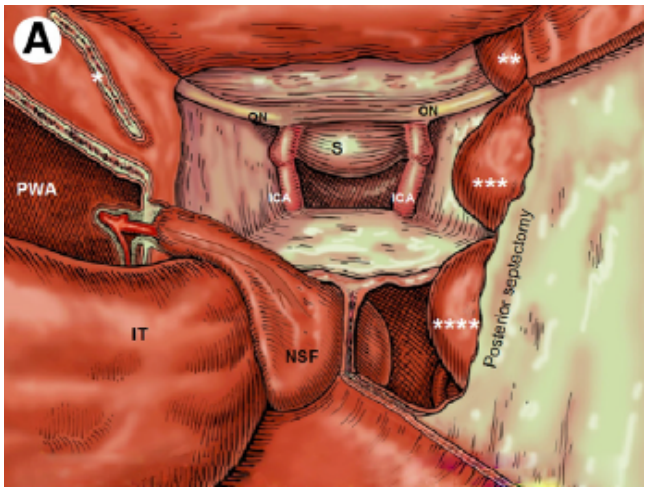


Relationship of cavernous sinuses and carotid artery to sphenoid sinus in cadaveric study (Rhoton)(44)

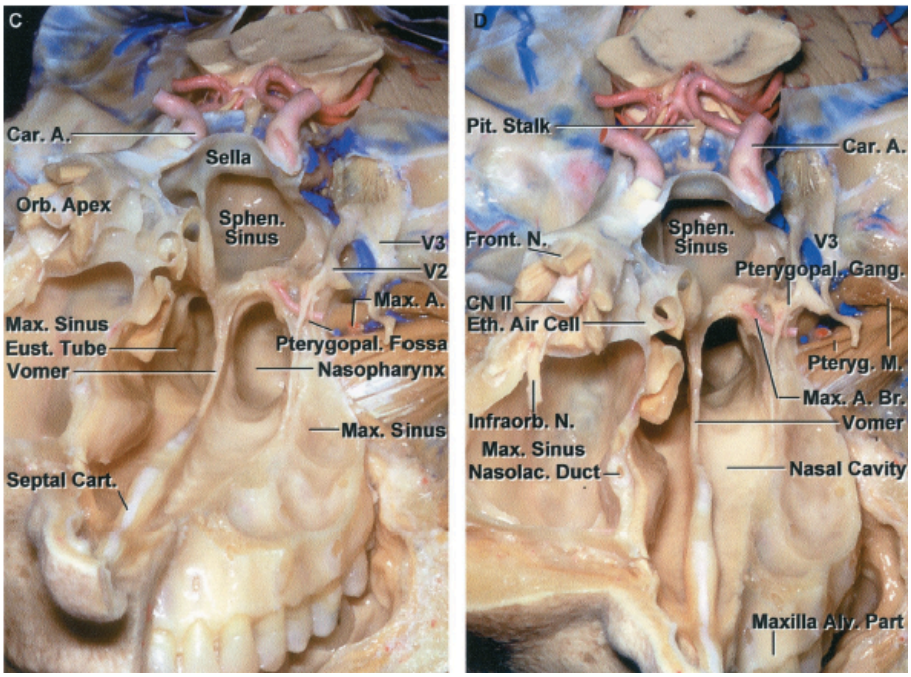
The cavernous sinuses are paired venous structures that lie lateral to the sphenoid sinus. They are contained within a dural envelope. They receive venous blood from the superior and inferior ophthalmic veins, superficial cortical veins, the sphenoparietal sinus and the superficial middle cerebral veins. They drain via the superior and inferior petrosal sinuses and emissary veins through skull foraminae. They also have connections to the basilar plexus posteriorly and the pterygoid plexus of veins via the inferior ophthalmic and inferior facial veins(45-47).



Posterior view of the relationship of the carotid arteries and inferior petrosal sinuses to the cavernous sinus(48)



Representation of Endoscopic right nasal view with a naso-septal flap (NSF) moved laterally and the view of the sella (S) beyond with the internal carotid arteries (ICA) laterally in the cavernous sinus. (IT) inferior turbinate, (PWA) posterior wall of antrum, (NP) Nasopharynx(49)



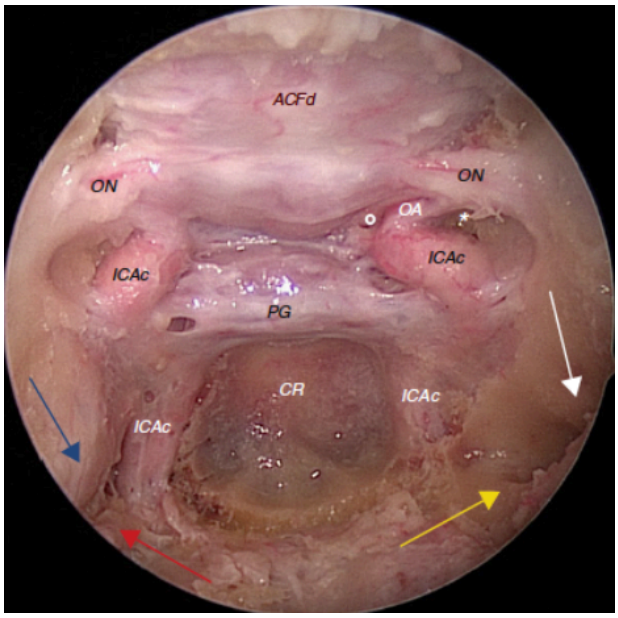
Anterior view of partially dissected cadaveric specimen showing trajectory through nasal cavity to sphenoid sinus and then to pituitary stalk and intracranial cavity (Rhoton). (50)

The carotid artery runs through the cavernous sinus, as does the abducens nerve(51). The lateral wall contains the oculomotor and trochlear nerves and the first and second divisions of the trigeminal nerve(52, 53). There are a number of pathologies that can occur within the sinus and the structures passing through it(54). Despite initial enthusiasm, surgery within the cavernous sinus itself has largely been abandoned because of concerns about bleeding and damage to vital neural structures, with mortality rates of 7-12% and morbidity above 50% in some series(24, 55-59). Irradiation with proton beam or gamma knife has generally been considered a preferable approach for tumours in this region(60-63).

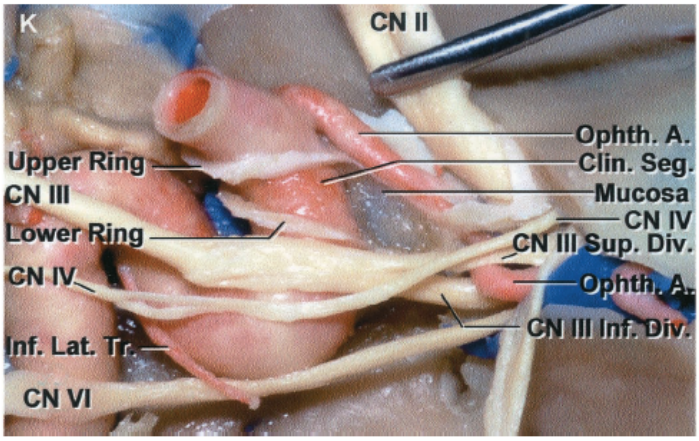
Internal Carotid Artery Anatomy

Within each cavernous sinus is the cavernous segment of the internal carotid artery(ICA). This is the first of three common ‘danger areas’ where the possibility of major vessel haemorrhage is greatest – the others being the anterior cerebral artery (ACA) complex and

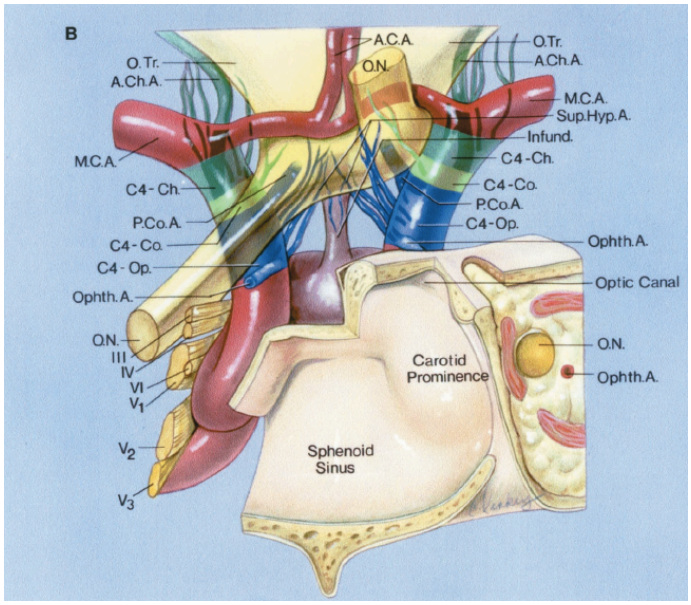
the basilar artery (BA) region. It is conventionally divided into three segments separated by two 'turns' or genua(51). There are two main branches that leave the carotid artery along these three segments. The meningohypophyseal trunk is the most proximal of these, which subsequently divides into the inferior hypophyseal artery (supplying the posterior pituitary), the tentorial artery of Bernasconi and Cassinari (supplying the tentorium, occulomotor nerve and trochlear nerve) and the dorsal meningeal artery, suppling the abducens nerve and clivus(2, 64). The inferolateral trunk is the more distal and divests from the parent artery approximately 6-8mm distal to the meningohypophyseal trunk. It supplies the inferolateral wall of the cavernous sinus and the first and second divisions of the trigeminal nerve(65, 66).



Endoscopic view of the anterior cranial fossa dura (ACFd) looking from anteriorly into the sphenoid sinus. ON-optic nerves, ICAc-cavernous carotid artery, PG-pituitary gland, CR-clival recess(67)



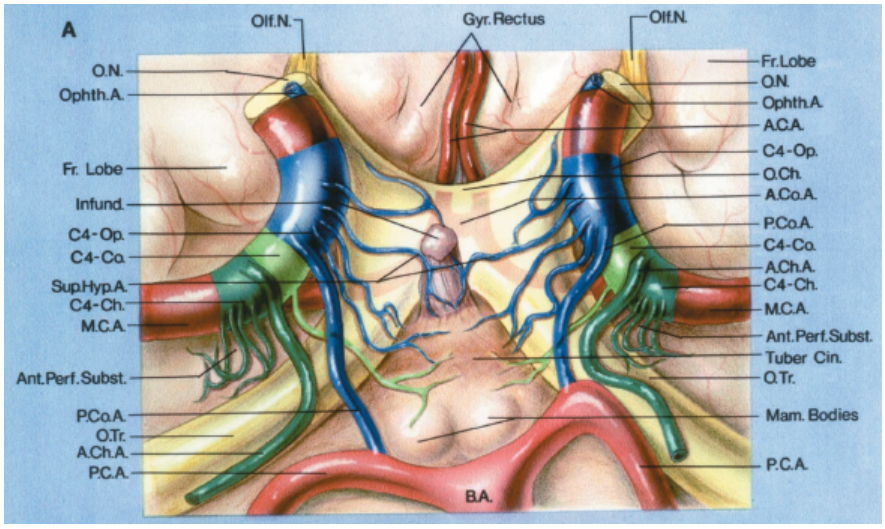
Carotid artery as it passes through the cavernous sinus (Rhoton) (53)



The cavernous segment of the carotid and the contents of the cavernous sinus from an anterior perspective(68).

The ICA then passes through the base of the skull and divides into the ACA and the middle cerebral artery (MCA) to supply brain parenchyma (the anterior circulation)(67). In up to 25% of cases, the posterior communicating artery (PCOM) is also supplied by ICA, either unilaterally or bilaterally – this is known as the 'foetal' or 'embryonic' configuration(69). This is clinically important because in the orthodox adult configuration, only the middle cerebral

artery (MCA) and anterior cerebral artery (ACA) are fed from the ICA and are thus at risk of infarction if this supply is interrupted(70, 71). In the foetal or embryonic configuration, the posterior cerebral artery (PCA) is also fed from this anterior circulation and is thus also at risk of infarction in an ICA occlusion(72).

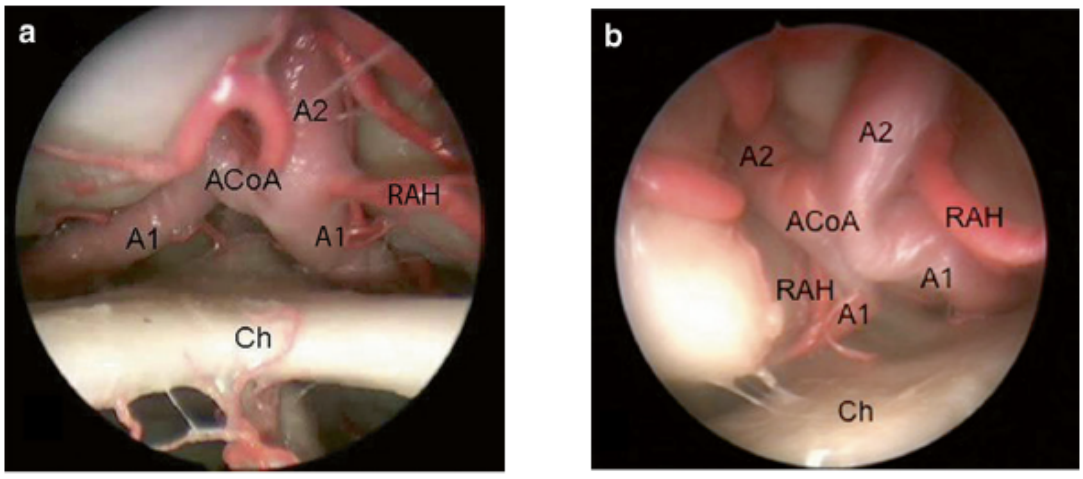


Circle of Willis viewed from inferior aspect demonstrating ICA, MCA, ACA, PCA and optic chiasm (Rhoton) (68)

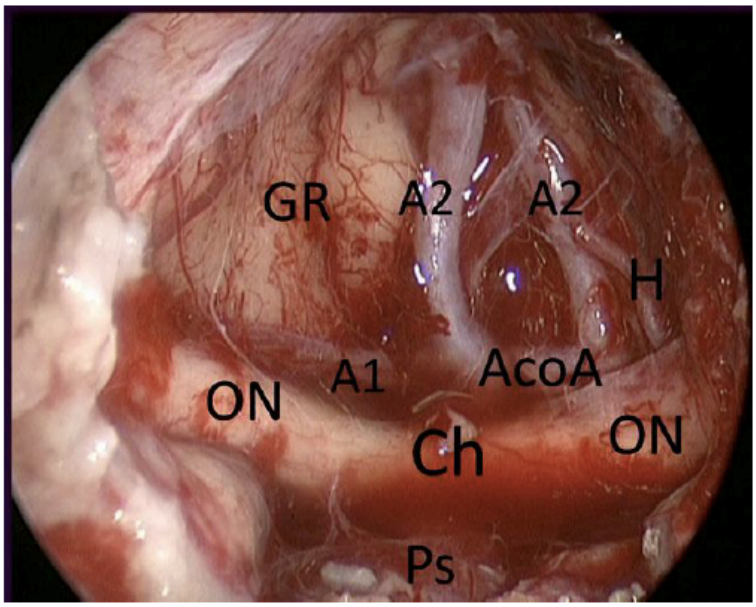
Anterior Cerebral Artery anatomy

The second major area of vascular concern in extended endoscopic neurosurgical procedures is the anterior cerebral artery (ACA) complex. The ICA gives off an anterior cerebral branch running anteriorly (the pre-communicating or A1 segment) before continuing on laterally as the middle cerebral artery(51, 67). The ACAs unite via the anterior communicating artery (ACoA) which forms part of the circle (polygon) of Willis (this may not be complete). In some individuals, there may be a significant discrepancy in the sizes of each ACA with the larger or 'dominant' one supplying the majority of the blood flow to the anterior communicating artery is important when deciding from which side to approach an aneurysm of the anterior complex(7, 73, 74). There may also be an absent anterior cerebral

artery on one side. In this situation, the ipsilateral ACA territory is fed by the contralateral A1 across the anterior communicating artery. This is known as an azygous ACA. The recurrent artery of Huebner is a small feeding artery to the caudate nucleus and anteroinferior internal capsule that comes of the distal A1 or proximal A2. Disruption to its flow may give the patient contralateral spastic hemiparesis and sensory loss(75). Endoscopic approaches to the floor of the anterior cranial fossa and the planum sphenoidale require drilling and tissue dissection directly under this complex. The ACAs and their perforating branches supply eloquent cortex and every effort is made to preserve the vessels. Dissection is complicated by the fact that anterior skull base pathology such as planum sphenoidale and tuberculum sella meningiomas often shift the anterior cerebral arteries from their usual anatomical position(73, 76, 77). Careful attention must be paid to the pre-operative imaging to ensure that the surgeon is aware of where they are likely to be encountered.



Two Views of the anterior cerebral artery complex in an injected cadaveric specimen (A1 – A1 segment anterior cerebral artery; A2 – A2 segment anterior cerebral artery; RAH – recurrent artery of huebner; ACoA – anterior communicating artery; Ch – Chiasm)(73)

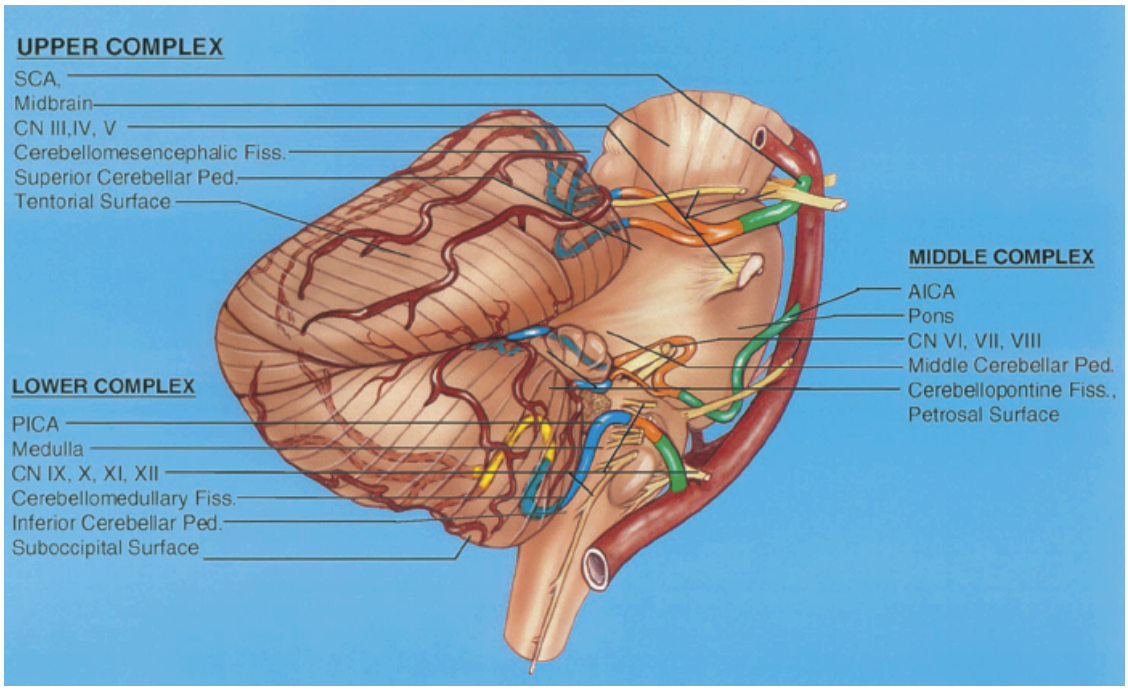


Endoscopic, endonasal view of the suprachiasmatic cisternal region post resection of tuberculum sellae meningioma (GR:Gyrus Recti; ON: Optic nerve; A1: Pre-communicating segment of the anterior cerebral artery; A2: Post-communicating segment of the anterior cerebral artery; Ch: Chiasm; Ps: pituitary stalk; AcoA: Anterior communicating artery; H: artery of Heubner)(78)

Basilar Artery Region and Posterior Circulation Anatomy

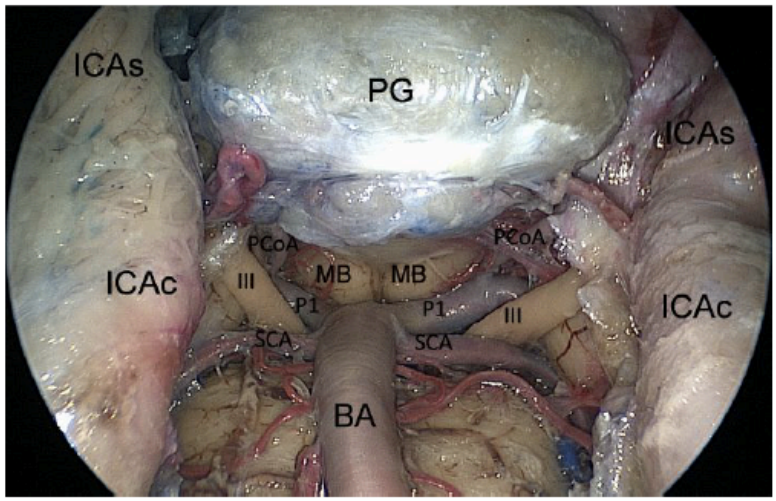
The posterior circulation may also be encountered in endoscopic skull base surgery. The paired vertebral arteries enter the cervical spine at the C6 level in the majority of patients. They travel superiorly through the foramen transversarium, lateral to the spinal cord, and then loop posteriorly and then anteriorly over C1 to enter to skull through the foramen magnum. They give off a posterior inferior cerebellar artery (PICA) and then unite to form the basilar artery (BA), which is generally at approximately the level of the ponto-medullary junction(2, 79, 80). The anterior spinal artery is given off at this point and runs down the anterior aspect of the spinal cord in the midline. The BA then runs in roughly the midline plane (but can deviate significantly to either side) up the anterior aspect of the brainstem, gives off the anterior inferior cerebellar arteries (AICA) and the superior cerebellar arteries (SCA) and then ends by dividing into the posterior cerebral arteries (PCA). There are

numerous brainstem perforators and branches to the labyrinthine apparatus given off along its course.

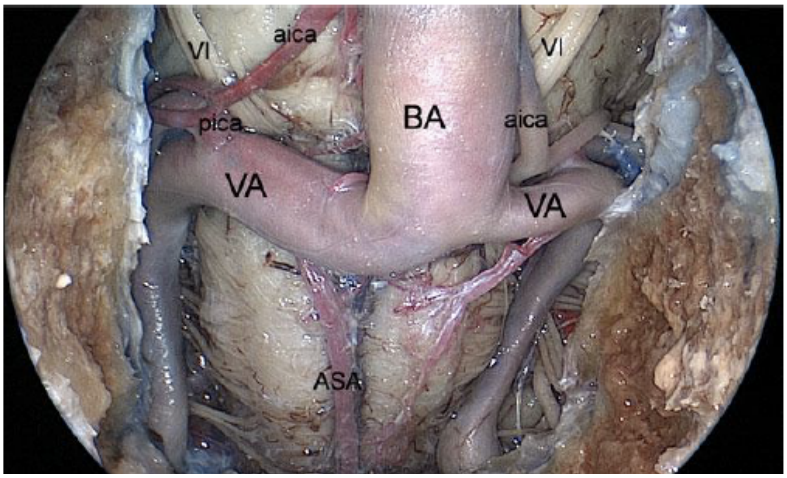


Brainstem and cerebellar perforators given off by the basilar artery (Rhoton)(79)

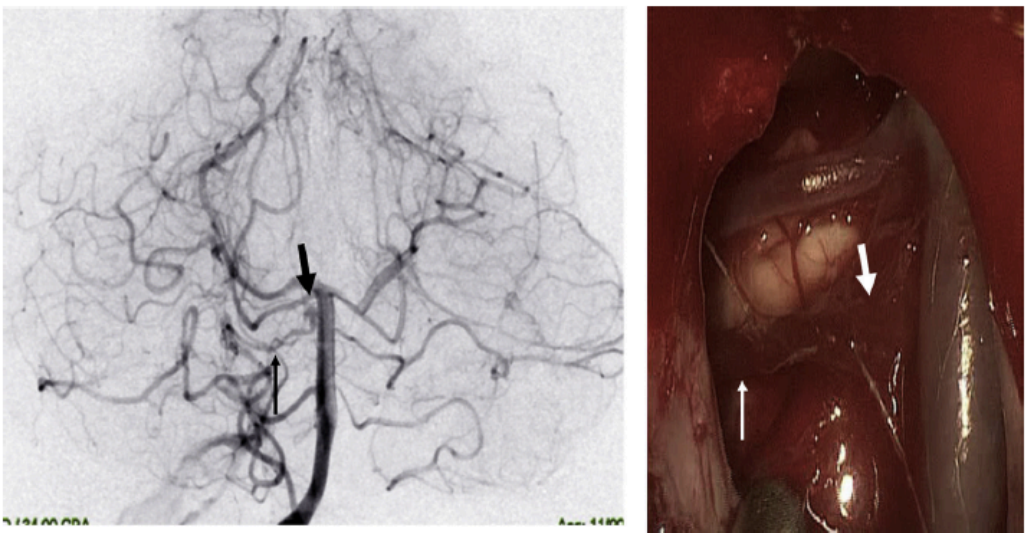
The passage of the basilar and its perforators are of interest to skull base surgeons as more extended endoscopic skull base approaches involve removing part or all of the clivus to access the posterior fossa. A number of pathologies exist in this area that may be amenable to endoscopic approach. These include aneurysms and vascular malformations which may not be amenable to endovascular treatment(7), chordomas that originate from the clivus, or to remove pannus from anterior to the spinal cord in the case of arthritic conditions and instability(18). Again, the caveats to endoscopic approaches apply – these are primarily for midline pathologies however more recently, these have expanded to include lateral conditions usually treated with retrosigmoid craniotomies(81).



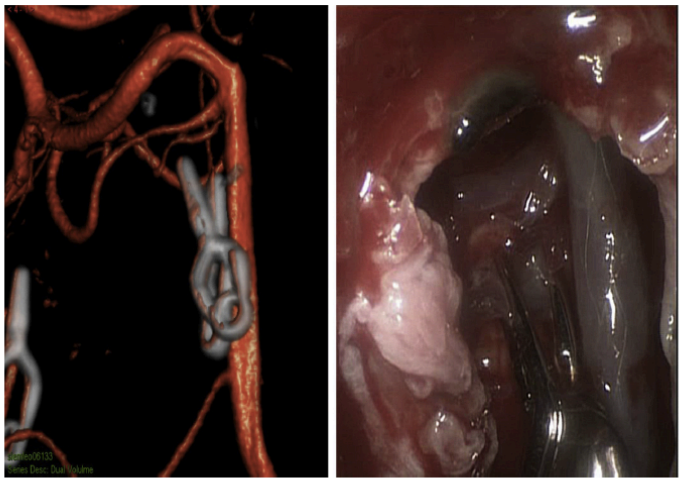
Endoscopic, endonasal view of the superior third of the retroclival area. (PG: pituitary gland; MB:Mamillary bodies; ICAs Internal carotid supracavernous segment; ICAc: Internal carotid cavernous segment; III: Third nerve; BA: Basilar artery)(78)



Endoscopic, endonasal view of the inferior third of the retroclival area. (VA: Vertebral arteries)(78)



(Left) Cerebral digital subtraction angiogram demonstrating 4mm right distal basilar perforator aneurysm feeding small arteriovenous malformation. (Right) Intraoperative image demonstrating basilar region aneurysm with endoluminal stent in situ(82)



3D CT angiogram and intraoperative image showing aneurysm clipping of basilar perforator aneurysm (82)

Bleeding from the BA in endoscopic approaches is difficult to control. It is an artery at least as vital as the carotid in terms of its supply of eloquent brain (primarily the brainstem) and complete occlusion is usually fatal or severely disabling(83). Bleeding may also occur from any number of small brainstem perforating vessels which are generally unable to be coagulated without causing neurological deficit(7, 84, 85). This is a deep approach, often at the limits of instrument length and ensuring that there is a clear operative and visual field is

vital. There is a clear need for a haemostatic agent that is able to stop bleeding from the BA or its branches without causing occlusion or parent vessel thrombosis.

Carotid occlusion

The brain does not store glucose and is therefore highly susceptible to damage in the event of disruption of its vascular supply. This is most commonly due to focal embolism or thrombus but may also occur as a result of global hypoperfusion and hypotension(86, 87). It makes up 2% of the adult human body weight but receives 25% of cardiac output and utilises 20% of total energy production(88). This energy is used primarily for neuronal signalling and maintaining ion gradients across cells utilising the sodium potassium ATPase pump(86). The circle of Willis connects the two hemisphere's vessels through the PCOM and ACoA. There is wide anatomical variation and most authorities agree that this is fully patent in only around half the population(89, 90). Scoring systems have been proposed to determine the potential function patency of the circle by radiologically measuring the ACOA and PCOM and PCA segment 1 to give a score however it remains to be validated in large case numbers(89).

In theory, acute unilateral ICA blood flow disruption should result in contralateral ICA and VA flow reaching the hemispheres through the circle of Willis(91). In practice, however, this is highly variable. Techniques to control haemorrhage in endoscopic skull base surgery must take this factor into account and maintain patency of flow through the ICA as much as possible. The evidence from neurosurgical literature regarding the temporary proximal clipping of feeding vessels indicates that interruption to the blood supply of <3 minutes is generally well tolerated, with the brain likely deriving oxygen and glucose from collateral

perfusion, however longer times risk permanent neuron and astrocyte death(92-94). Care should also be taken when extrapolating this data to entire anterior circulation or whole hemisphere models which would occur with sudden, complete ICA occlusion. The risk of cerebral ischaemia or infarction post sacrifice of the carotid artery is difficult to quantify however even in patients who have passed a balloon occlusion test (where a balloon is inflated in the carotid artery to occlude flow, the patient kept awake and monitored for neurological change), rates of neurological injury can be as high as 4.8%(95-97). This injury may not be immediately apparent and there is a risk of ongoing development of delayed cerebral ischaemia of 1.4% per year(98). In open cranial surgery for aneurysm or extra-cranial/intracranial bypass, temporary clips may be applied to the parent artery proximal to the aneurysm to allow manipulation of the fundus and dissection around the aneurysm neck(80, 99). This temporary clipping is tolerated to varying degrees depending on a number of factors including collateral circulation, systemic blood pressure and blood rheology. In some instances, ischaemia and infarction may occur(100, 101). Woertgen and colleagues performed an audit on 292 patients treated for aneurysm. 29% demonstrated an ischaemic lesion on CT post-operatively. 58% of these patients had undergone temporary clipping as part of their surgery. Temporary clipping time correlated with increasing vasospasm and the development of infarction (34% vs 22% $p<0.006$)(100).

Haemorrhage control

Bleeding in skull base surgery

Bleeding in skull base surgery is a feared complication. If one takes the 'Swiss cheese' model of root cause analysis and applies it to surgery in this region, one can see a number of

factors or ‘slices’ lining up to potentially contribute to, not just bleeding in this area, but increasing the difficulty in stopping it once it occurs(102-105). The skull base is an anatomically constrained area with brain parenchyma, neural and vascular structures, bony prominences and canals, acute angles and dural reflections all existing within a few millimetres of each other. In addition to this, there are a wide range of pathologies that can occur in this area. These include neural tumours, nerve-sheath tumours, dural-based tumours, bony skull lesions, cavernous sinus lesions, and, importantly, vascular aneurysms and malformations given the location under the brain of the circle of Willis(6-8, 29, 102-114).

The control of haemorrhage in endoscopic approaches is of concern to surgeons; and has long been recognised as such. Small working corridors and the unique sensitivity of neural structures to damage from both compressive forces and toxic metabolites of red blood cell breakdown make it crucial that surgeons are prepared to deal with this complication. The endoscope lens must remain free of blood to avoid the surgeon operating ‘blindly’. To achieve this, suction must be introduced into the field and further instruments to control bleeding are then utilised. Space and angle restrictions in endoscopic skull base surgery mean suturing and haemostatic clamps are not usually a realistic option. It is also much more difficult to introduce traditional bipolar cautery, although endoscopic skull base instruments with curved and angled tips have been designed(115). It is obvious that the two-surgeon, four-hands technique is of great help in this situation. It is important that the approach is planned so that there is adequate space to introduce and manipulate multiple instruments concurrently.

Increasing use of anticoagulant medications including aspirin (thromboxane A2 inhibitor), clopidogrel (ADP receptor inhibitor), dabigatran (direct thrombin inhibitor), warfarin (vitamin K carboxylation inhibitor) and apixaban (GPIIbIIIa inhibitor) in an aging population makes achieving haemostasis even more difficult unless these medications can be ceased pre-operatively. This is sometimes difficult given comorbid conditions and, occasionally, the requirement for urgent surgery(116). In addition, the underlying parenchyma being operated on is often abnormal, with tumours, trauma, and infections with subsequent hyperaemia. This facilitates further bleeding and damage to neural structures.

Carotid artery injury in endoscopic endonasal skull base surgery

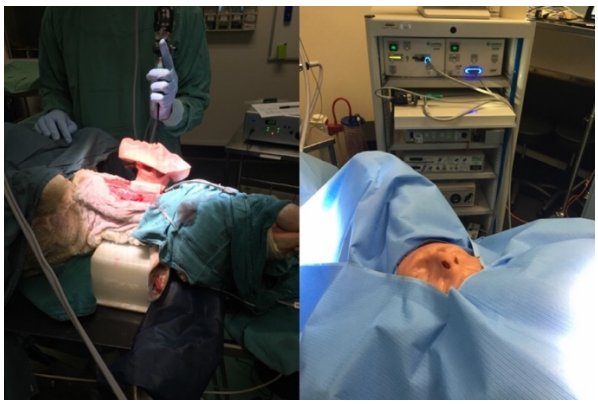
Incidence

The incidence of major vessel injury in uncomplicated midline endoscopic skull base surgery varies in the literature but is quoted as being between 0.16% and 1.1%(117-123). This is in contrast to the much lower rate in endoscopic sinus surgery (<0.25%) where the sphenoid sinus is either not entered or not drilled extensively(120). These rates are much higher in the case of extended endoscopic approaches (EEA) where the instruments and endoscope may come into more direct contact with the carotid artery or require more extensive bone removal. These EEA generally extend the surgical corridor more rostral or caudal and may, through the use of angled scopes, also extend the boundaries of the approach laterally. Couldwell et al & Frank et al and Gardner et al report rates of 5-9% in series of craniopharyngiomas, chordomas and chondrosarcomas, which, by virtue of their location and tumour consistency, invariably require more bone removal or dissection(117-120, 124-126). Surgeons who have been involved with greater than 500 transphenoidal approaches

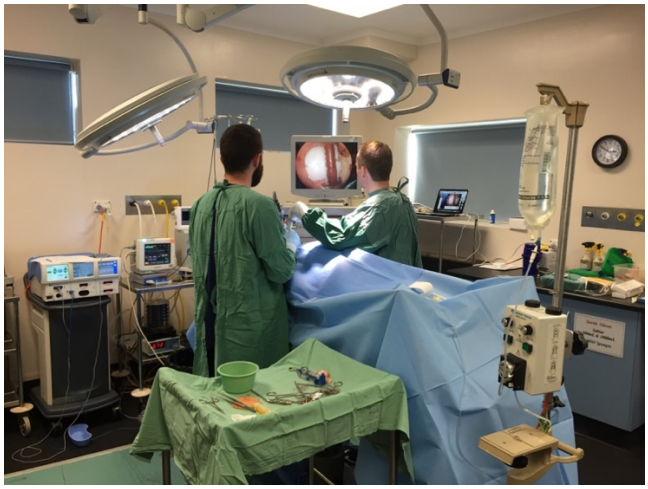
are thought to have a 50% chance of having seen a carotid artery injury(127). Given the increasing use of endoscopic transphenoidal surgery to access more complex pathologies, it is reasonable to assume that rates will only increase despite our knowledge of predisposing factors.

Initial planning and management

The most important initial management step occurs before the operation even begins and requires that the surgeon be proactive in assessing the likelihood of the injury occurring. Tumours that encase the carotid, or are at least adherent in a plane >120 degrees are at much higher risk and these cases should be discussed in a multi-disciplinary meeting with skull-base otolaryngologists, skull-base neurosurgeons, radiologists, endovascular radiologists & neurosurgeons and ophthalmologists(128). The value of developing a team-based approach to skull base surgery cannot be over-stressed, and extended endonasal cases should not be attempted before developing a collaborative team able within the institution. Additionally, team-based training in vascular injuries courses on animal models, such as that developed by Valentine and Wormald is invaluable and allows surgical teams to practice managing this stressful scenario(117-119).



Endoscopic trainer utilised on sheep carotid artery injury model(129)



Training on the animal model of endoscopic haemorrhage control(129)

Risk Factors

It is important to review the pre-operative imaging closely to determine if there are any pre-disposing factors that may increase the risk of ICA injury and allow modifications to surgical technique at any pre-determined 'danger points'. These factors may be divided into anatomical factors, tumour factors and patient factors.

Anatomical factors

The posterior wall of the sphenoid sinus is dehiscent in 4-22% of cases, leaving only mucosa or dura to surround the carotid. Even if present, the wall should not be considered thick enough to protect the artery from the drill or other instruments(130). Bone windows on pre-operative CT may assist with this but should not be relied on. The carotids may also deviate towards the midline. The ICA are usually at least 12mm apart but have been described as close together as 4mm in their cavernous segment and, rarely, even touching each other(131). These so called 'kissing carotids' have been considered as having contributed to ICA injury in a number of case reports(53). CT or MR angiography may be performed but, even on plain MRI, flow voids may be seen on axial slices and the distance

between these measured. There may also be bony septations and spicules within the sphenoid and cavernous sinuses which, whilst not dangerous in themselves, may either require the use of instrumentation to remove, with consequent risk of ICA injury by either bone fragments or the instruments used to remove them. Cavernous segment ICA aneurysms make up to 12% of total intracranial aneurysms in some case series and require the surgeon to carefully plan any endoscopic approach(66, 74, 96, 132). It is advisable to consider treating these prior to attempting any endoscopic endonasal skull base approaches(133-135). Rupture of this intra-operatively may cause torrential bleeding and, potentially, the development of a carotid-cavernous fistula (CCF). These may be treated with flow diverting stents with or without coil insertion into the fundus, however it is important to recognise that this will require the patient to take antiplatelet therapy for at least 3-6 months post stent insertion which may factor into further surgical planning.

Tumour factors

Tumours may themselves adhere to the ICA and any extension into the cavernous sinus should be approached with caution, especially if this extends >120 degrees around the ICA. There is a risk of tumour adherence to the ICA. This not only predisposes the ICA to injury when the tumour is dissected off the carotid, but this dissection may result in vasospasm as the carotid is handled(128). This may manifest as neurological injury or as elevated blood pressure as the patient attempts to maintain cerebral perfusion pressure with impaired autoregulation. The size of the tumour is an independent risk factor. Tumours that require extensive bone removal for exposure will tend to require exposure of more of the ICA.

There also appears to be an increased risk of carotid injury with pituitary tumours secreting growth hormone or adrenocorticotrophic releasing hormone (ACTH)(136, 137). This may be

due to tumour size or invasion but also due to as yet undefined influences of Insulin-like growth factor-1 (IGF-1) or cortisol on the arterial wall(120).

Patient factors

Previous transphenoidal surgery may result in aberrant anatomy and adhesions. It may also be difficult to ascertain the precise amount of anatomical disruption and bone removal that has previously occurred. Previous radiation to the region may result in scarring and adhesions with loss of normal tissue plans and/or increased friability of vessels. Bromocriptine therapy appears to increase the risk of ICA injury, possibly due to adhesions and fibrosis and acromegaly patients have a tendency towards more ectatic and tortuous carotid arteries(120, 128).

Factors contributing to increased risk of ICA injury

Anatomical	Sphenoid wall defect Carotid proximity Bony septations and spicules Cavernous segment ICA aneurysm
Tumour	Encasement of ICA Surrounding >120 degrees ACTH secreting tumours
Patient	Previous surgery Previous radiotherapy Bromocriptine therapy Acromegaly

Theatre setup

Ideally factors such as anatomical variation or the possibility of tumour erosion into the artery or cavernous sinus will have alerted the surgeon to the danger of carotid artery injury however this is not always possible or indeed reliable(138, 139). The entire surgical team

must always be alert to the possibility of injury. A pre-operative briefing involving all staff that identifies the risks involved and the plan should such an injury occur is a highly valuable step. The patient should always be cross matched with compatible blood readily available, especially in more extended endonasal approaches. Anaesthetic staff should be aware of the possibility of ICA injury and have a plan in place in the event that it occurs. Large bore IV access and invasive arterial monitoring should be considered prior to it being required. Neuro-navigation should be utilised as, whilst it does not replace a thorough knowledge of anatomy, it gives the surgeon greater confidence in identifying anatomical structures intraoperatively. A Doppler ultrasound device with an angled probe may also be useful to confirm the location of the ICA intraoperatively.

Theatre staff should have patties, gauze and oxidised cellulose (surgicel snow(ethicon) is generally preferred for its absorptive capacity) on the setup for all cases and the endoscope should have a lens cleaning system attached. There should be two 10 French suction devices on the setup. These suction devices should be checked to ensure that they work and do not require changing immediately prior to any bone removal and at regular intervals. Most authors advocate the use of blunt instruments, such as suction Freer dissectors and pituitary ring curettes, when working adjacent to the ICA and that bone removal should take place with diamond rather than cutting burrs(7, 117-122, 125, 128, 140-142). These should all be ready on the instrument tray at the commencement of the case. It may also be advisable when using the kerrison punch that a twisting or pulling motion is not used but that the sharp edges of the instrument are allowed to completely transect the bone before removal. The lower blade of the Kerrison should be kept in contact with the bone when approaching

the carotid as folding the vessel wall between the Kerrison upper and lower blades is the most common cause for vascular injury when removing bone over the carotid(128).

Injury types

It is important to be aware of the different injury types that are seen in carotid vessel injury. These range from linear incisions to stellate or large wall defects caused by kerrison-type punches or high-speed drill injury. Whilst initial management and resuscitation is the same, the likelihood that different techniques may work varies accordingly and decision making should reflect this(122).

Initial management

The immediate management of carotid artery injury in endoscopic surgery is a team-centered approach involving coordination between nursing, anaesthetic and surgical staff(138). Communication with the anaesthetic staff early is vital and they should immediately begin volume resuscitation of the patient before their haemodynamic parameters are affected. It is important to maintain normotension or even mild hypertension to maintain cerebral perfusion despite the temptation to lower the blood pressure to assist haemostasis. Theatre staff should check the suction to ensure that the bag isn't becoming full which may lead to a stoppage of suction when most required. Theatre staff should also activate the on-call angiography team.

ICA injury is much easier to manage with a 2 surgeon, 4 hands approach. Consideration should be given to obtaining a second surgeon's assistance if they are not already in the room. A third surgeon may also be utilised to harvest muscle from the abdomen or lateral

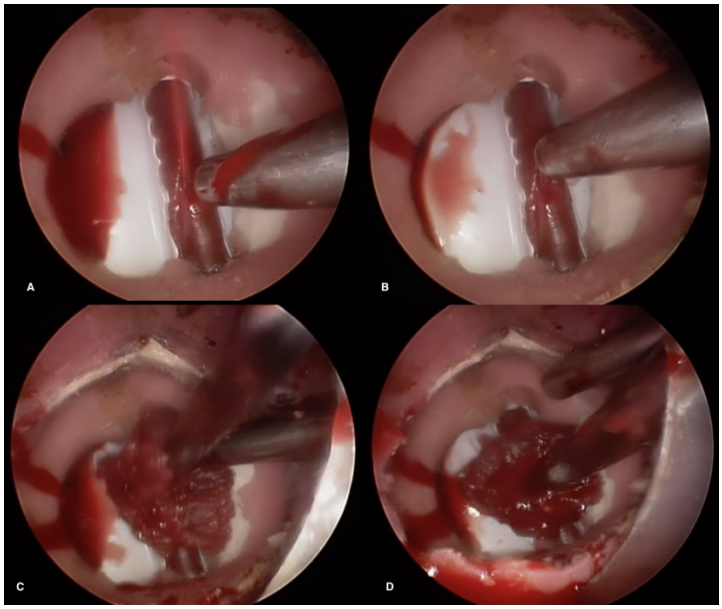
thigh. Visualisation is key to management and the endoscope lens must remain free of blood. To achieve this, suction must be introduced into the field, generally down the side closest to the bleeding with the endoscope introduced down the opposite nostril to just proximal to the posterior septal edge, allowing it to be used as a shield from the blood jet(143, 144). The sucker is used to direct the flow of blood away from the lens. The second sucker should be placed adjacent to the endoscope to ensure it is kept clear of blood. This can then be moved in further to hover over the bleed point. It is obvious that the two-surgeon technique is of great help in this situation and planning the approach such that there is adequate space to introduce and manipulate multiple instruments concurrently is key. Routine removal of the posterior septum to create a single posterior cavity is helpful in this regard as the surgeon may use both nostrils to pass instruments and endoscope.

Once visualisation has been achieved, pressure can be applied to the bleeding point with patties, gauze or surgical snow(Ethicon) as a temporising measure. It is tempting to pack the entire nasopharynx in an attempt to stop the bleeding but this should be avoided as much as possible as it impedes access to the bleeding point. Depending on the point reached in the operation, this practice may stop the bleeding from exiting the nose but blood may be directed intra-cranially with detrimental results. Over-packing may also completely occlude the ICA and any other vessels exposed with potentially devastating results. In theory, acute unilateral ICA blood flow disruption should result in contralateral ICA and vertebral artery flow reaching the hemispheres through the circle of Willis. In practice, however, this is highly variable. Techniques to control haemorrhage in endoscopic skull base surgery must take this factor into account and maintain patency of flow through the ICA as much as possible. The evidence from neurosurgical literature regarding the temporary proximal

clipping of feeding vessels indicates that interruption to the blood supply of <3 minutes is generally well tolerated with the brain likely deriving oxygen and glucose from collateral perfusion however longer times risk permanent neuron and astrocyte death(92-94). Care should also be taken when extrapolating this data to entire anterior circulation or whole hemisphere models which would occur with sudden, complete ICA occlusion.

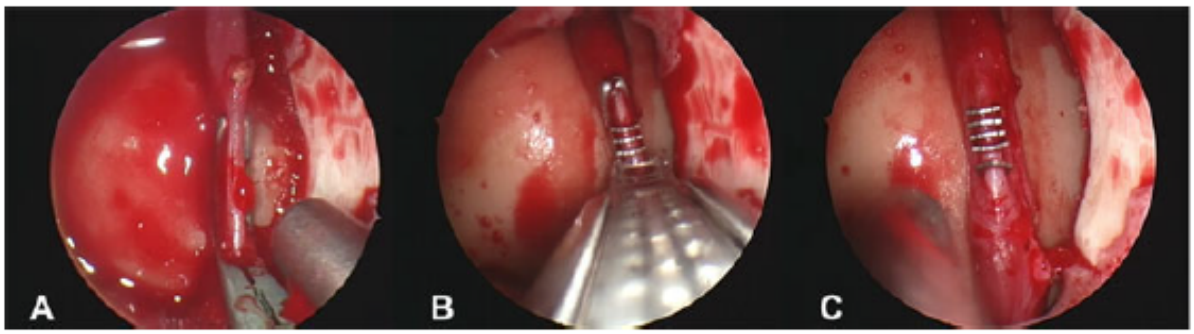
Haemostatic options

Traditionally, 1 to 2cm^3 of muscle has been harvested from the abdomen or thigh. This is crushed and placed over the bleeding point and held in place for at least 5-7 minutes (although this may take up to 12 minutes to adhere)(117-120). It is important to hold this in place with sufficient pressure to stem the bleeding but not to completely occlude the artery. The crushed muscle is thought to activate a platelet/fibrin plug which seals the artery whilst the high flow within the lumen maintains patency of the parent vessel(145). No attempt should be made to remove the muscle once haemostasis has been achieved. This muscle patch should be reinforced with a previously raised septal flap if the carotid is in the nasal cavity and covered with oxidised cellulose and fibrin glue if intracranial(128). Nasal packing may then be placed however it is important that this is not packed too tightly as this may occlude distal ICA flow. This method is suitable for both linear and stellate or jagged type injuries(122).



Haemorrhage control in a sheep model. A – Blood jet from ICA, B – Sucker in contralateral nostril used to direct flow away from endoscope lense, C – Crushed muscle patch being applied, D – Crushed muscle held in place for 5-7 minutes(128).

If the injury is clearly able to be visualised and accessed with instruments, then direct vessel closure may be attempted. This is usually more applicable for linear injuries. Padhye et al have shown in a sheep model of haemorrhage that a curved T2 aneurysm clip may be applied to the wall of the vessel without occluding the lumen(122). Direct closure may also be achieved with a 'pincer' type metal clip (AnastoClip – LeMaitre Vascular). The vessel walls are held together by a Wormald endovascular clamp and the clips applied sequentially along the injury.



A – Wormald vascular clamp across carotid laceration in a sheep model of haemorrhage. B – AnastoClips being applied with clamp in situ. C – Anastoclips post clamp removal(122)

Bipolar cautery devices have been designed specifically for endoscopic endosnasal surgery and attempts have been made to use this technique on carotid injury(115). Whilst this may work for smaller bleeds, there is the risk of the cautery device becoming adherent to the bleeding vessel with subsequent tearing when attempts are made to remove the instrument. There is some experimental evidence to suggest a higher rate of secondary bleeds and complete vessel occlusion than other methods and its use is not recommended in ICA bleeding(128).

Other haemostatic patches and glues

There are a number of other commercially available products that have been trialled with varying degrees of success. A high flow/high pressure bleed will tend to wash away thrombin-based powders such as Floseal(Baxter) or Surgiflow(Ethicon) and fibrin/thrombin glues(117-120).

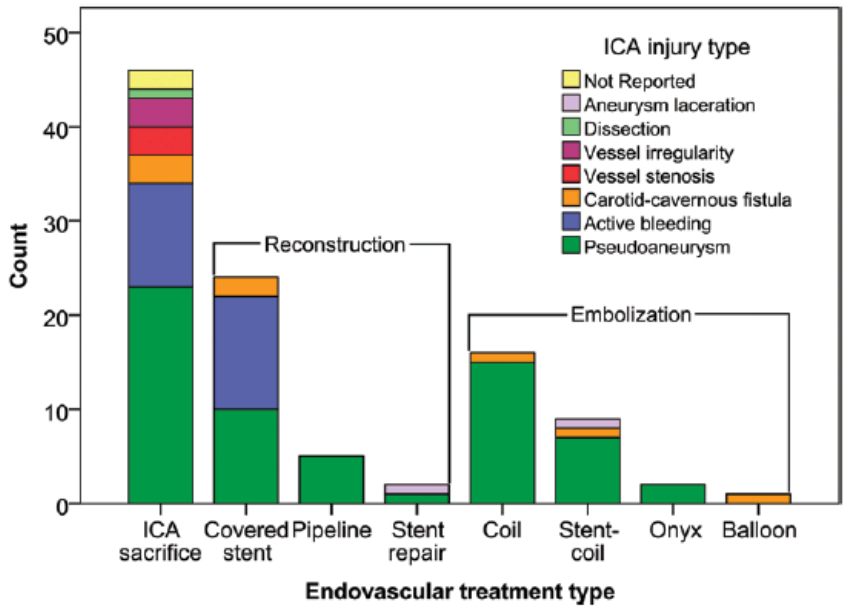
Endovascular control

If haemostasis is not able to be achieved with these methods, then endovascular intervention may be urgently required to occlude the ICA(146). It is important that endoscopic techniques are attempted first as endovascular intervention tends to result in artery sacrifice. Balloon or coil occlusion should be performed at the site of injury to prevent ongoing haemorrhage from antegrade or retrograde filling(146). It is also important to avoid balloon or coil migration and subsequent occlusion of the ophthalmic artery (a complication discussed later). It is important to emphasise that endovascular intervention at this point is a life-saving procedure and a pre-sacrifice balloon test occlusion is unlikely to change management at this stage, unlike in the elective setting(147).

Post-operative management

Once haemostasis has been achieved, the case should not proceed further as there is risk of further injury or dislodgment of the muscle patch or clips. The patient should undergo a formal angiogram to determine if a dissection, pseudoaneurysm or carotid-cavernous fistula has formed(138). It is advisable to perform this immediately under the same anaesthetic to avoid blood pressure fluctuation or a valsalva manoeuvre being performed by the patient on emergence and extubation, which may potentially cause the patch to 'blow off' the vessel. The surgeon should attend the initial formal angiogram to determine if the ICA is patent, especially if large amounts of nasal packing has been left in situ. If this is 'over-packed' the patient may need some of this removed to allow distal flow in the ICA. It is important to note that whilst we have discussed the obvious and immediate ICA bleed, a smaller, unrecognised injury may occur and manifest in the weeks or months following surgery(148).

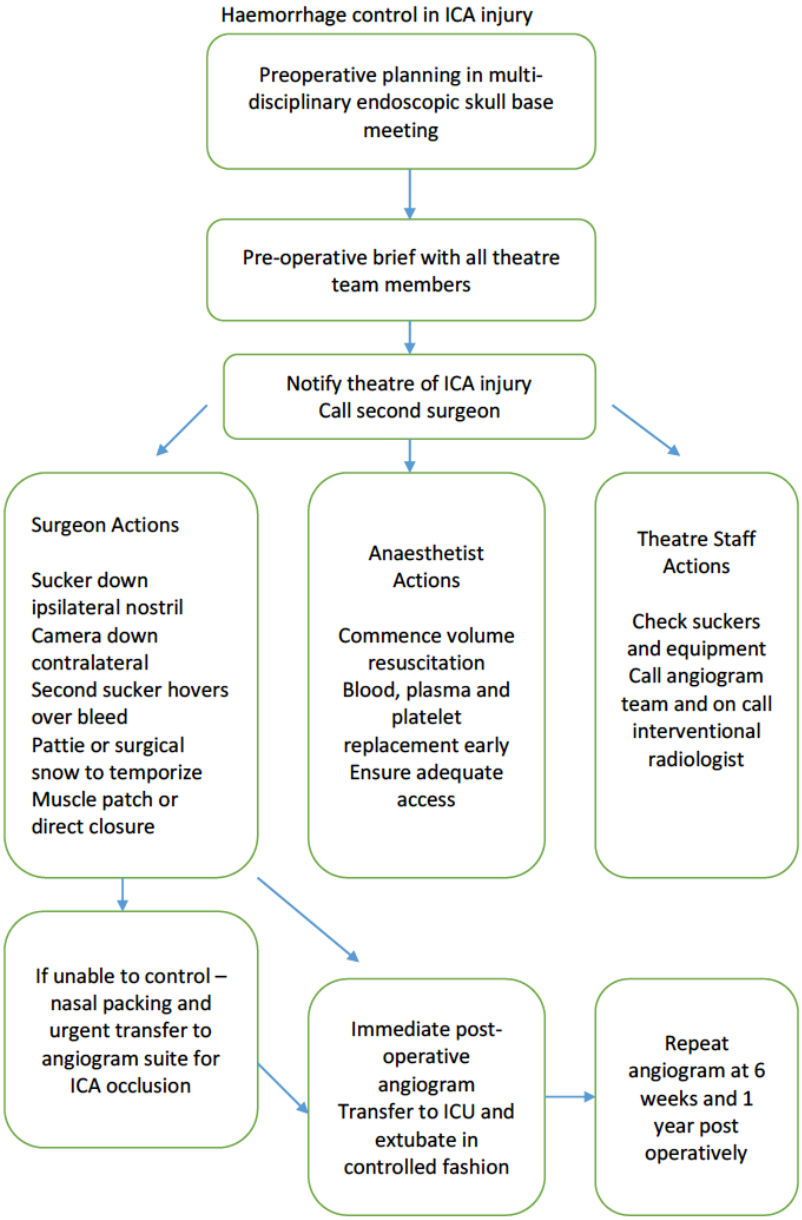
The decision to extubate the patient depends on whether any vascular anomaly is discovered at the initial post-operative angiogram. If none is discovered then a reasonable option is to wake the patient slowly in ICU, avoiding blood pressure fluctuations and ensuring cerebral perfusion is maintained. Sylvester et al performed a retrospective institutional and literature review of the types of ICA injury and the subsequent endovascular treatment modalities(147).



Types of ICA injury and subsequent treatment modalities (from Sylvester et al) (147)

If an arterial wall injury such as a dissection flap is seen on formal angiogram, it is safe to assume that the intima has been disrupted with potential for thrombosis to occur. Some authors advocate intravenous heparin administration at the time of injury(138). Much of the rationale behind this has been extrapolated from stroke literature which does tend to limit external validity when applying this data to the ICA injury patient population. The CADISS trial, designed to determine the optimal treatment for carotid dissection was unable to make a recommendation regarding whether antiplatelet agents or anticoagulant agents should be used(149). Our practice has been to wait until the immediate post-operative angiogram to make a judgement regarding the risks and benefits of anticoagulation at this point.

Flowchart: Haemorrhage control in ICA injury



(150)

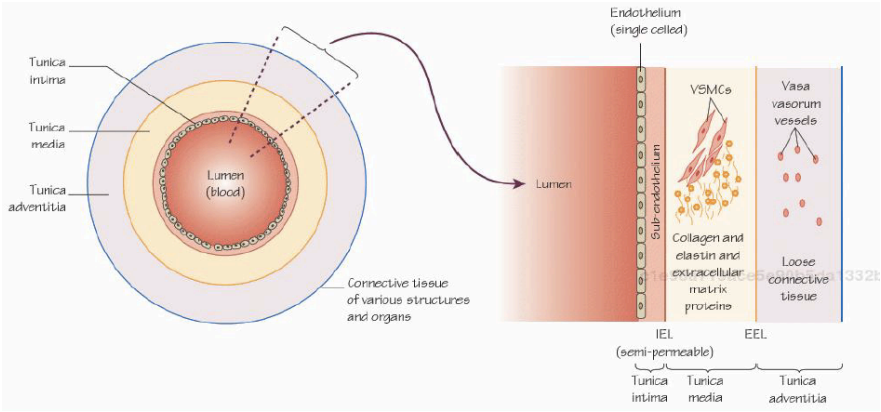
Complications After Arterial Injury

Arterial anatomy and response to injury

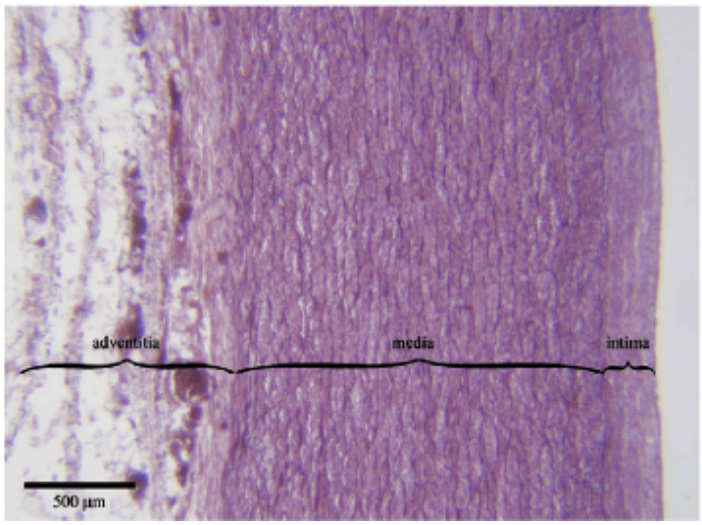
There are three layers to the carotid artery. The tunica intima, the tunica media and the tunic adventitia. The endothelial layer of the arterial wall prevents the circulating blood from interacting with procoagulant proteins such as tissue factor and collagen(151). In the

event of disruption of this endothelium, whether by surgical injury or atherosclerotic plaque rupture, underlying collagen and von Willebrand factor are exposed and platelets undergo activation and adherence(152). In the event of a laceration or tear of all three layers, as can occur in an injury during endoscopic surgery, blood escapes until the surgeon is able to apply pressure to the wound and close the defect. The anastoclip and primary closure with suture are two ways in which this can be performed(108, 121, 122, 140). It is important when closing the arterial wall to ensure that all three layers are included in the closure as the possibility exists of a dissecting pseudoaneurysm forming if the intima or media are not tightly opposed(153, 154).

Evidence for the pathophysiological mechanism of this is extrapolated from histopathological studies of spontaneous and traumatic carotid dissections(155). Blood may track between the intima and media layers and generate a false lumen. Pulsatile flow peaking at systolic pressures may force blood along this neo-layer. This may eventually thrombose or may rupture out into the surrounding tissues. In arteries where there are branch vessels, the dissection may occlude the take-off of these vessels and cause distal ischaemia. This propagation is usually anterograde but may occur in a retrograde fashion as well(156). Thrombosis of the aneurysm is not benign. The thrombosis may cause intraluminal clot initiation via exposure of the luminal contents to pro-thrombotic stimuli. This may cause the entire lumen to become blocked. There may also be extensive disruption of vascular smooth muscle layers(123, 135, 157).

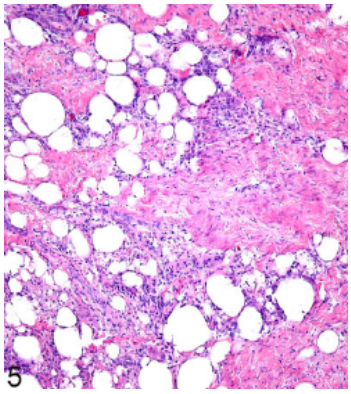


Anatomy of the arterial wall(158)

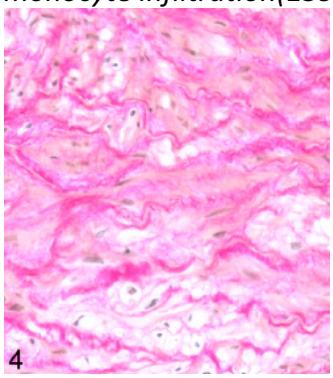


H&E *Histology of human carotid artery wall demonstrating three layers with media showing large amounts of smooth muscle cells (155)*

Histology of pseudoaneurysm is not well described however there are cases described in veterinary pathology. The adventitial side wall of the pseudoaneurysm contains fibroblasts in a myxomatous matrix. These form whorls and small to medium blood vessels are present. Smooth muscle cells that usually form the vascular wall are replaced with collagen fibres in non-linear orientations and there are large numbers of neutrophils, multi-nucleated giant cells and moderate amounts of adipose tissue(159).



H&E of pseudoaneurysm in Fresian horse demonstrating fibrosis, fat and inflammatory monocyte infiltration(159).



H&E stain of pseudoaneurysm in Fresian horse demonstrating disorganised collagen fibres(159)

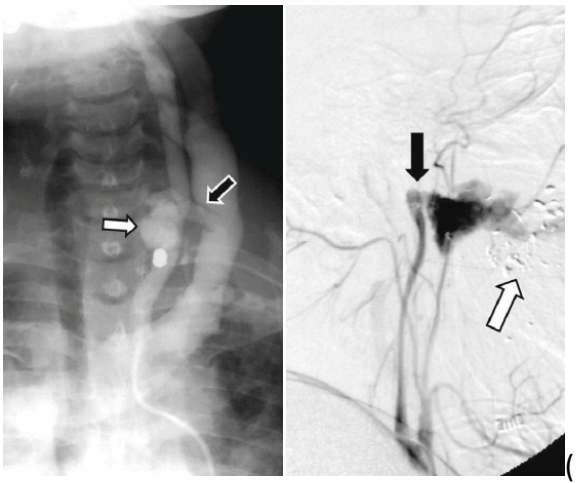
Histology and immunohistochemistry studies have been performed to determine the precise sequence of events in arterial wall remodelling and healing(160). Bauriedel et al used a rat carotid artery model of injury to show the changes that occur at 0, 4, 24, and 48 hours and 4, 7, 14, and 28 days post injury. Their experiment was designed to delineate the exact cells involved to determine if these could be inhibited to prevent stent thrombosis post angioplasty. Rats underwent a balloon angioplasty to damage the endothelium of the vessel wall. 6 rats were then sacrificed at each time point and their artery wall examined. They demonstrated the development of a neointima at 4 days post injury. Intimal cells arranged themselves in concentric rings over the course of 7 days. Immunohistochemistry demonstrated high reactivity for dendritic cells at the 7-day mark. By the 28-day mark, the

neointima displayed evidence of alpha-smooth muscle actin positive - Smooth muscle cells. Whilst this is not a sharp surgical injury, it does provide some evidence for the time course of neo-intimal regeneration(160).

Pseudoaneurysm

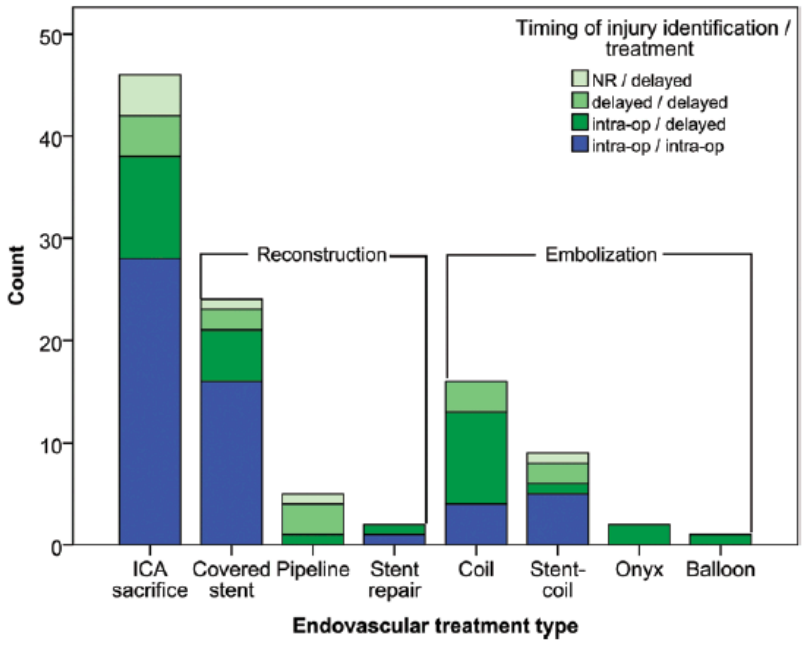
After an ICA injury, there is the potential for the development of a pseudoaneurysm(161). The putative mechanism of development is damage to the vessel in the initial phase with subsequent arterial pulsations forcing blood between layers (sometimes known as a 'false aneurysm') or, more commonly in this context, between the damaged vessel and the patch or packing which has been applied(123, 125, 162, 163). This grows larger over time as the systolic pressure forces further tracking of blood along this dissection layer(164). The most common aetiology worldwide is in penetrating cranial trauma such as stabbing or shrapnel/bullet wounds and the majority of the literature supporting treatment is extrapolated from this(165, 166). Post ICA injury, it is generally considered that the incidence of subsequent pseudoaneurysm development is in the order of 10-35% however some series report up to 66%(7, 111, 121, 125). This is partly a function of the low case numbers in each case series reported and partly because no reliable animal model of this exists. A pooled analysis of case series would seem to indicate that a reasonable estimate would put the rate somewhere between 10-35% across all patients with a patent ICA(7). A recent review of carotid pseudoaneurysm after transphenoidal surgery identified 23 cases in the literature from 1975-2010(167). Intraoperative arterial haemorrhage occurred in 17 (77%) of these patients. The remaining 23% (5 patients) did not have intraoperative bleeding evident but 4 of these patients presented with epistaxis at an average of day 12 post-operatively. The average time between operation and pseudoaneurysm diagnosis was

64 days for those with intraoperative haemorrhage and 83 days for those without. The mortality rate was 9% with one patient dying of epistaxis and another of intracranial hypertension day 1 post stenting(167). Treatment mechanism was heterogeneous as would be expected for a pooled case series over such a long time frame and ranged from conservative management, stenting, coiling, microsurgical clipping and complete carotid occlusion (41% of patients). The authors of this review advocate weekly MRI/MRA post-operatively in the event of carotid injury for 4 weeks with monthly MRI/MRA post this(167). The Adelaide experience has been to obtain a formal angiogram on the day of injury, which is then repeated at 1 week post-operatively and then again at 6 weeks and 1 year post-operatively(58, 121, 122, 168-170). Crucially, as the authors of this review point out, any bruit, ophthalmoplegia or epistaxis in patients who have undergone transphenoidal surgery should prompt a search for a vascular injury(147).

 (171)

Catheter angiogram of common carotid on gunshot victim. Bullet fragment (white) overlies common carotid pseudoaneurysm (left). Digital subtraction angiogram of transected internal carotid post gunshot with contrast extravasation (right).(171)

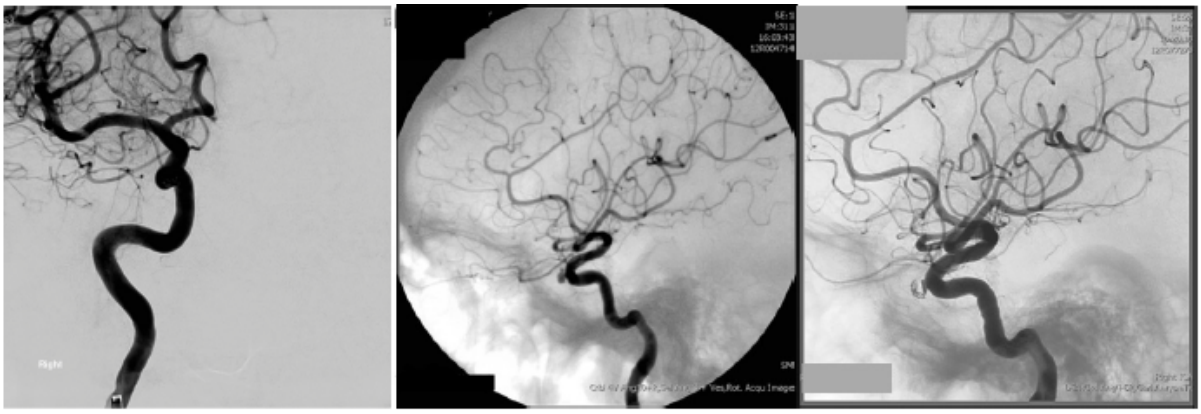
Sylvester et al's review of the literature on ICA injury demonstrates the spectrum of timing in injury recognition(147).



(147)

The treatment of pseudoaneurysm is dependent on size, location, and whether it has ruptured prior to treatment. Given that even a balloon test occlusion cannot reliable predict an individual patient's reliance on blood flow from a particular carotid artery, consideration should be given to preserving the carotid if possible(172). It is possible to perform an endovascular sacrifice of the carotid artery by completely occluding it with coils however this leaves the patient at risk of developing an infarction distal to this(138). Other options include coil occlusion of the pseudoaneurysm with or without stenting of the parent vessel or direct open repair, and remodelling (although this is generally not possible in the cavernous segment of the carotid)(122, 148, 156, 157, 169, 170, 173-175). It may also be possible to use a flow diverting stent that directs flow beyond the pseudoaneurysm and causes stasis and subsequent thrombosis within the aneurysm(173, 176). It is important to be aware of the location of the ophthalmic artery in relation to the pseudoaneurysm. The ophthalmic typically leaves the ICA a few millimetres beyond any injury to the cavernous segment and stent migration may occur, occluding this artery and resulting in potential

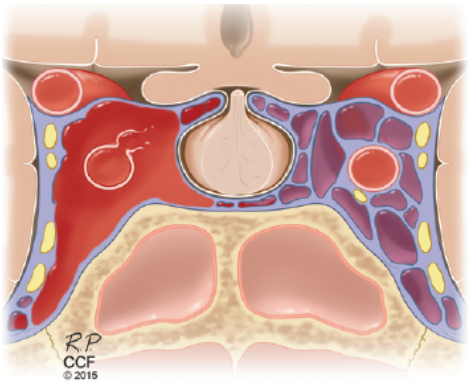
blindness or even death(53, 68). Additionally, rates of stroke following carotid stenting can be as high as 4.5% in the first 30 days and patients are generally treated with dual antiplatelet agents (aspirin and clopidogrel) or similar agents which may delay or preclude further surgery(134, 135, 177-180). A more invasive approach to revascularisation is an extra-cranial to intracranial (EC-IC) bypass with a conduit being sutured from the external carotid to the middle cerebral artery allowing retrograde filling of the MCA and ACA and subsequent sacrifice of the now redundant ICA(181).



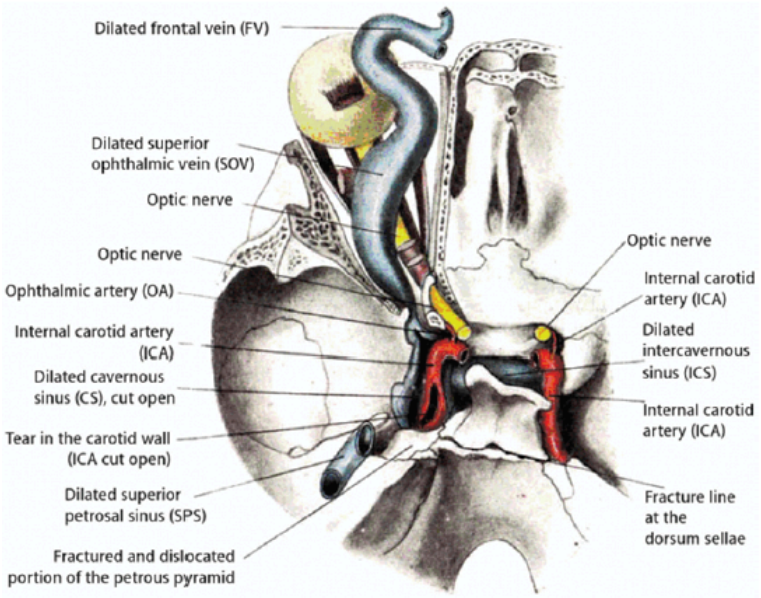
Pseudoaneurysm post ICA injury. A – initial angiogram with no aneurysm, B – Angiogram at 1 week showing cavernous segment pseudoaneurysm, C – Coiling of pseudoaneurysm(150)

Carotid-Cavernous fistula

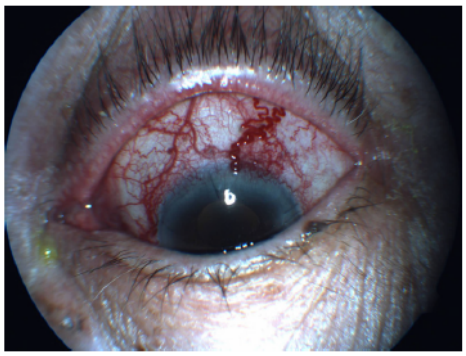
In this situation, arterial blood escapes from a defect in the ICA and enters directly into the venous channels of the cavernous sinus. Signs of this include proptosis, chemosis, conjunctival injection, ocular bruit, blindness due to venous hypertension, ophthalmoplegia due to cranial nerve defects, pulsatile tinnitus, venous infarct of the cortex and pain(182). CT or MRI demonstrates characteristic features of proptosis and 'corkscrewing' of engorged vessels however diagnosis requires formal angiogram and demonstration of early venous filling as blood exits the ICA(123, 182-184). Treatment is performed via endovascular approach with obliteration of the fistula via coils, glue, stenting or combination of these(24, 58, 182-185).



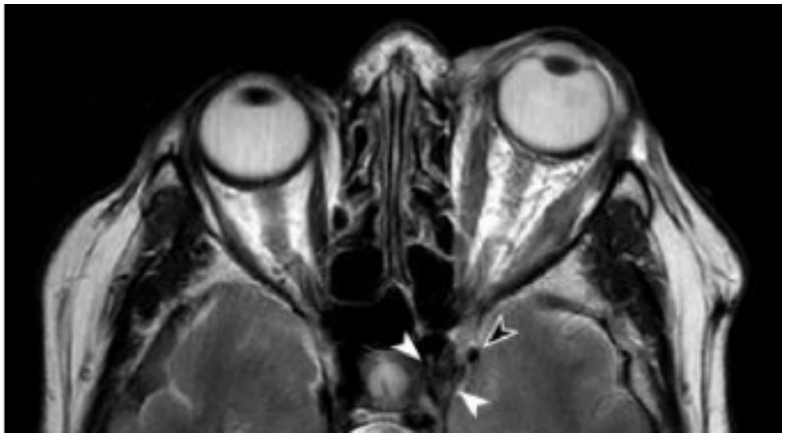
Representation of a coronal section through a cavernous sinus with extravasation of blood from the ICA into the venous channels of the cavernous segment(186).



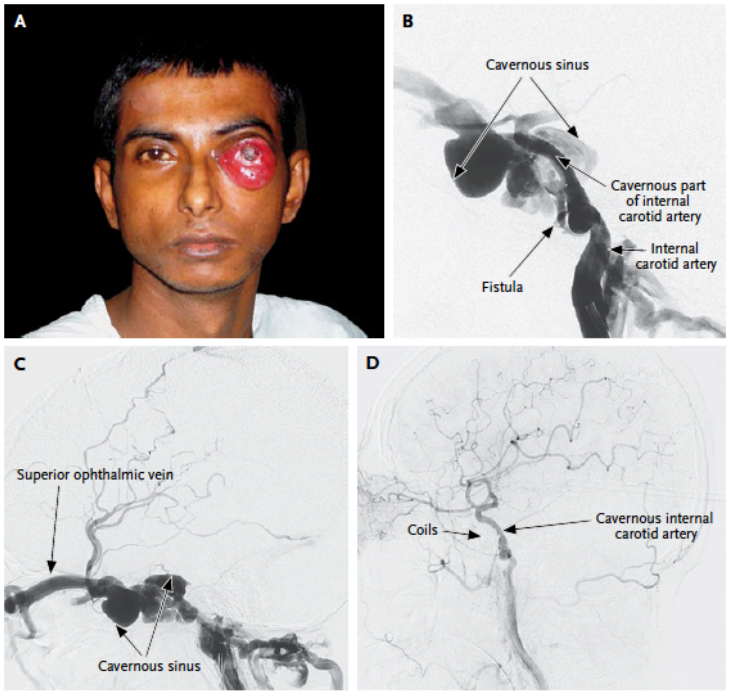
Axial representation demonstrating dilation of the superior ophthalmic vein secondary to CCF with proptosis of the eyeball(186).



Conjunctival and episcleral vessel injection in carotid-cavernous fistula.(182)



Axial T2 MRI of orbits showing left sided venous engorgement and proptosis in carotid-cavernous fistula(182).



A case of carotid-cavernous fistula of unknown aetiology demonstrating marked proptosis, chemosis and injection(A). The initial angiogram is shown at (B) and (C) demonstrating filling of the cavernous sinus in arterial phase and then an angiogram post coiling to obliterate the fistula (D)(185).

Summary

ICA injury is a potentially devastating complication of endoscopic endonasal surgery. It is crucial to have a plan in place before this potentially disastrous situation occurs. Good communication and teamwork is vital to ensure that the situation is rapidly brought under

control. It is helpful to have trained for this eventuality as a team and ensure that roles are clearly defined before this situation is encountered. The muscle patch is certainly the simplest and most studied haemostatic material utilized in this setting however in the event that the injury is able to be both visualized and accessed by instruments, direct vessel closure with AnastoClip (LeMaitre vascular) or a curved T2 aneurysm clip may be attempted. It is important that whatever technique is used, that vessel lumen patency is maintained and ongoing flow to allow distal perfusion of the brain occurs. Endovascular sacrifice of the vessel is a last resort and one that may potentially lead to neurological deficit, even if one had performed a balloon test occlusion previously. It is important to realize that stopping the haemorrhage is merely the first step in the management of these patients and they will require ongoing angiographic follow up to ensure that they do not develop pseudoaneurysm, carotid-cavernous fistulae or have a delayed ICA rupture. It is crucial that endoscopic, endonasal skull base operations, even those that are considered comparatively simple, are performed by a dedicated skull base team and that all cases are discussed and individualized risk analysis performed pre-operatively.

Haemostasis and Platelet activation

Platelet anatomy and function

Effective Coagulation and subsequent haemostasis results from a balance of multiple inhibiting and facilitating factors. These include the endothelial wall cells of blood vessels, platelets, leukocytes, coagulation cascade, and the milieu of temperature and blood pH(152). The precipitating event for non-pathological coagulation initiation is vessel wall damage(187). This causes activation of endothelial cells and subsequent platelet activation.

Platelets 'roll' along the damaged cells and, using von Willebrand adhesion factor to bind to newly exposed subendothelial cells, aggregate in platelet clumps(152). Post this event, a mesh of platelets and trapped leukocytes form a scaffold for development of a fibrin clot as part of the coagulation pathway(188).

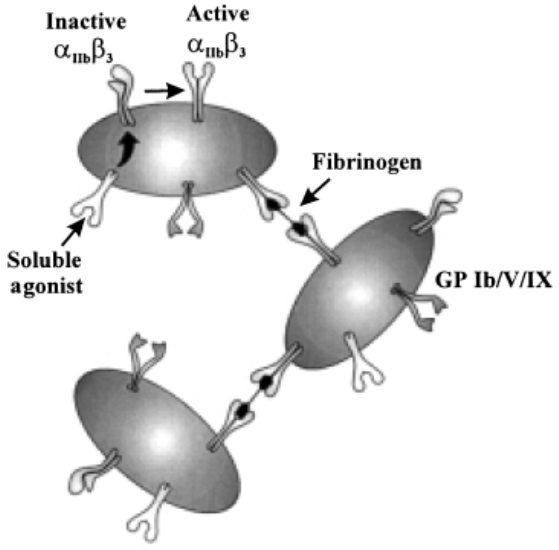
The interaction of platelets with the damaged endothelial wall is a key component of the pathophysiology of many atherosclerotic and ischaemic disease but it also serves a valuable role in haemostasis. The normal platelet count is 150-300x10^9L. Platelet life span is generally considered to be 8-10 days which accounts for the usual surgical practice of ceasing irreversible antiplatelet agents 7-10 days prior to elective surgery. Platelets have a size ranging from 2-5 micrometers and a mean cell volume of 5 femtolitres(189).

The platelet has an outer layer known as the glycocalyx. This is comprised of glycoproteins (GP). It is the binding of these glycoproteins to substances within the bloodstream and on the exposed and damaged endothelial walls that activates and aggregates platelets and allows them to form a plug. Platelets' response to exposure to activating factors may be reversible or irreversible. The reversible responses are shape changing and adhesion. The irreversible are release reactions and aggregation. Adhesion and shape change are the first step towards plug formation(189).

There are multiple methods and phases of activation but the most prominent is the exposure of platelets to collagen, which is found almost everywhere in the body apart from the inner wall of the vascular endothelium. When endothelium becomes damaged, the normal laminar flow of the blood vessel becomes disrupted. Platelets adhere to exposed

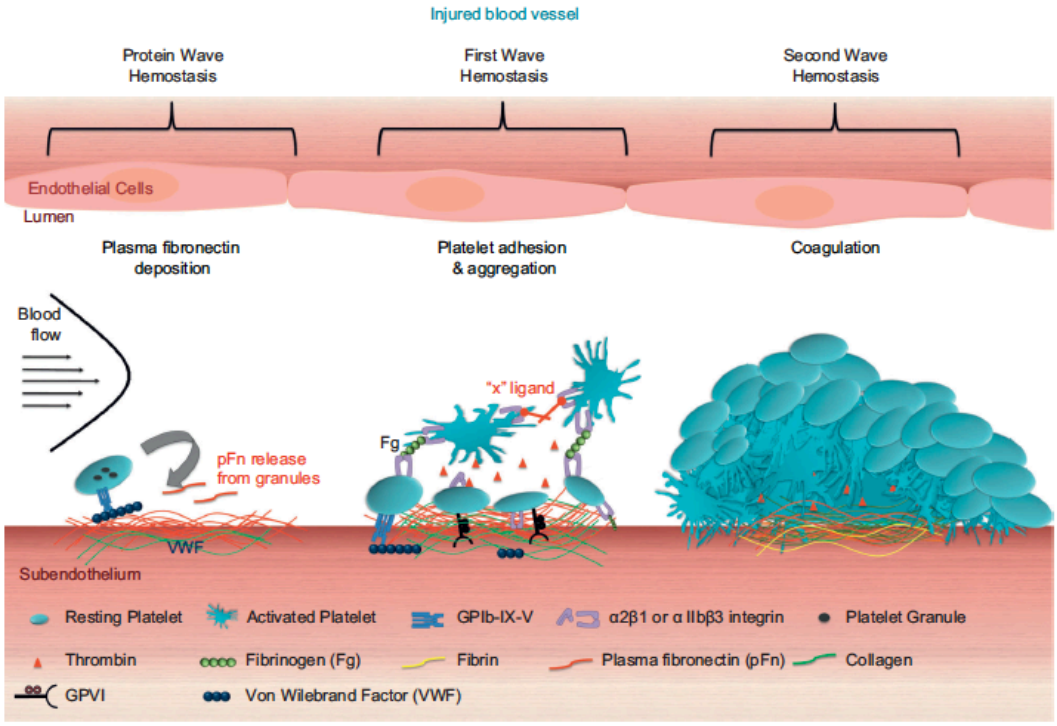
collagen and extracellular membrane proteins on damaged endothelium despite this extensive 'shear stress' caused by rapidly flowing blood(190, 191). They do this via a number of mechanisms, the most prominent being GPIb-IX binding to vWF and GPIIbIIIa binding to fibrin and fibrinogen(189).

On the platelet membrane surface, GPIb-IX (a glycoprotein receptor) is coupled to the platelet cytoskeleton by actin-binding protein(192). When GPIb-IX is exposed to vascular endothelium, it immediately attaches to von Willebrand factor (vWF), which is layered on collagen fibres in the damaged endothelium. Two collagen receptors, GPVI and GPIIbIIIa stablise this attachment. GPIb-IX and GPVI bind together and activate GPIIbIIIa complex which binds fibrinogen and fibronectin to the damaged area(192-195). This GPIb-IX binding to vWF triggers actin filament formation and acts to further the production of a scaffold with other activated platelets along with secretion of products stored in platelet organelles. As further platelets express GPIIbIIIa on their surface, they aggregate together to serve as a platelet plug(196).



Traditional model of platelet activation and aggregation. Integrin α IIbß3 is activated and binds to fibrinogen, cross linking platelets. Activated GPIIbIIIa is identified on flow cytometry

by binding of PAC1 to the exposed fibrinogen site (197)



Phases of the mechanisms of platelet-induced haemostasis. Platelet fibronectin (pFn) is released from intracellular platelet granules. Platelets then bind to vWF and collagen exposed on the vessel wall. This activates GPIIbIIIa with fibrinogen binding and platelet plug formation(152).

Once platelets bind to the subendothelial matrix, they change their shape and become more spherical compared with their usual discoid shape when circulating in blood(195, 198). This shape change also involves the projection of pseudopods, which increase platelet surface area for adhesion. This adhesion is mediated by many factors(199). High levels of localised extracellular calcium (Ca+) and magnesium (Mg+) ions cause 'reversible aggregation' whereas irreversible aggregation occurs when activated platelets release arachidonic acid derivatives from their internal membrane and pro-aggregation substances such as adenosine diphosphate (ADP), adenosine triphosphate (ATP), Serotonin and Ca+ from their dense granules (organelles within the platelets only released upon activation)(189). The

basic structure of aggregation is of vWF and fibrin binding to receptors on two or more platelets simultaneously and holding them together – crosslinking them(189).

Fibrinogen

Fibrinogen in a soluble glycoprotein that exists in blood plasma. It is comprised of three polypeptide chains. It is cleaved into fibrin by the action of thrombin, a serine protease(200). Damaged cells release tissue factor. This initiates the extrinsic pathway of coagulation, the end result of which is the development of a cross-linked fibrin meshwork or 'clot' via thrombin production. This extrinsic pathway does not result in sufficient thrombin in and of itself however. The small amounts of thrombin produced initially cause subsequent further activation of platelets and triggers a further coagulation pathway, the intrinsic pathway. This intrinsic pathway activation results in the conversion of prothrombin to thrombin. This thrombin causes the development of a stable fibrin clot via catalysis of fibrinogen to fibrin and activation of factor XIII and inhibits subsequent fibrinolysis.

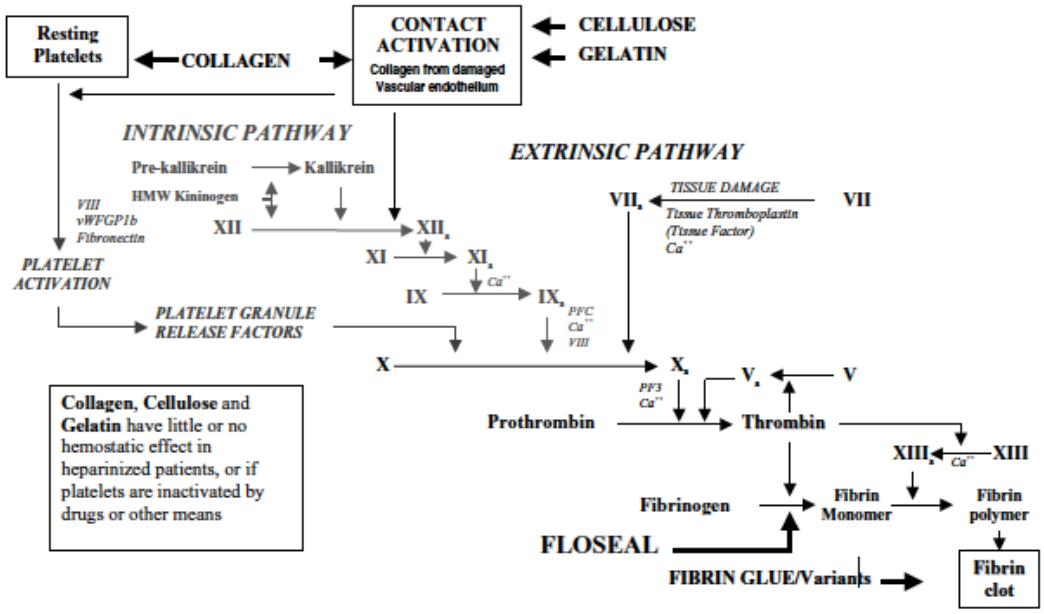


Figure 1. *Coagulation cascade and hemostatic technologies.*

The coagulation cascade with several haemostatic agents shown at the stage in which they primarily cause effect(201)

As more becomes known about the way platelets become activated and subsequently aggregate, new therapeutic targets become available for anti-platelet drug design. Common agents include clopidogrel (a thienopyridine-class Adenosine Diphosphate (ADP) receptor antagonist), aspirin (a Cyclooxygenase-1 (COX-1) inhibitor) and Abciximab/Ticagrelor (GPIIbIIIa antagonists)(202).

The efficacy of these drugs is still debated however there is significant evidence for the efficacy of GPIIbIIIa antagonists as agents in the treatment of acute coronary events by blocking the final step in the pathway of platelet aggregation(203-206). These should all ideally be ceased 7-10 days prior to elective neurosurgery however the indications and the relative risk/benefit for each patient must be taken into account(203, 207-209).

There are a number of pro-coagulant factors that occur as a result of platelet activation and aggregation that cause a trend towards clot formation by the coagulation pathways. The intrinsic coagulation pathway is potentiated by membrane phospholipids released from platelets. This leads to eventual thrombin formation(189). Several factors expressed on platelet surface appear to play a role in preventing the clot from being broken down. Platelet factor 4 inhibits heparin activity. P-selectin is a glycoprotein usually found only in granules within the platelet. It translocates to the platelet surface when the platelet is activated and mediates platelet-leukocyte interactions to include them within the platelet-clot plug. The fact that P-selectin is found only on the surface of activated platelets makes it useful in flow cytometry studies as it may be 'tagged' and serve as a marker for platelet activation(210).

Tests for platelet activation and aggregation

Given the important role platelets play in both physiological and pathological responses to damaged endothelium, and the subsequent formation of clots, it is useful to be able to look for markers of activation and aggregation, especially if these were able to be detected in peripheral blood in the setting of acute coronary syndrome or stoke. Platelet activation may be detected by a change in shape and aggregation. It is also possible to measure specific metabolites released by platelets into plasma(189). Platelet aggregation may be measured via platelet aggregrometry when agonists such as ADP and collagen are added. Light is passed through platelet rich plasma and compared with platelet free plasma. There is some uncertainty if this in vitro technique reflects in vivo conditions however(189, 211). Platelet alpha granule contents such as thromboglobulin and platelet factor-4 may be measured in vivo by ELISA and radioimmunoassay however there are a number of

endogenous and exogenous factors that make measuring this problematic, not least of which is that normal levels are not known(212). A better reflection of in vivo platelet activity is thought to be achieved through the use of flow cytometry(213).

Flow Cytometry

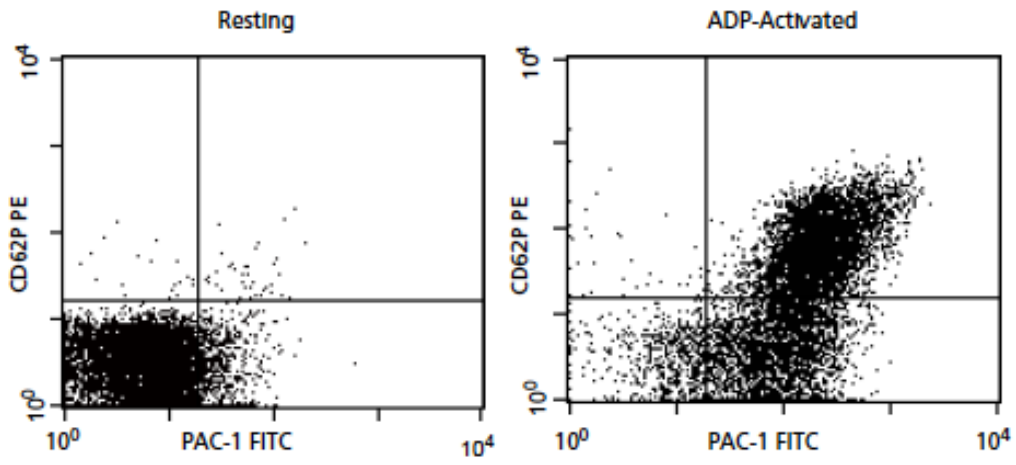
Flow cytometry is used to determine changes in platelet membrane structure and glycoprotein expression which act as surrogate markers of activation. It is also be used to demonstrate structural deficiencies in certain proteins denoting platelet function disorders, and the relative function of antiplatelet agents(189, 214). The benefit of flow cytometry is that it utilises whole blood and is thought to more accurately reflect in vivo conditions(215).

Monoclonal antibodies or fluorescent stains are utilised to identify and detect specific antigens on the membrane of activated platelets, platelet membrane-bound proteins (GPIIbIIIa or P-selectin) or shape changes in activated platelets(216, 217). GPIIbIIIa undergoes a conformational change with a new epitope formation when the platelet is activated and this is able to be detected by a laser when a tagged monoclonal antibody conjugated to a fluorescent dye is added(215). Whole blood is incubated with these antibodies. It is able to detect activation in platelets with high sensitivity (down to 0.8% of the total number of platelets)(189). Flow cytometry is able to give an accurate percentage of platelets activated in any given sample by using a laser to count the number of labelled platelets as they pass by a detector.

Antibodies demonstrating platelet activation in flow cytometry

PAC1

GPIIbIIIa is a receptor for fibrinogen and von Willebrand factor that is required for platelets to aggregate. When activated, it undergoes a conformational change exposing the fibrinogen binding site(218). PAC1 antibody is able to bind to this site thus labelling active platelets when analysed via flow cytometry(216). PAC1 expression is generally decreased with clopidogrel treatment. Clopidogrel blocks the ADP P2Y12 receptor site and prevents platelet activation(215). Aspirin (acetylsalicylic acid) does not affect CD62 or PAC1 expression post ADP stimulation(215).



Flow cytometry demonstrating PAC1 positivity (right) on ADP stimulated platelets compared to non-stimulated (left)(219).

CD62

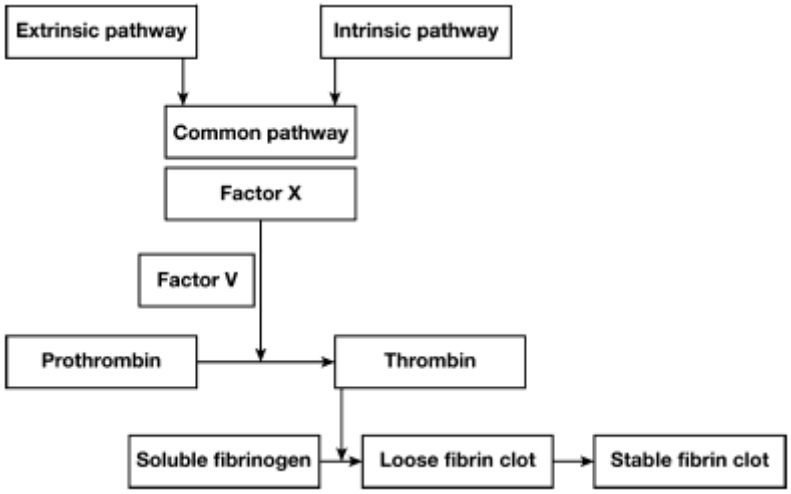
P-selectin (P-SEL) is a protein component of the alpha-granule membrane of resting platelets and is only seen on the platelet surface membrane after platelet activation(218). It is thus a marker of platelet degranulation which only occurs with platelet activation. In vitro, this is irreversible. In vivo, platelets lose P-selectin rapidly(216). CD62 antibody binds to P-SEL and demonstrates activation(220).

Commercially available haemostatic agents in neurosurgery

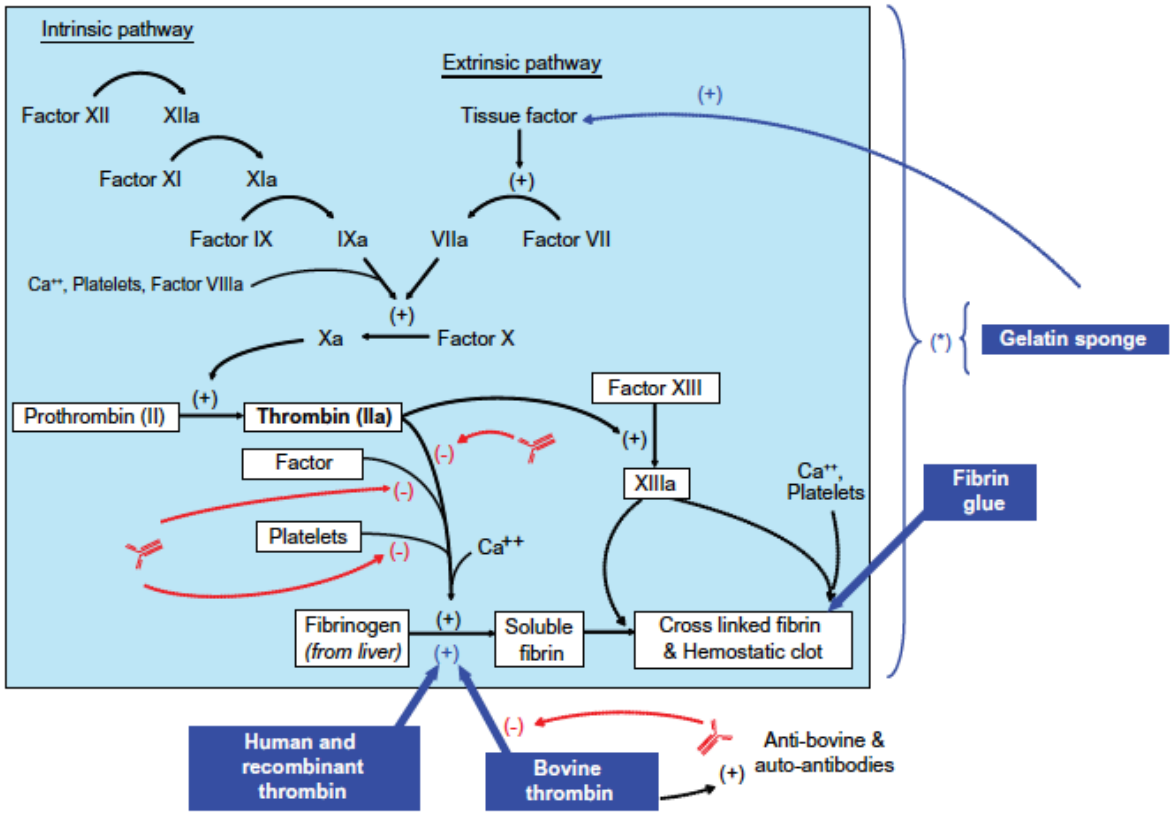
Neurosurgery involves operating within the central nervous system on structures that have no capacity for regeneration. There are eloquent areas within the cortex and brainstem that are irreplaceable in terms of neurological control of human functions, both physical and cognitive. Additionally, there are some areas where collateral blood supply is minimal or absent so sacrifice of feeding vessels is a last result as doing so will result in stroke in areas they perfuse. Conventional methods such as warm irrigation for small capillary bleeding in brain parenchyma, bipolar cautery (in which bipolar electrical current is used to weld cut ends of a vessel together), and very gentle simple compression are suitable for open cranial operations and have some applicability to endoscopic skull base surgery. Major haemorrhage is, however, unlikely to be stopped with such methods. Novel agents have been developed to attempt to influence or promote various parts of the coagulation cascade. These use the pre-existing natural pathways but insert foreign promoters to both mimic and increase the local coagulation response. These products include gelatin-thrombin matrix powders, fibrin/thrombin patches and fibrin glue sealants. These agents have the effect of amplifying the natural coagulation pathway(201).

Topical Thrombin

Topical thrombin exerts its effect by converting soluble fibrinogen to fibrin, allowing a fibrin clot to form and subsequently stabilise. There are a number of forms commercially available.



Simplified coagulation pathway demonstrating the place of thrombin and its role in developing a polymerized fibrin clot(221)



Mechanism of action of Thrombin(222)

Floseal[Baxter]

Floseal is a bovine gelatin/human-sourced thrombin combination. The bovine gelatin is arranged into microspheres with a diameter of approximately 500 micrometers. The

thrombin is derived from plasma prothrombin that is then mixed with calcium chloride to convert it to thrombin. These are both sourced from within the United States. This presents a small but real risk of infectious disease transmission despite screening. This includes bovine spongiform encephalopathy (BSE – a transmissible form of neurodegenerative prion disease) however the absolute risk is likely to be exceedingly small(223). There have been no reports of adverse immune reaction other than a single case report of anaphylaxis attributed to the gelatin component in a 14 year old undergoing scoliosis correction(224).

The gelatin-thrombin powder matrix is mixed in a syringe with a flexible applicator in 5-10ml quantities and, once applied, conforms to whatever cavity shape required. It is then covered with a moist cottonoid or gauze and allowed to exert local effect for 2 minutes. It swells in contact with blood so providing a localised tamponade effect. From the point of view of coagulation activation, the thrombin catalyses the formation of a fibrin clot and allows subsequent onflow coagulation effects. Excess matrix is removed with irrigation at the end of the 2 minutes(225). There is a small risk of oedema from the granulomatous inflammation subsequent to Floseal application however this is minimized by removal of excess matrix post fibrin clot formation(226).

Floseal does not require the presence of functional platelets and, by providing topical thrombin, bypasses much of the upstream intrinsic coagulation cascade. It will not, however, work in the presence of a fibrinogen deficiency(201). Whilst Floseal has been used since 1999 in the United States and 2008 in Australia, the majority of evidence for its use comes from animal studies(227) and case series(26, 227-230). The first prospective, randomized evidence for its efficacy was a study of 309 patients across ten hospitals(201).

The study compared Floseal with a gelatin sponge with thrombin in an effort to demonstrate equivalent results. Surgeries were spread across cardiac, vascular, spinal and orthopaedics. If bleeding was unable to be controlled via conventional means (sutures, pressure, bipolar cautery) patients were randomized to Floseal or gelatin sponge with thrombin (control). Success was defined as haemostasis at 10 minutes following application(201). At 3 minutes, Floseal was significantly more effective at controlling bleeding (Floseal 85% vs. control 48% p<0.001). At 10 minutes, the haemostasis success rate was similar when all specialties were pooled (floseal 95% vs. control 83% p<0.001)(201).

TABLE 3
Treatment Successes, by Surgical Specialty for First Lesion Only

Surgical Specialty	Treatment Group	Number of Patients	Number of Successes	Percent Successes	CMH P-value[b]
All Patients	FloSeal	156	149	96	<0.001
	Control[a]	153	118	77	
Cardiac	FloSeal	48	45	94	<0.001
	Control	45	27	60	
Vascular	FloSeal	43	40	93	0.036
	Control	46	35	79	
Spinal	FloSeal	65	64	98	0.042
	Control	62	56	90	

[a]Control = Thrombin-soaked-Gelfoam.
[b]p-value from Cochran-Mantel-Haenszel test for row mean scores, adjusted for investigational site.

Percentage of patients achieving primary outcome of haemostasis at 10 minutes(201)

Floseal has also been used in endoscopic nasal/sinus surgery. Cappabianca authored a qualitative experience with 29 patients with bleeding from various sources. He described Floseal as a very useful adjunct to haemorrhage control in this setting(230)

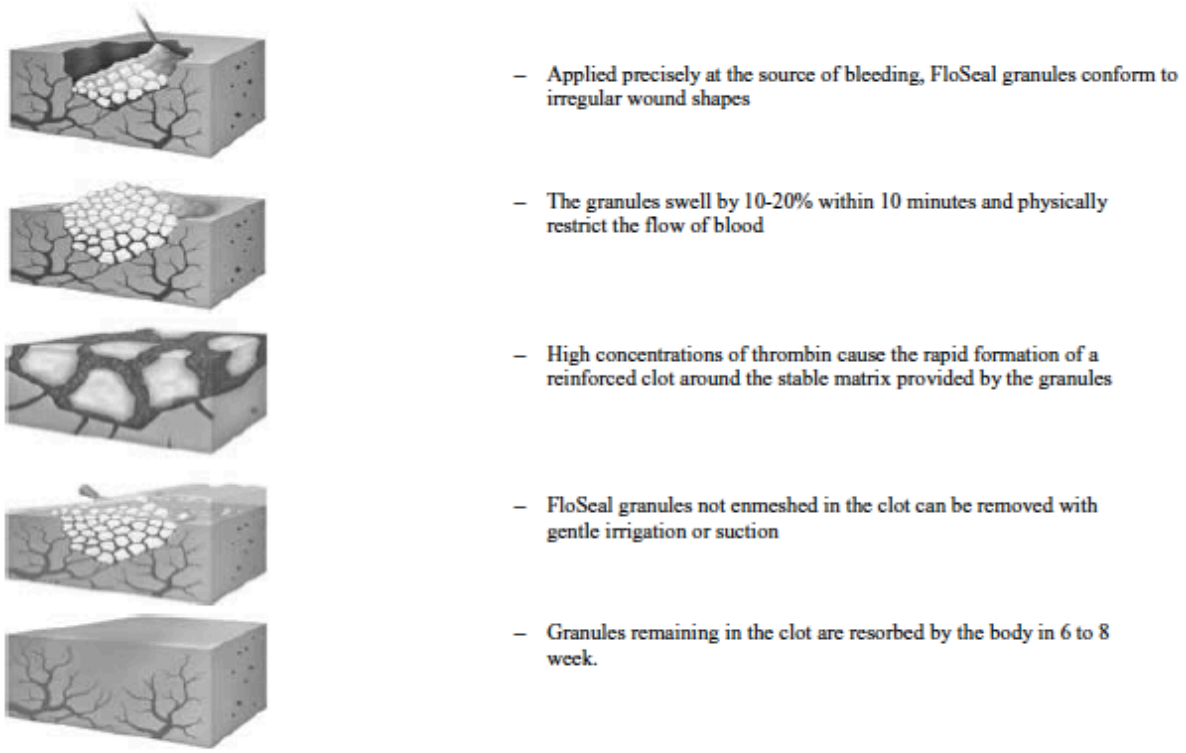


Figure 2. *FloSeal—mechanism of action.*

Floseal[Baxter] macroscopic mechanism of action(201)

Floseal is used extensively for areas of bleeding within the brain parenchyma. Gazzeri describes a neurosurgical indication for floseal in a series of 31 patients with primary intracerebral haemorrhage. The rationale for this approach was that instead of a wide craniotomy and potentially destructive corticotomy to access the clot and inspect all quadrants of the cavity to ensure haemostasis, a much small corticotomy could be performed if Floseal was then used to fill the cavity. These 31 patients underwent craniotomy and evacuation of the intracerebral clot with a minimally-sized craniotomy. The cavity was filled with Floseal for 3 minutes and then irrigated out with wash. An 80% reduction in clot volume and mass effect was achieved and only one patient required reoperation for rebleed 2 days later which was performed using the same technique(229).

Removal of bone flap and clot evacuation with bipolar and suction through small corticotomy(229)

Injection of Floseal into the cavity and subsequent irrigation out after 3 minutes.(229)

It does, however, appear important to remove as much of the excess Floseal as possible without disrupting the fibrin clot. A more recent study of rat brains treated with Floseal demonstrated granulomatous reaction and inflammation 28 days post use(227).

Surgiflow[Ethicon]

Surgiflow with thrombin is comprised of porcine gelatin and human-derived thrombin (2000IU). These are packaged separately and mixed prior to application. The powder may swell up to 20% post application. The manufacturer's advice is that Surgiflow not be used in conjunction with autologous blood recollection circuits as the gelatin collagen particles have been demonstrated to pass through scavenge filters of ~40micrometers(231). The porcine

gelatin has also been observed to be a nidus for infection and may potentiate bacterial growth. The gelatin/thrombin has, like floseal, been shown to cause a granulomatous foreign body reaction(231). There is a variable immune response in patients on whom Surgiflow has been used. The baseline safety study showed anti-porcine collagen antibodies developed in 6/206 patients compared with a single patient who had these at the commencement of the study(231). The risk of clinically apparent adverse events does not, however, appear to differ between bovine and porcine origin thrombin as assessed in a phase III randomized, double-blinded, controlled trial of 305 patients (porcine n=153 vs. bovine n=152)(232).

Adverse Effects of topical thrombin

Clinically apparent adverse effects have been reported with the use of topical thrombin, specifically of bovine origin(233-235). Factor V antibodies may develop as a response to exposure. These inhibitors block the normal coagulation function. Approximately 126 cases have been reported worldwide, approximately 2/3 of which are post bovine-thrombin exposure. 33% of these developed clinically apparent bleeding problems(233). If asymptomatic, no treatment is required. Mild to moderate bleeding is treated with oral steroids and more severe cases with plasma exchange, Intravenous immunoglobulins (IVIG) or cyclosporine A(236).

Ballard et al pooled the results of 8 trials that utilized thrombin in humans with a total of 552 patients(233). 5 of these 552 (0.9%) developed antibodies to recombinant thrombin at 29 days post use. None of these patients suffered an adverse effect secondary to this. The patient's native thrombin continued to function normally in coagulation tests. Further 2.2%

of the patients in the pooled analysis had pre-existing antibodies recognizing recombinant thrombin despite never having been exposed to this previously. The researchers were able to determine this because recombinant thrombin was not available at this time other than as a study drug for these trials(233).

There is also some evidence that topical thrombin use may significantly increase the risk of deep vein thrombosis formation. Safaee et al published a single institution review of 467 patients undergoing craniotomy for meningioma(237). 2.6% of these patients suffered a thromboembolic event (DVT/PE). In univariate analysis, the authors found that higher tumour grade and higher body mass index (BMI) were positively associated with the chance of developing thromboembolic complications. In multivariate analysis, they found that BMI and the use of >10ml of topical thrombin agent (Floseal[Baxter]) was associated with increased risk of DVT/PE(237). Interestingly, the cases in this paper were performed by two surgeons, one of whom did not put patients on any form of DVT prophylaxis and one who did so but only after 72hrs. There are no strict protocols within the Australian neurosurgical community, but accepted practice is to commence DVT prophylaxis either immediately post-operatively or within 24 hours after a post-operative CT or MRI scan to ensure that there is no haematoma formation(238, 239).

There are also isolated case reports of venous air embolism associated with the use of Floseal(240). This complication occurs when operating with the head at a higher level than the heart. If a venous sinus is opened, air (and potentially Floseal) may be sucked into the sinus and embolise to the right atrium. This may cause a decrease cardiac output and potentially cardiac arrest and death. Cardinal signs are a decrease in blood pressure and end tidal CO2 reflecting the decreased blood flow through the lungs available for gas exchange.

It does not, however, appear to be a common event and is a potential risk at any point when surgeons operate near the cranial venous sinuses irrespective of the use of haemostatic agents.

Table 2 Commercially available topical hemostatic agents [2]

Product	Manufacturer	Description	Indication
Thrombin-containing products			
Thrombin-JMI	King Pharmaceuticals, Bristol, TN	Bovine thrombin	Aid in hemostasis whenever oozing blood or minor bleeding from capillaries and small venules is accessible, and control of bleeding by standard surgical techniques is ineffective or impractical.
Evithrom	Johnson & Johnson, Somerville, NJ	Lyophilized human pooled thrombin	Aid in hemostasis whenever oozing blood and minor bleeding from capillaries and small venules is accessible, and control of bleeding by standard surgical techniques is ineffective or impractical.
Recothrom	Zymogenetics, Seattle, WA	Recombinant thrombin	Aid in hemostasis whenever oozing blood and minor bleeding from capillaries and small venules is accessible, and control of bleeding by standard surgical techniques is ineffective or impractical.
FloSeal™ Hemostatic Matrix	Baxter Healthcare Corporation, Hayward, CA	Flowable bovine gelatin matrix and licensed human thrombin	In surgical procedures (other than ophthalmic) as an adjunct to hemostasis when control of bleeding by ligature or conventional procedures is ineffective or impractical.
CoStasis	Cohesion Technologies Inc., Palo Alto, CA	Flowable bovine collagen and licensed bovine thrombin	In surgical procedures (other than neurological, ophthalmological, and urological) as an adjunct to hemostasis when control of bleeding by ligature or conventional procedures is ineffective or impractical.
Surgiflo	Johnson & Johnson, Somerville, NJ	Porcine gelatin with or without thrombin	In surgical procedures (except ophthalmological) for hemostasis, when control of capillary, venous and arteriolar bleeding by pressure, ligature, and other conventional procedures is ineffective or impractical.

Summary of commercially available topical thrombins(241).

Fibrin Sealants and Gels

There are several commercially available fibrin-based sealants(242). These are generally composed of varying ratios of human plasma-derived thrombin and fibrinogen. Their efficacy has been established in multiple anatomical sites and across many flow rates of bleeding in both animal models and human trials(242-250). Commercial preparations have been marketed as both haemostatic agents and as sealants to prevent cerebrospinal fluid (CSF) leaks(16, 26, 251-253).

Evicel[Ethicon]

Evicel[Ethicon] is a haemostatic agent approved for use in the United States and Australia as an adjunct for haemostasis. It consists of 55-85 mg/ml fibrinogen and 800-1200 IU/ml human thrombin in frozen solution. It is provided in separate vials which are mixed in a sterile applicator. They are stored in a refrigerator and may be mixed and ready to apply in <1 minute. It is dripped or sprayed onto the wound. This may be assisted with an air pump as the provided cannula has a tri-lumen to ensure that the products mix at the tip. The manufacturer advises caution when using the air pump to avoid the occurrence of an air embolism(254). It is derived from human plasma so the theoretical risk of infectious disease transmission remains, however this is screened for viral and bacterial pathogens. A randomized vascular surgical study demonstrated a statistically significant improvement in achievement of haemostasis when compared to manual compression in end-to-end femoral anastomoses (83.3% vs. 39.7% P<0.001) at 4 minutes(245). It is currently undergoing human clinical trials in Australia to expand its indication as a dural sealant in cranial and spinal neurosurgery(255).

Evicel was original marketed under the trade-name Quixil, and contained tranexamic acid, however this was found to be potentially neurotoxic so was removed from the formulation. This did not affect the haemostatic effect of the fibrin/thrombin combination because plasminogen is also removed from the fibrinogen component by chromatographic techniques and, therefore, tranexamic acid is not required as a stabilizer(256). Fibrin sealants are broken down and metabolised by endogenous fibrinolytic activity. Healing wounds induce plasmin production and activity and the clot undergoes fibrinolysis and eventual phagocytosis(256).

Tisseal[Baxter]

Tisseal[Baxter] is a combination of human thrombin, human fibrinogen and a synthetic fibrinolysis inhibitor – aprotinin – to prevent premature degradation of the clot(257, 258). It can be used directly on the brain, dura and spinal cord and it has been described as an adjunct to cavernous sinus surgery in which it is injected into the cavernous sinus prior to tumour dissection to minimise peri-capsular bleeding(243, 259). It will adhere to a wet surface however the flow rates of torrential arterial bleeds mean that it is unable to provide adequate haemostasis unless it can be held in situ as part of a patch(260). There is evidence to show that it reduces time to haemostasis and blood loss in vascular anastomosis lines(260, 261). A randomized non-blinded trial of 17 patients was performed to determine whether topical fibrin sealant reduced anastomosis suture line bleeding during carotid endarterectomy with polytetrafluoroethylene (PTFE) patch closure. Time taken to achieve haemostasis at the suture line and intraoperative blood loss were measured. The median time to achieve haemostasis was 5.5 min (range 4-31 rain) in the treatment group and 19 min (range 10--47 min) in the control group ($P<0.005$). Operative blood loss was lower in the treatment group (median 420mi, range 300-500mi) than in the control group (median 550ml, range 350-1200ml) however this was not statistically significant(261). These findings have subsequently been replicated in a cardiac surgical trial along aortic graft suture lines(262).

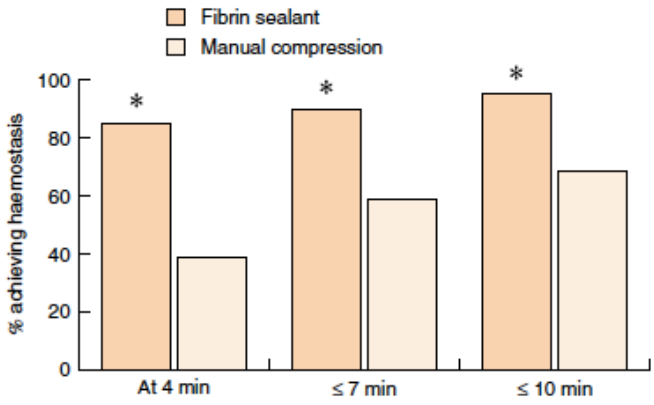
Mechanism of action of Fibrin products

Fibrin sealants augment the final stages of the endogenous coagulation process. Thrombin activates the conversion of fibrinogen into fibrin, which occurs by the splitting of fibrinogen into fibrin monomers and fibrinopeptides. The fibrin monomers aggregate and form a fibrin

clot. Factor XIIIa, which is activated from Factor XIII by thrombin, crosslinks fibrin. Calcium ions are required for both the conversion of fibrinogen and the cross-linkage of fibrin(242, 243, 247, 248). This crosslink stability has been shown in a number of studies where Evicel[Ethicon] was compared with Tisseal[Baxter]. The resultant Evicel fibrin clots had more tensile strength than Tisseal on skin adhesive tests (0.25N vs. 0.08-0.11N $p \leq 0.006$)(263) however other studies have shown less clear results(241, 242). This difference in clot stability may be due to the fact that the fibrin component of Evicel has detectable levels of factor XIII in the formulation (9IU/ml) whereas the Tisseal had undetectable levels(264). Measures of resistance to stretch also favoured Evicel over Tisseal (mean 38 kPa vs. 11 kPa $p \leq 0.001$) as did tensile strength (135 kPa vs. 25 kPa $p \leq 0.001$)(263).

Haemostatic properties of Fibrin Gel Sealants

In a vascular surgical prospective randomised controlled trial, Evicel (75 patients) was compared with manual compression only (72 patients) in patients undergoing end to side femoral or upper extremity arterial anastomoses(245). These grafts were polytetrafluoroethylene-based. Evicel was significantly more effective at achieving haemostasis than manual compression alone at the 4-minute mark (85% vs 35%). The incidence of treatment failure was also lower in the Evicel group(245). Graft thrombosis or occlusion occurred in 8% of the Evicel group compared with 1% in the manual compression group in the early post-operative stage out to 12 days. When reassessed at 5 weeks postoperatively, an additional 3% of Evicel group patients had suffered a graft occlusion compared with 7% of the manual compression group. 9% of the Evicel group (7 patients) and 4% of the manual compression group (3 patients) required further surgical intervention(245). These complications did not differ significantly between groups(245).



Proportion of patients achieving haemostasis at 4, 7, and 10 minutes (Evicel vs manual compression(245)

This positive haemostatic effect was also shown in a study comparing Evicel (when formulated as Quixil) to Kaltostat (a calcium alginate dressing) in carotid endarterectomies(265). Primary outcomes were median time to haemostasis and mean blood loss. Quixil was placed onto the suture line. The interim analysis was so compelling after 20 patients in favour of Quixil that further recruitment was ceased. Median time to haemostasis 2.5 vs. 17 mins p≤ 0.001) and mean blood volume loss 24.5ml vs 203ml (P≤ 0.001)(265).

Evicel has also been trialled in endoscopic endonasal surgery using post-operative mucosal bleeding rates as an outcome measure(266-268). Post-operative bleeding occurred in 0-5% of patients in the Evicel groups compared with 23-37% of patients in the nasal packing alone groups. This also had the advantage of allowing the patient to nasal-breath immediately post-operatively in the absence of nasal packing(268). It is important to note that this was

purely looking at small mucosal and capillary bleeds and not high flow, high pressure arterial haemorrhage.

Evicel displays no neurotoxicity. A study using a rabbit model placed Evicel subdurally and observed behavioural characteristics for 14 days with no abnormal events occurring(254). Histological and CSF studies did not demonstrate any inflammation(254, 256). There is the theoretical risk of transmission of blood-borne diseases with fibrin sealants of human derivation. This risk is minimised by screening of donors, inactivation of viruses and testing of pooled plasma(269-271). This is considered to effectively remove the risk of Human Immunodeficiency Virus (HIV), Hepatitis A, Hepatitis B and Hepatitis C however may not be effective against Parvovirus B19. The risk of transmission of HIV or the hepatitis Viruses is thought to be negligible and the risk of Parvovirus B19 is thought to be <1 in 100 000(256).

Table 1 Commercially available fibrin-based sealants [2]

Product	Manufacturer	Description	Indication
EVARREST	Ethicon, Inc, a Johnson & Johnson Company, Somerville, NJ	Human fibrinogen and human thrombin sealant patch	For use with manual compression as an adjunct to hemostasis for soft tissue bleeding during open retroperitoneal, intra-abdominal, pelvic, and non-cardiac surgery.
Quixil/Crosseal	OMRIX Biopharmaceutical Ltd., Kiryat-Ono, Israel	Two vials containing human thrombin and human fibrinogen	For use in obtaining liver hemostasis and in orthopedic surgery
TachoSil	Nycomed GmbH, Linz, Austria	A ready-to-use surgical patch composed of a dry collagen sponge made from horse tendons, and on one side coated with human fibrinogen and thrombin	An adjunct to hemostasis in cardiovascular surgery when control of bleeding by standard surgical techniques (such as suture, ligature, or cautery) is ineffective or impractical.
Evicel	Johnson & Johnson, Somerville, NJ; OMRIX biopharmaceuticals Ltd. Kiryat Ono, Israel	Fibrin sealant—human pooled	An adjunct to hemostasis for use in patients undergoing surgery (liver and vascular surgery are also separately indicated) when control of bleeding by conventional surgical techniques is ineffective or impractical.
CryoSeal Fibrin Sealant System	Thermogenesis, Rancho Cordova, CA	Fibrin sealant—human	An adjunct to hemostasis on the incised liver surface in patients undergoing liver resection when control of bleeding by standard surgical techniques is ineffective or impractical.
Vitagel	Orthovita, Malvern, PA	Fibrin sealant- individual units of plasma, bovine collagen, and bovine thrombin	For use during surgical procedures (except neurosurgery and ophthalmic surgery) as an adjunct to hemostasis when control of bleeding by ligature or other conventional procedures is impractical or ineffective.
TISSEEL	Baxter Healthcare Corporation, Westlake Village, CA	Fibrin sealant- human pooled	An adjunct to hemostasis in surgeries involving cardiopulmonary bypass and treatment of splenic injuries. TISSEEL is satisfactory for use in fully heparinized patients undergoing cardiopulmonary bypass. Also indicated as an adjunct to prevent leakage from colonic anastomosis following the reversal of temporary colostomies.

Summary of commercially available fibrin sealants(241)

Haemostatic Patches for Topical Haemostasis

Patches impregnated with haemostatic agents are widely marketed(272). The patch assists in rapid application and helps to hold the agent in situ. Much of the research involving these patches has been performed by the military in the treatment of battlefield injuries with the potential for exsanguination prior to definitive surgical management(273-276).

Modified Rapid Deployment Haemostat [MRDH; Marine Polymer Inc; Boston, Mass]

This is a purified, fully acetylated poly-N-acetyl glucosamine nanofibre material in a 4x4inch non-absorbable sponge. This has been demonstrated to have red cell and platelet specific

receptor interactions to decrease time to haemostasis(194, 277-281). This is applied directly to the site of bleeding and compression applied for ten minutes(282). This is a large bandage and used primarily to staunch haemorrhage from bullet and blast injuries in an 'immediate response' setting to allow the patient time to reach definitive surgical care. It swells on contact with blood and would be unsuitable for use in an endoscopic environment, especially one so constrained as cavernous ICA bleeding.

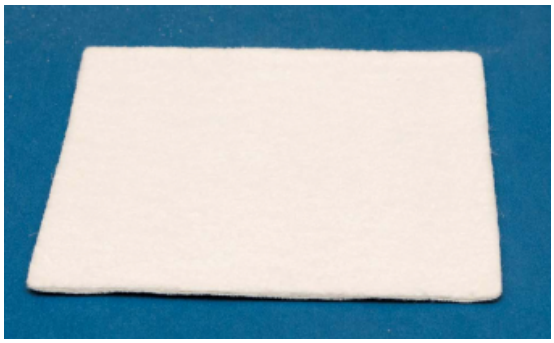
Syvek [marine polymer technologies]

Syvek is a soft, white, sterile, non-woven pad of poly-N-acetyl glucosamine fibres derived from algae. It is attached to a foam backing and is marketed commercially in both the United States and Australia as a sealing dressing for the femoral artery post angioplasty(283, 284). The glucosamine fibres induce vasoconstriction mediated via endothelin release and activate plasma clotting proteins and platelets. Studies in cardiology patients demonstrate its efficacy with only a 1.4-2% failure rate in >600 patients(285). Syvek has regulatory approval for use in the United States and Australia in an external fashion only. Animal studies on rat aorta have demonstrated that Syvek causes arterial vasoconstriction in the presence of an intact endothelial layer. When this layer is removed, the vasoconstriction effect is lost(286).

Evarrest [Ethicon]

Evarrest is a fibrin sealant patch indicated for use with manual compression as an adjunct to hemostasis for control of bleeding during adult liver surgery and soft tissue bleeding during open retroperitoneal, intra-abdominal, pelvic, and non-cardiac thoracic surgery in adults when control of bleeding by standard surgical methods of hemostasis (e.g., suture, ligature,

cautery) is ineffective or impractical(287). The Patch consists of human fibrinogen and human thrombin embedded in a flexible composite patch component. The patch is absorbable. It contains 8.6 mg per square cm of human fibrinogen and 37.5 Units per square cm of human thrombin. Upon contact with a bleeding wound surface, the biological components embedded in the patch component are hydrated, and the subsequent fibrinogen-thrombin reaction initiates the last step in the conversion of fibrinogen into fibrin monomers that further polymerize to form a fibrin clot(287, 288). Hemostasis is achieved when the formed fibrin clot integrates with the patch component and adheres to the wound surface thus providing a physical barrier to bleeding. Evarrest has been shown to have a statistically significant difference compared to usual standard of care in the proportion of subjects achieving haemostasis at 4 minutes after identification of the target bleeding site and randomization (82.5% versus 29.5% ($p < 0.0001$), with no re-bleeding requiring treatment any time prior to the initiation of wound closure(287-291).



Evarrest patch(292)

Tachosil [Baxter]

Tachosil is a topical fibrin sealant patch (9.5x4.8cm) that consists of human fibrinogen and human thrombin coated onto an equine-derived collagen sponge. The patch is absorbable and contains human fibrinogen 3.6 to 7.4 mg (5.5 mg) per cm^2 and human thrombin 1.3 to

2.7 Units (2.0 U) per cm^2(291). It is thought that it works in a similar fashion to other thrombin/fibrin patches by activation of fibrinogen into fibrin monomers with subsequent polymerization into a clot and platelet activation. The fibrin polymers and platelet activation lead to subsequent activation of the coagulation cascade with further adherence to the wound surface via further thrombin mediated fibrin polymerization and conglutination of the patch's collagen matrix and the wound surface, forming a tight seal(293). The human origin of the fibrinogen and thrombin mean a theoretical risk of CJD and B19 Parvovirus however the donor blood is screened for these(294, 295).

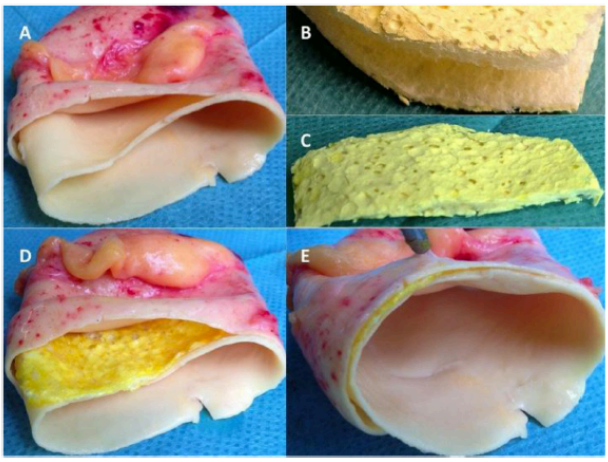
Tachosil patch(292)

Tachosil is indicated for use in cardiac and hepatic surgery as an adjunct to haemostasis where it is placed as a sheet upon the bleeding surface(291). The literature is replete with case reports and small series of novel use. Several cardiac case studies report its use to seal ventricular bleeding post infarction and rupture and at least one case of its use to seal a high pressure ruptured coronary artery(296-299). It has also been trialed to prevent the development of pericardial adhesions post cardiac surgery(300, 301), been used in swine liver and spleen injury models(299, 302-304), gynaecological surgery for uterine wrapping and myomectomy bleeding(305, 306), in renal artery aneurysm surgery(307, 308) and post nephrectomy surgery(309, 310).

Tachosil has also been used in neurosurgery in humans. A large case series at the University of Helsinki retrospectively analysed the use of tachosil in 100 neurosurgical patients as both a haemostat and as a dural sealant with a mean follow up of 4 months. Indications for neurosurgery varied but included tumour surgery (53%), cerebral aneurysm (31%), Arteriovenous malformation (4%) and cavernoma (4%). The remainder were spinal tumours and chronic subdural haematoma(293). The authors describe its use in a number of settings from stopping small arteriole bleeding to reinforcing suture lines as a dural sealant. The most applicable from a haemostatic perspective was its use to repair the superior sagittal sinus in vertex and parafalcine meningioma surgery. Conventional teaching is that the anterior 1/3rd of the sinus may be resected without neurological deficit occurring but that resecting the posterior 2/3rds will result in venous infarction of the cortex and potentially death(28, 115, 311). In this series, the authors describe laying the tachosil in strips cut to size over dural defects when resecting meningiomas with a patent superior sagittal sinus. There were no instances of post-operative haematoma resulting from this practice and no evidence of thrombosis of the sinus as a result of its use(293).

The authors describe some technical points or nuances of relevance to endoscopic skull base surgery. Firstly, wetting the tachosil prior to application made the patch too flaccid to handle properly prior to application, especially down a narrow surgical corridor. Secondly, the instrument used to apply the patch should be cleaned of blood as the patch tended to stick to the instrument in such cases. This was a problem when withdrawing the instrument as it occasionally pulled the patch off the bleeding point with further haemorrhage. To obviate this, the authors covered the tachosil with fibrillar oxidized cellulose with good results(293).

Tachosil has been used in the treatment of type-A aortic dissections. A small case series of 12 patients from Germany describes a dual layer of tachosil being inserted between the two layers of aortic dissection before a new aortic ring was sutured in place(312). This was designed to ensure adhesion between the dissected layers and prevention of further propagation of the dissection distally. There was no evidence of recanalization of the false lumen once it had been treated with tachosil insertion and no evidence of neurological impairment in the 10 survivors of the procedure and perioperative period. There were two deaths – one of cardiac tamponade from suture line leak at day 29 and one patient who awoke with hemiparesis, was sent to a neurological rehab facility and died 6 months post the procedure from unknown causes(312).



Human aorta(A) with tachosil folded into the dissection(D,E)(312)

Tachosil is bound to equine collagen. Whilst the incidence of the development of antibodies to the human fibrin and thrombin is less than 1%, a paper reporting the use of tachosil in a hepatic haemorrhage model reported that the incidence of development of antibodies to equine collagen was 26%(293, 313). This did not result in any known clinical harm. It does

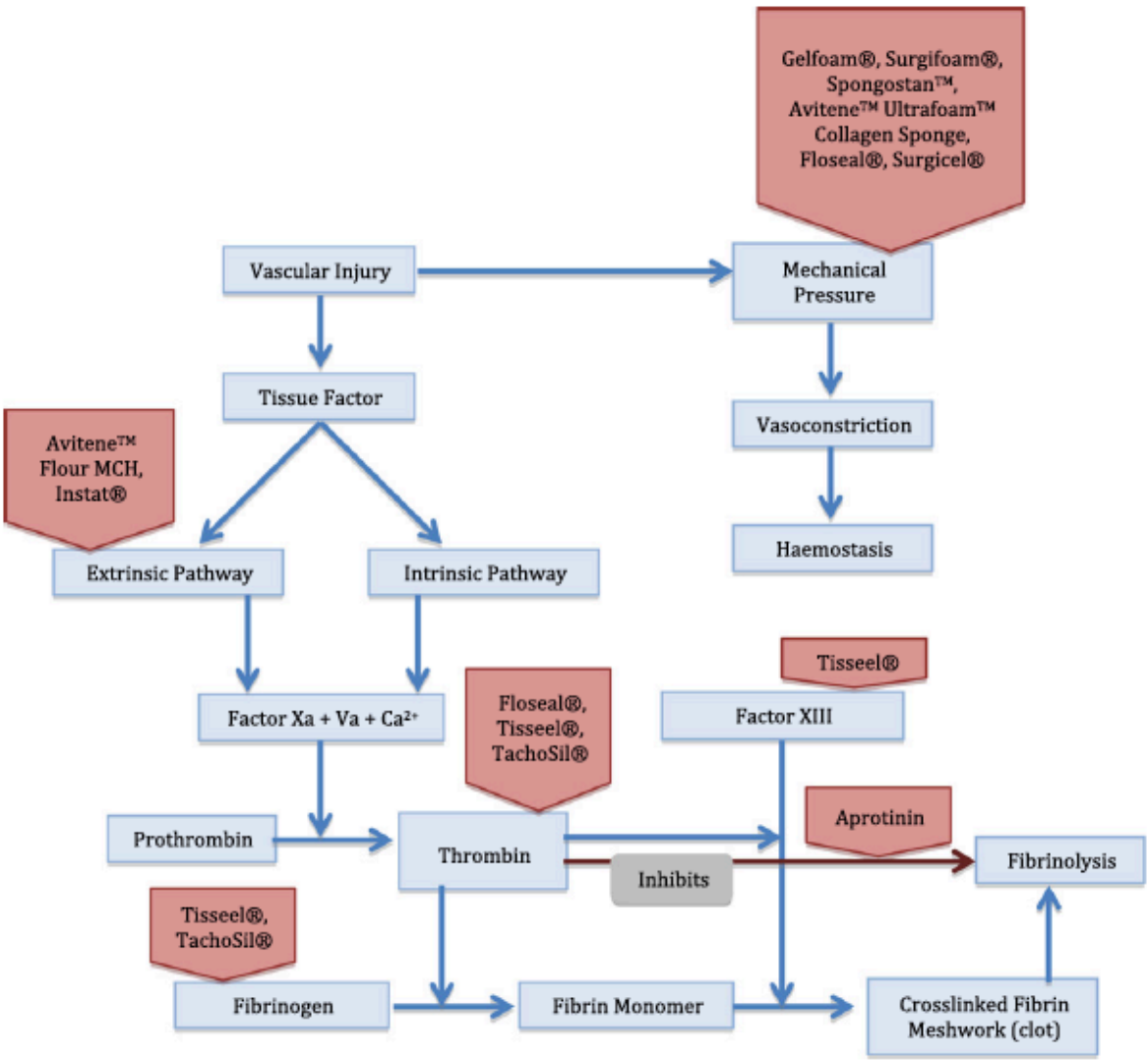
not appear to carry an increased risk of systemic or localized thromboembolic complications(234). It is important to acknowledge that given the proposed trial of this product on the carotid artery, which feeds the brain with its paucity of collateral flow, it must be established that thromboembolism does not occur.

Crushed muscle as a haemostatic agent

Crushed muscle is currently considered to be the gold standard in haemostasis in the event of carotid or large vessel injury in endoscopic surgery(120, 121, 145). It is postulated that a mix of vessel spasm and activation of a fibrin/platelet plug results from the application of this crushed muscle(314). A platelet aggregation study by Rajiv et al demonstrated concentration-dependent aggregation of platelets increasing as the concentration increased from 0.1mg/ml to 0.8mg/ml(145).

Other Agents

Other agents include sealants that have no effect on the coagulation system itself but rather form a physical barrier to blood or cerebrospinal fluid. These include cyanoacrylate absorbable sealant that polymerises on contact with tissues and glutaraldehyde bovine albumin glue(315-317). Research has also been performed on systemic agents that may influence the coagulation pathways. Tranexamic acid to prevent fibrinolysis and recombinant factor VII have been trialed however their use is generally more appropriate in situations with systemic coagulation deficits and consumptive pathologies such as disseminated intravascular coagulation in major trauma(318, 319).



Schematic demonstrating the primary mechanism of action for commercially available neurosurgical haemostatic agents from Yao et al (223)

Table 2
Intra-operative haemostasis methods in neurosurgery

Haemostasis method and examples[a]	Mechanisms of action	Comments
Mechanical compression	■ Physical barrier to bleeding ■ Provides stasis to allow clot formation	■ Conventional haemostasis method ■ May damage delicate underlying and surrounding neural tissue ■ May be difficult to apply in some procedures
Bipolar cautery	■ Spasm of vessel walls ■ Dessication of tissue ■ Thrombus formation	■ Often adequately achieves haemostasis ■ May place adjacent neural tissue at risk of thermal injury
Warm water irrigation	■ Suggested mechanisms: ○ Oedema of surrounding tissue → mechanical compression ○ Vasodilatation → decreasing intraluminal pressure	■ Often used in diffuse oozing ■ Can be used in conjunction with other haemostatic adjuncts
Bone wax	■ Physical barrier to bleeding ■ Providing stasis to allow clot formation	■ Used to stop bone bleeding ■ Can cause a range of adverse reactions including impaired bone healing, allergic reaction and granuloma formation ■ *Preparation time: Ready to use out of the package*
Absorbable gelatin sponges ■ Gelfoam® ■ Surgifoam® Absorbable Gelatin Sponge ■ Spongostan™ ■ AviteneTM UltrafoamTM Collagen Sponge	■ Absorption of blood and fluids → swelling of sponge → mechanical compression ■ Stable site for clot formation	■ Can swell and cause potential problems in confined spaces so excess material should be removed after haemostasis achieved ■ *Preparation time: Ready to use out of the package*
Microfibrillar collagens ■ Avitene™ Flour MCH ■ Instat®	■ Promotes platelet aggregation and adhesion	■ Does not work in severe thrombocytopaenia ■ Requires an intact coagulation cascade ■ Can swell and cause potential problems in confined spaces so excess material should be removed after haemostasis achieved ■ *Preparation time: Ready to use out of the package*
Oxidized regenerated cellulose ■ Surgicel®	■ Reacts with blood to precipitate an artificial coagulum ■ Provide substrate for further clot formation	■ Thought to be bactericidal due to its acidic quality ■ Can swell and cause potential problems in confined spaces so excess material should be removed after haemostasis achieved ■ *Preparation time: Ready to use out of the package*
Microporous polysaccharide hemospheres ■ Arista®	■ Absorb fluid and small molecular blood components → concentrates platelets and coagulation proteins on its surface and thereby enhance fibrin clot formation	■ Requires an intact coagulation cascade ■ Rapid degradation of haemostatic product as it is made from starch, therefore cause less granulomatous inflammatory reactions ■ *Preparation time: Ready to use out of the package*
Thrombin ■ Used in conjunction with other haemostatic agents like: Floseal®, Gelfoam Plus®	■ Catalyzes the conversion of fibrinogen to fibrin ■ Activates factor XIII to stabilize the clot ■ Activates fibrinolysis inhibitor	■ Often used in conjunction with other haemostatic adjuncts ■ Acquired factor V antibodies may develop, which has potential to cause life-threatening bleeding ■ Risk is reduced with recombinant human thrombin ■ *Preparation time: 30 s to reconstitute thrombin in calcium chloride solution*
Fibrin sealants ■ Tisseel®	■ Action of thrombin as above with extrinsic boost of fibrinogen, factor XIII ■ Contains aprotinin which is a potent antifibrinolytic	■ Requires no bleeding for activation ■ Does not rely on intrinsic coagulation cascade ■ Primarily used in prevention and treatment of cerebrospinal fluid leak in neurosurgery ■ *Preparation time: If not pre-thawed will require 5–12 minutes of thawing in the sterile field*
Fibrinogen and thrombin coated collagen sponge ■ TachoSil® patch	■ Action of thrombin above with extrinsic boost of fibrinogen ■ Action of collagen sponge as above	■ Require no bleeding for activation ■ Does not rely on intrinsic coagulation cascade ■ Primarily used in prevention and treatment of cerebrospinal fluid leak in neurosurgery ■ *Preparation time: Ready to use out of package*
Gelatin-thrombin matrix sealant ■ Floseal®	■ Gelatin component swells up to produce mechanical compression and platform for clot formation ■ Action of thrombin above	■ Less effective in fibrinogen deficiency ■ Can swell and cause potential problems in confined spaces so excess material should be removed after haemostasis achieved ■ *Preparation time: 2 minutes*
Glutaraldehyde/bovine albumin ■ Bioglue®	■ Polymerises to form a firm adhesive bond	■ Used in prevention and treatment of cerebrospinal fluid leak in neurosurgery ■ *Preparation time: 30 seconds – 1 minute*

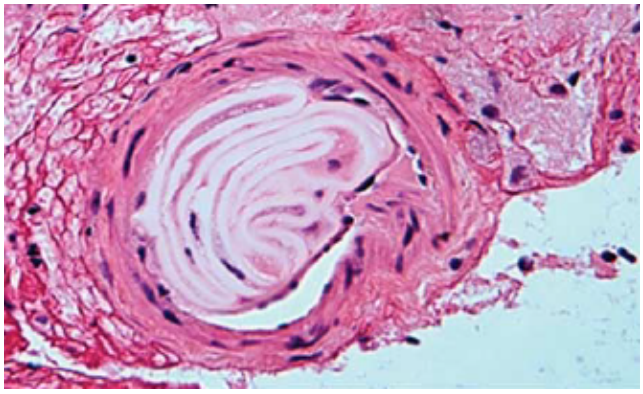
→ = leading to.
[a] The manufacturer details of each producted: Arista® (Medafor, Minneapolis, MN, USA); Avitene™ Flour MCH (Davcol, Warwick, RI, USA); AviteneTM UltrafoamTM Collagen Sponge (Davcol); Bioglue® (Cryolife, Kennesaw, GA, USA); Floseal® (Baxter, Hayward, CA, USA); Gelfoam® (Baxter); Instat® (Ethicon, Somerville, NJ, USA); Spongostan™ (Ethicon); Surgicel® (Ethicon); Surgifoam® Absorbable Gelatin Sponge (Ethicon); TachoSil® patch (Nycomed, Linz, Austria); Tisseel® (Baxter).

(223)

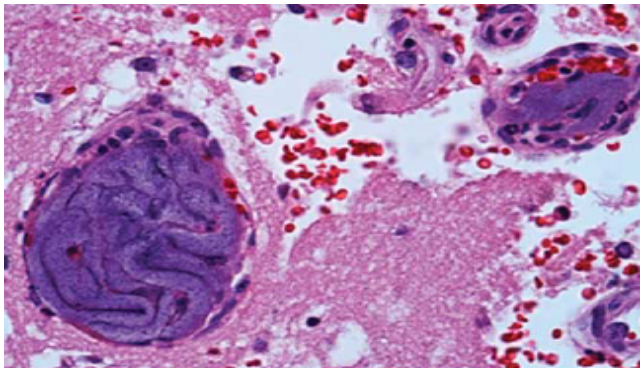
Cerebral thromboembolism

Any haemostatic agent placed directly on an open arterial injury runs the risk of becoming an embolic phenomenon and lodging distally in the arterial tree. There are a number of case

reports of foreign bodies becoming lodged in the cerebral vasculature, sometimes quite delayed post the initial event(320, 321). The most common of these are high velocity projectiles such as shotgun pellets(165, 322-324). These have caused a number of vascular pathologies, the most common being strokes and fistulae(325). There are however, also several reports of iatrogenic thromboembolism of hydrophilic polymer coating of vascular stents, glues and cyano-acrylates that were intended for use in obliterating arteriovenous malformations and aneurysms(326-328). These embolic phenomena have resulted in death and histopathological examination at autopsy has revealed this embolised foreign material completely occluding the cerebral vasculature(327).



Embolised hydrophilic polymer coating within the vasculature of the temporal lobe(327)



Embolised hydrophilic polymer coating within the vasculature of the occipital lobe(327)

There are also isolated case reports of venous air embolism associated with the use of Floseal on superior sagittal sinus wounds(240). This are assumed rather than actually proven events but it highlights a potential issue and the potential importance of a carrier matrix to keep any thrombotic material localised to the area in which it is required. It is important to note, however, that haemostatic agents such as fibrin/thrombin powders, gels and oxidised cellulose are regularly used on bleeding vessels within the cerebral cortex with no evidence of clinical ill-effects, distal embolism or stroke(229, 230, 329-333). It is important to ensure that any new haemostatic agent does not embolise distally, especially to an organ that is exquisitely reliant on continuous blood flow and has unreliable collateral circulation.

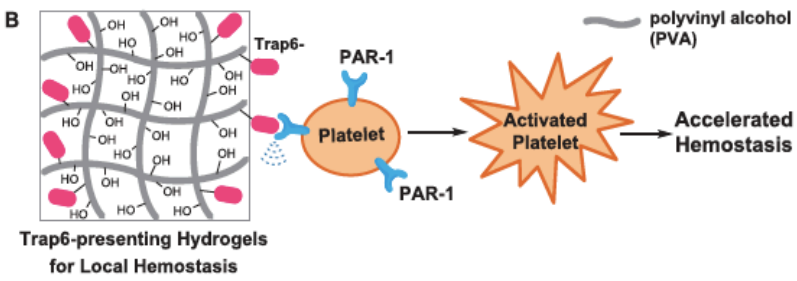
Synthetic Chemical Engineering in Haemostasis

As the coagulation and platelet activation and aggregation/clot formation pathways become more clearly defined, synthetic agents are better able to be engineered to activate or augment intrinsic mechanisms(334). The majority of them are derived from human or animal (generally bovine or ovine) sources and consist of purified or refined forms of substances such as thrombin and fibrin. These act as 'additives' to augment the patient's innate response. This is important as one of the rate limiting steps to haemostasis is delivery of autologous components to the area involved and the consumption of these components once they are incorporated into the platelet mesh and subsequent clot(318, 319).

These substances do, however, suffer from the disadvantage of a relatively short shelf life, storage conditions that range from frozen to room temperature, and comparatively long preparation times (2-10 minutes). There is also the theoretical risk of disease transmission given their source, despite this being exceedingly low given that they are screened and, in

the case of animal source, usually from a specifically bred and genetically-typed herd(201, 223, 225, 227, 230, 330). Seconds can count in major haemorrhage and tissue engineering and biomaterials design work has been focussed on eliminating some of these storage and preparation issues(334). The ideal haemostat would be stored at room temperature and be able to be readily applied within a matter of seconds from its storage state without activating substances needing to be added.

Qin et al have demonstrated an elegant potential solution to this problem. They have covalently bonded thrombin-receptor-agonist-peptide-6 (TRAP-6) to a poly-vinyl alcohol (PVA) hydrogel(334). Once platelets become activated, they express GPIIbIIIa on their surface to act as a binding site for fibrinogen. This activation is mediated by Protease activated receptors 1 and 4 (PAR-1 and PAR-4)(335). Thrombin proteolytically cleaves the N-terminal domain of PAR-1 to become a new N-terminal ligand domain, TRAP-6. This triggers the signalling activation pathway for PAR-1(336-338). By covalently bonding TRAP-6 to PVA hydrogel, the clotting time was reduced by 45% when compared to physiological clotting time. It was also able to cause platelet activation as measured by multiplate analysis whereas PVA itself had nil or negligible effect(334). Both these substances are able to be stored at room temperature and humidity.

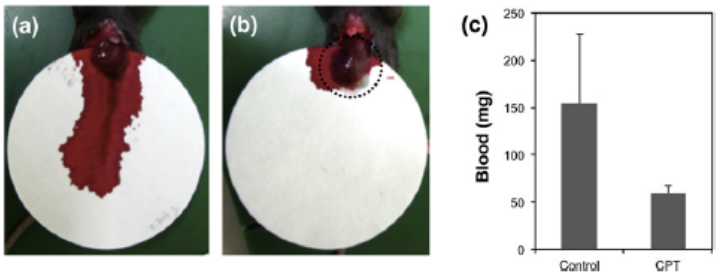


Mechanism of action of TRAP-6/PVA combination(334)

Hydrogels

Hydrogels are networks of polymer chains that are hydrophilic and high in water content (usually >90%). They are very absorbent and flexible due to this water content. Hydrogels form a net-like structure with multiple voids which increase their water carrying capacity. An in-situ hydrogel is one which is liquid at room temperature but becomes a gel under specific conditions, whether this is due to ionic-cross linking, temperature, or pH(339). These hydrogels may act as a base material whose properties can be manipulated for a desired effect. Gong et al, for example, developed a hydrogel polymer with antimicrobial properties(340). This is due to the addition of a long lipophilic alkyl chain that penetrates bacterial membranes and causes autolysis and cell death(340). Recent research has focussed on the use of hydrogels as dressings promoting wound healing, as scaffolds for drug delivery that are able to release their therapeutic drug load only under certain physiological conditions, and as contact lense components(339, 341-343).

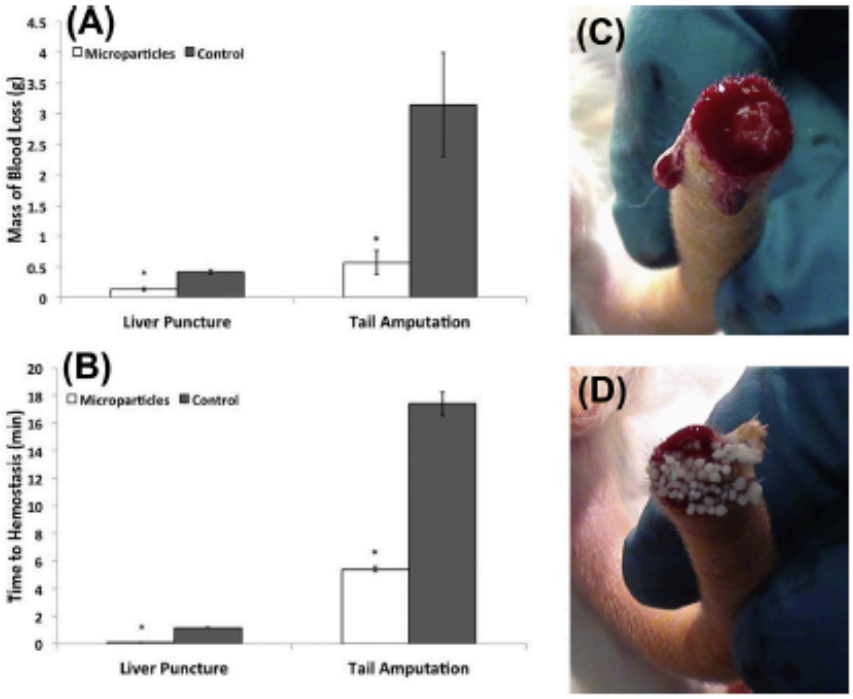
Lih et al combined chitosan with poly-ethylene-glycol which allows this to form an in situ hydrogel(316). They then evaluated the adhesive strength and wound healing ability of this hydrogel in vitro and in vivo in a mouse model of liver haemorrhage. With regard to haemostasis in vivo, the hydrogel reduced blood loss by 2/3rds (154mg vs. 59mg)(316). The in situ hydrogel gelated within 5 seconds and demonstrated an adhesive strength of 3-20 times that of a fibrin glue used as a comparator(316). This adhesiveness was thought to be due to the interaction of positively charged amino groups of the chitosan with negatively charged sialic acid residues on the mucus membrane of porcine skin used as the test substance(316).



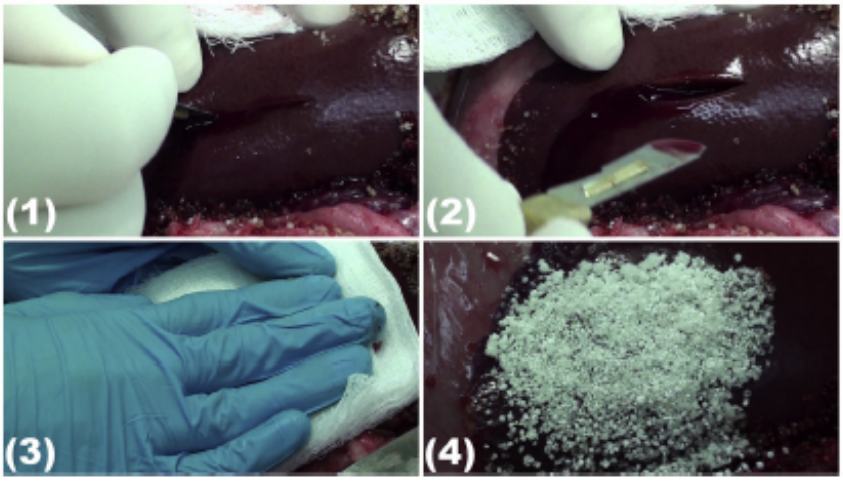
Comparison of haemostatic ability in a rat liver injury model of hydrogel (a) and control (b) with total blood loss after 3 minutes (316)

Original formulations of chitosan-based hydrogels suffered from the inability to gelate quickly enough for use as a haemostatic agent. Nie et al have developed a chitosan/ε-polylysine hydrogel with a fast crosslinking rate by the addition of maleimide group that acts as a peptide crosslink. The gelation time was reduced from 60 minutes to 15seconds +/-3sec(344).

Behrens et al manufactured a N-(3-aminopropyl)methacrylamide hydrochloride (APM) hydrogel that was dehydrated and formed into spheres(345). These were then mixed with ovine blood. Their baseline size ranged from 500-1000 micrometers. Once mixed with blood, they increased in size to 1400-2900 micrometers. This equated to a 1600% increase in mass and a 1687% increase in volume. These particles were used on a mouse liver and tail amputation model and resulted in a 300-500% decrease in blood loss. These particles formed a macroscopically visible aggregate at any point of contact with bleeding(345).



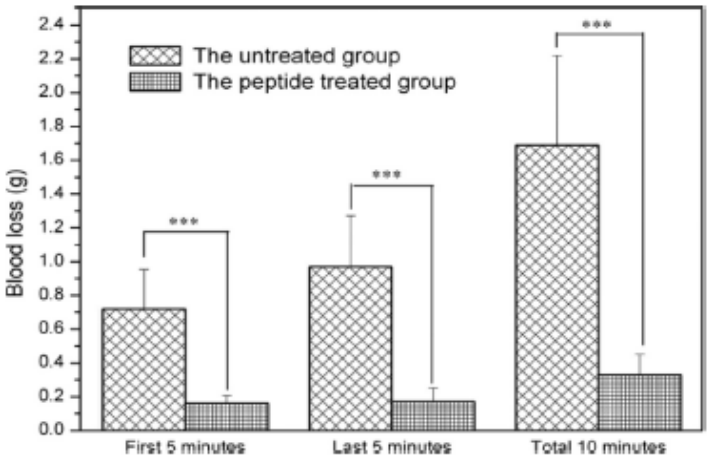
Mass of blood loss in rat liver and tail amputation model with hydrostatic particles vs gauze controls and photographs of proximal tail amputation site showing absence of bleeding in the hydrostatic particle group (345)



Photographs demonstrating the liver laceration (1) and (2), followed by gauze pressure over hydrostatic particles (3) and post gauze removal (4) (345)

Wu et al developed a hydrogel based on RADA-16 (a well-described nano-peptide used as an experimental haemostat in animal models). This was applied to an iliac crest defect and a middle auricular artery laceration in rabbits. The hydrogel formed a "*red jelly-like half-solid*

state" which formed a mesh with 100 nanometre pores(346). Blood loss in both experimental injuries was significantly reduced (0.16g vs. 0.72g). A small amount of this nano-peptide hydrogel was placed within the paravertebral musculature of rabbit. This was then harvested at 2, 4 and 8 weeks. H&E staining revealed relatively little inflammatory reaction with only a few foreign body giant cells identified(346).



Blood loss of untreated controls and peptide study groups in Wu et al(346)

Whilst this hydrogel may be applicable and efficacious in smaller arterial injuries in animals, this gel is unlikely to achieve haemostasis in a carotid injury in humans given the much higher flows and pressures involved.

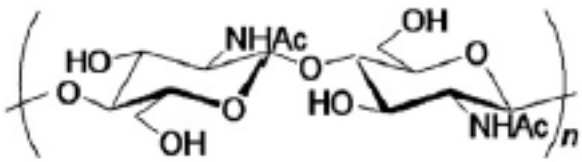
Kim et al developed a hydrogel with a dopamine poly-ethylene-glycol base which gelates at human body temperature within a few seconds(347). This is based on the properties shown by mussel adherent proteins that allow mussels to adhere to wet surfaces under a variety of conditions with high binding strength. This was tested on a rat liver model of haemorrhage and demonstrated an ability to significantly decrease blood loss (22.675mg vs. 71.16mg in

the control group)(347). Again, this is in a low flow/low pressure model and it remains to be seen if this has any applicability to higher flow/pressure bleeding in skull base surgery.

These experiments go some way towards validating the concept of engineering 'haemostasis activators' that can immediately be used to control bleeding. It remains to be determined the effect that these substances will have on tissues and whether they provide long term control. Further, animal studies will be required to not only examine their efficacy, but to ensure that complications such thromboembolism, infection, intracellular insoluble protein inclusion and pseudoaneurym do not occur.

Poly-N-acetyl Glucosamine (pGlcNAc) (Chitin/Chitosan) as a haemostatic agent

Poly-N-acetyl Glucosamine (pGlcNAc) is a carbohydrate polymer derived from animal, fungal and algal sources. In its natural form, it is a major component of crustacean exoskeletons, squid pens, the wings of insects, marine algae, or fungal mycelial mats(279, 348-350). It consists of polymers in a crystalline form. These polymers are arranged in either parallel lines (where they may be termed β-chitin) or antiparallel lines (where they may be termed α-chitin) which are held together by a combination of van der Waal forces and hydrogen bonds. They are able to be formed, via temperature variation and various solvents, into nano-fibres measuring 60-100 micrometers in length and 20-80 nanometres in diameter. The marine form, derived from microalgae, forms a beta-pleated sheet(350-353). The exoskeleton and fungal forms are usually of a more complex form incorporating deacetylated analogues and other proteins(354).



Chitin

(355)

Chitin is a heterogeneous mix of polymers with 10-20% N-acetyl residues and 80-90% glucosamine residues(356). This heterogeneity poses a problem in utilising the pGlcNAc in medical settings where standardisation and predictability of action is key. This problem can be mitigated somewhat by combining chitin with alkaline substances such as sodium hydroxide. This is termed 'deacetylation' and the resultant product is known as chitosan(357). The form taken by chitosan varies depending on the degree of hydration, the complexity of the original chitin mix, and the counter-ion mix(355, 358).

pGlcNAc has also been produced from microalgae sources. These are unique because the polymer fibres are bound to each other by interchain hydrogen bonds in parallel structure. These fibres have a diameter of 2-4 nanometres and are composed of approximately 80 polymers per fibre. They are able to be dissociated from their polymer form by hydrogen bond breaking solvents and reassembled in an antiparallel fashion to form a sheet or hydrogel(356).

In vitro studies

pGlcNAc is thought to increase red blood cell aggregation and endothelial-dependant vasoconstriction. It has also been shown to activate platelets(200) and has an antimicrobial action against a range of pathogens(200, 359-364). The beta-chitin form of pGlcNAc, with its

parallel fibres derived from microalgae is more effective in tests of haemostasis than the alpha-chitin form of pGlcNAc, which has antiparallel fibres(365). Deacetylation of chitin to chitosan is thought to reduce its activation of the intrinsic coagulation pathway(356). Studies have demonstrated less red cell agglutination and platelet aggregation(354).

Thatte and colleagues performed in vitro studies on alpha and beta forms of pGlcNAc and demonstrated that contact with beta pGlcNAc caused total and irreversible activation of platelets. P-selectin was expressed on the platelet surface and there was a morphological shift in the platelets with pseudopodia demonstrated(366). The authors propose a three-step activation mechanism(366)

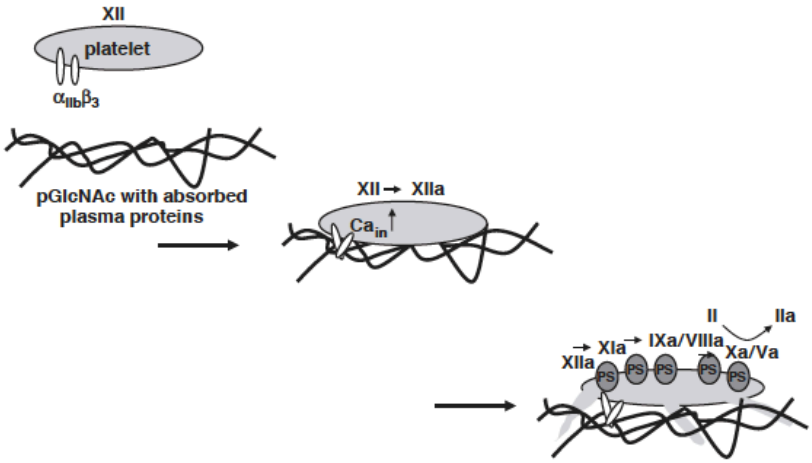
1. pGlcNAc binds to immobilised plasma proteins such as fibrinogen and/or platelets directly bind to pGlcNAc
2. Integrin-mediated platelet activation activated the intrinsic coagulation pathway via Hageman activation factor (Factor XII). This generates thrombin and forms a stable clot. This clot is further stabilised by the tendency for platelets to aggregate on pGlcNAc matrices and generate vasospastic substances such as thromboxane and serotonin
3. Clot retraction via platelet mediators and local vasospasm accelerates wound healing

Valeri and colleagues used thromboelastography to demonstrate platelet activation. A mixture of platelet poor plasma, platelet rich plasma or platelet rich plasma with red cells to a haematocrit of 20% was mixed with a pGlcNAc slurry. There was an increase in the

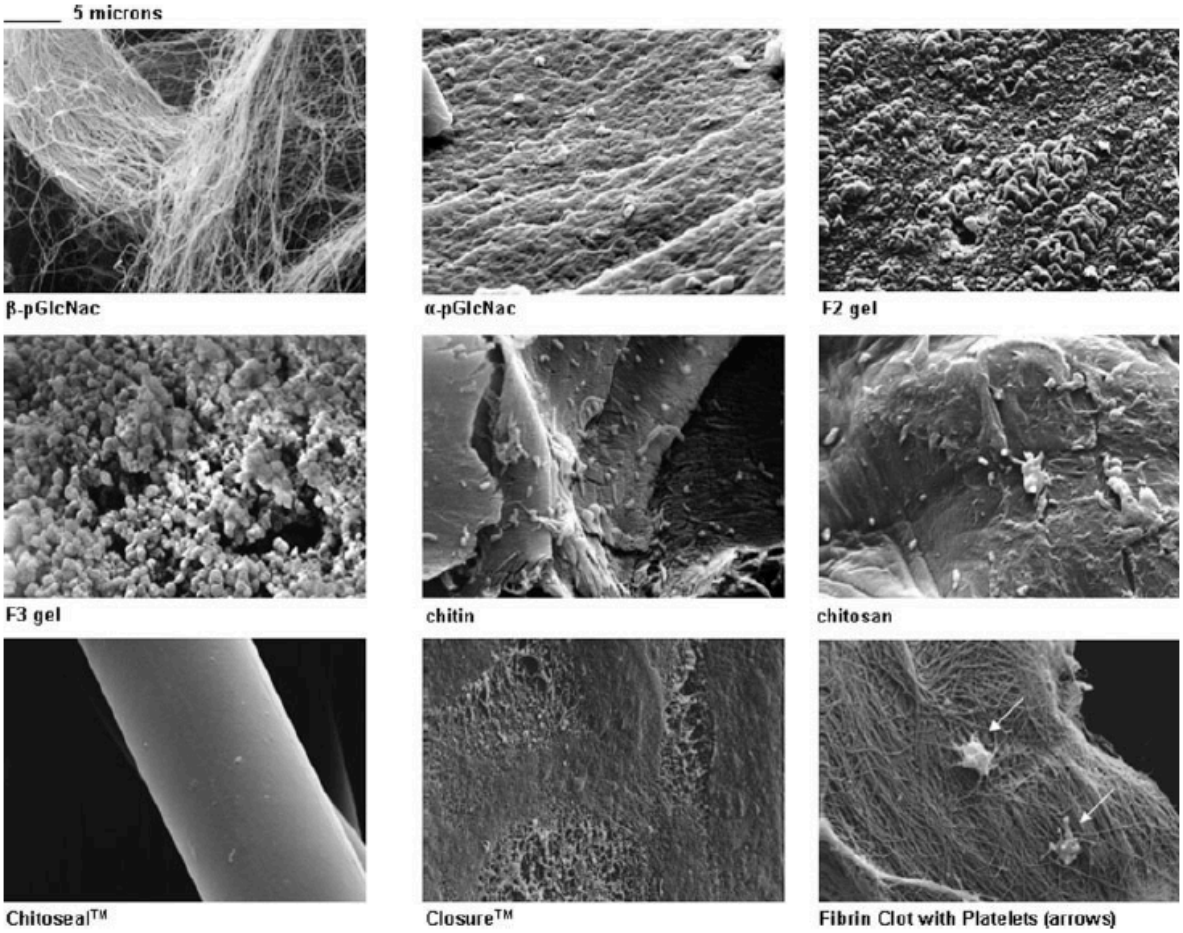
production of thromboxane A2 by platelets, an increase in platelet Factor X and Annexin V and increased platelet microparticles, all suggesting increased platelet activation(280).

Fischer et al performed a series of experiments to determine the mechanical events that lead to platelet activation when exposed to algae-derived pGlcNAc. They identified the plasma and surface proteins involved, the effect of integrin inhibitors and intrinsic coagulation pathway turnover on fibrin polymerisation and the intracellular signalling processes that activate platelets. Their conclusion was that the effect was multifactorial but they did determine that GP1b, a platelet protein that ordinarily binds to von Willebrand's factor, binds strongly to the pGlcNAc fibres to prevent platelet shearing and allow further binding to fibrin(194).

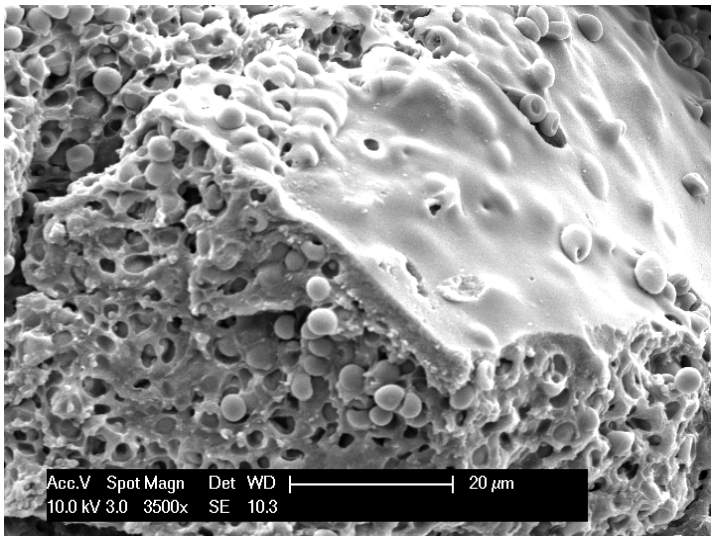
Follow up work by the same group using thromboelastography demonstrated that beta pGlcNAc (marine algae source) accelerated fibrin polymerisation to the extent that it negated the effect of GPIIbIIIa inhibition. Blood treated with eptifibatide (a GPIIbIIIa inhibitor) inhibited platelet aggregation. When beta pGlcNAc fibres were added, this inhibition was reversed. The kinetics of blood treated with aspirin or clopidogrel where unchanged compared with normal blood when mixed with pGlcNAc fibres(354). The authors suggest that the ability of pGlcNAc to reverse the GPIIbIIIa inhibition may be due to its exposure to platelet phosphatidylserine which activates the intrinsic clotting mechanism to produce thrombin and cross-linked fibrin polymers(192, 280, 354).



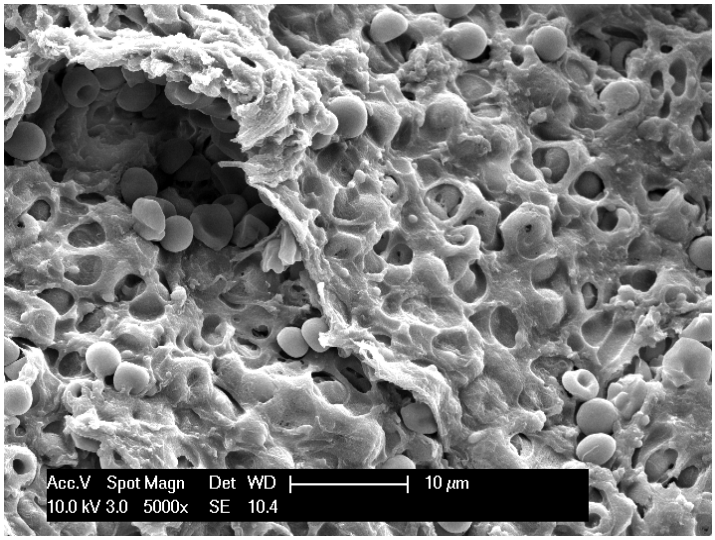
pGlcNAc binds to platelets. The resultant rise in intracellular calcium results in phosphatidyl serine being expressed on the platelet surface. There is also activation of peripheral platelet factor XII with activation of the intrinsic coagulation cascade(354)



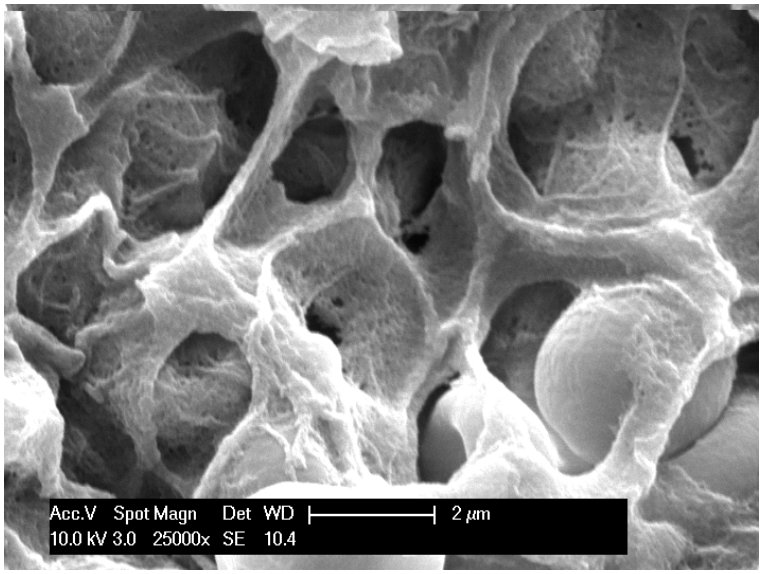
Scanning Electron Microscope photos of pGlcNAc products. The lowest right image shows activated platelets adhering to a fibrin clot(356)



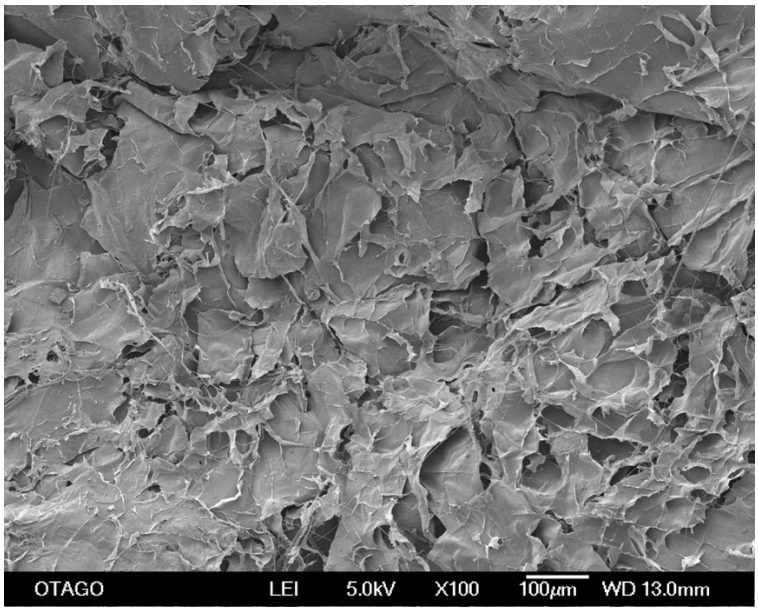
Scanning electron microscope image of a Chitosan-based patch(syvek) covered in ovine erythrocytes (Jukes unpublished data).



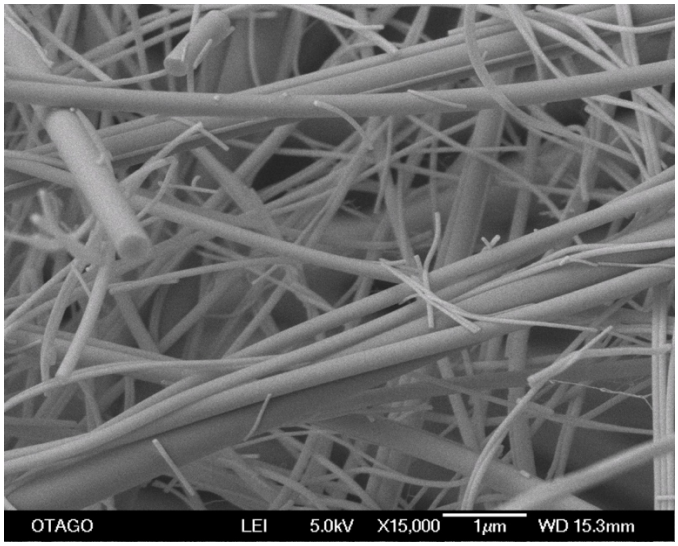
Scanning electron microscope image of a Chitosan-based patch(syvek) covered in ovine erythrocytes (Jukes unpublished data).



Scanning electron microscope image of a Chitosan-based patch(syvek) covered in ovine erythrocytes (Jukes unpublished data)



Scanning electron image of beta-chitin synthesised as a haemostatic patch (Jukes unpublished data)



Scanning electron image of beta-chitin synthesise as a haemostatic patch (Jukes unpublished data)

In vivo studies

Jegatheeswaran and colleagues performed in vivo experiments to determine the effect of pGlcNAc- derived chitosan on haemostasis in an ovine hepatic injury(367). They performed partial liver injuries and subsequently hepatectomies on pigs and demonstrated a non-significant decrease in time to haemostasis when compared with manual pressure (control) with a median time of 2 minutes less in the chitosan group(367). There was no significant difference in haemodynamic parameters or blood loss volume.

Horio et al performed a similar experiment on liver injury in rats, comparing hydrogel-mixed chitosan sponges to fibrin/thrombin patches (TachoComb)(368). They demonstrated no difference in haemostatic efficacy between these groups in non-heparinised rats however the chitosan sponges had a significantly higher haemostatic efficacy in the heparinised rats, with all rats in this group achieving haemostasis within 5 minutes and all rats in the control group exsanguinating(368).

Brandenburg et all used liquid chitosan (2mg/ml) in the brain of cats to determine efficacy and safety. Liquid chitosan was applied to a corticotomy 10mm wide and 5mm deep. Haemostasis was achieved in an average time of 4 minutes and 10 seconds. There was no increase in inflammation over and above that of controls in histological examination of the specimens when brains were sectioned at 6-8 weeks post injury indicating that chitosan is potentially safe for use in the central nervous system(369).

Chitosan is thought to increase granulation around wound sites. As it is broken down, it depolymerises and releases N-acetyl-D-glucosamine which stimulates fibroblasts and increases hyaluronic acid production at the wound site(355, 361, 370). This allows increased delivery of oxygen to surrounding tissues and increased neutrophil activation(200).

Chitin/Poly-N-acetyl glucosamine allergies

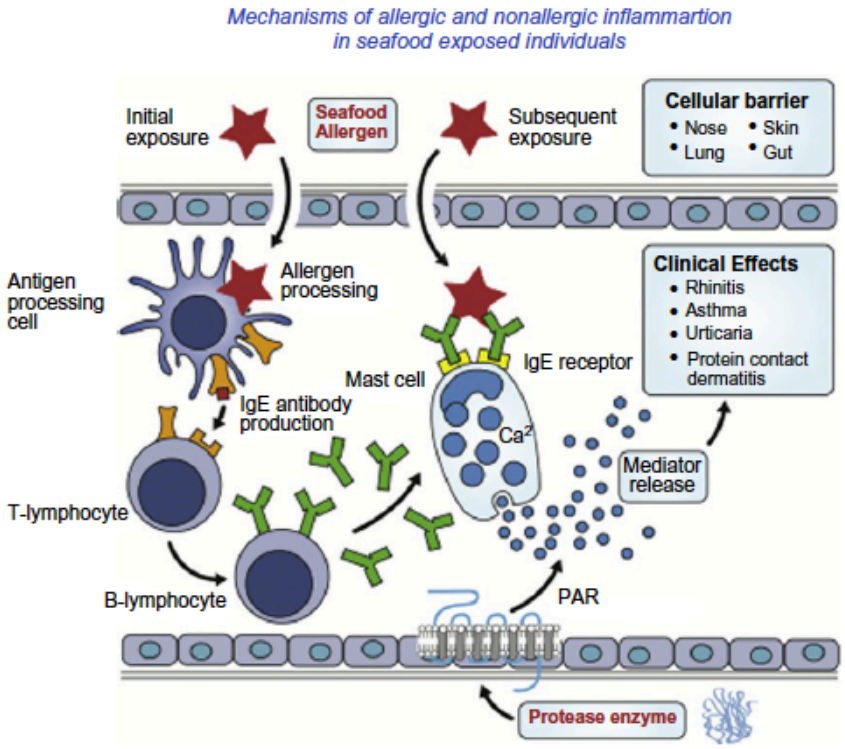
Given pGlcNAc is derived from crustaceans/molluscs/shellfish, it is important to ensure that it does not result in allergic reactions in those patients susceptible to this. The true incidence of shellfish allergy is unknown but population studies have placed it somewhere in the range of 0.5%-3.8%(371). Reactions vary from the potentially life-threatening anaphylaxis to mild skin reactions. There are over 50,000 different species of shellfish and over 100,000 species of molluscs described(371). The precise allergen is often difficult to determine and further testing is rarely performed. Once a shellfish allergy has been diagnosed, the advice is often simply to avoid the entire shellfish family(371-373). There are several individual allergens that have been identified including Tropomyosin (TM), arginine kinase (AK), myosin light chain (MLC), and a calcium-binding sarcoplasmic protein (SCP). Chitin itself, in purified form, is not generally considered immunogenic when not associated with these proteins(374). It is important to note that chitin in vivo in shellfish, molluscs and

fungi is part of a complex structure and not found in a purified form. It is generally covalently linked to proteins and, in fungi, linked to glucans. This is very different to the regulated and highly associated structure of purified chitin used in vitro(362).

Chitin is known to be involved in host tissue responses to allergy(374, 375). Workers in shellfish processing plants are known to have higher levels of allergy and 'shellfish asthma' is a recognised disease by the centre for disease control with a prevalence of 2-36%(376). These workers are also known to have a higher level of allergy to mites and insects, likely reflecting the homogeneity of the protein structures in their shells(376). There is some evidence that the chitin in fungal cell walls induces a T-helper cell type 2 (Th2) response and this has been hypothesised to contribute to chronic rhinosinusitis however this is not yet definitively established(377).

Reese et al demonstrated chitin accumulation in IL-4 cells involved in macrophage activation – essentially acting as a co-factor for activation and non-specific tagging of pathogens(374). It is also known to be a strong Th1 cell adjuvant in inducing mycobacterium immunity(362). Chitin, when introduced into the lungs of mice, causes an allergic response with both basophil and eosinophil migration within 6 hours and upregulation of the gene coding for an enzyme that breaks down chitin (chitinase)(374). There is, however, some conflicting evidence that chitin itself may actually down-regulate adaptive type 2 allergic responses to subsequent allergens and be beneficial in humans(378). Chitin fibres introduced into the sinuses have been shown to decrease the host response to the lipopolysaccharide from Enterobacter agglomerans. This may result in a trend towards less rhinorrhoea however the

difference in the literature is thus far non-significant(378). In studies of quantitative measures of circulating inflammatory cells, the presence of chitin in the spleen reduces expression of allergen-associated interleukins IL-4, IL-5 and IL-10(362, 379, 380).



Allergy mechanism via IgE mediated mast cell degranulation in response to environmental allergens(376)

From the point of view of drug design and biomaterials modification, there has not been a reported case of allergic reaction to purified chitin/chitosan in either humans or animals, no matter what the method of administration(362, 373, 381). Muzarelli, in a review of marine drugs derived from chitin makes the assertion that *"it is concluded that crab, shrimp, prawn and lobster chitins, as well as chitosans of all grades, once purified, should not be considered as "crustacean derivatives", because the isolation procedures have removed proteins, fats and other contaminants to such an extent as to allow them to be classified as chemicals regardless of their origin."(362)*. Once chitin has been deacetylated to chitosan or fully

depolymerized to pGlcNAc, these new structures have their own composition and characteristics that mean they may be safe to use in patients with shellfish allergies(362).

Peptides in Medicine

Nanomedicine

Nanomedicine was defined in 1983 as "*the ability to influence, in a positive or negative way, any process, cell or organ, to maintain function; in other words, to stop loss, maintain function, or increase function*" and refers specifically to work on structures that are <100nm in size (382). The challenges that this poses are immense and involve controlling structures on an atomic scale(383). Given many disease processes involve damage to cell structures, the ability to administer a substance that either provides a scaffold for ordered cellular regeneration or delivers growth factors that stimulate such recovery is attractive, especially if this scaffold were comprised of materials that were able to be broken down without causing long term damage once their function had expired(383, 384).

Peptides in the context of nano-medicine are amino acid chains that are amenable to self-assembly into a 3-dimensional structure – that is, they form secondary structures according to their amino acid sequence under certain external conditions(365, 383). They are, as Loo et all have pointed out, "*versatile building blocks for fabricating supramolecular architectures*"(365). The development of these has come about through increased understanding of the protein chains found in naturally occurring substances. Peptide self-assembly is a complex interaction of forces including hydrogen bonding, ionic forces, hydrophobic forces, van der Waals interactions, and electrostatic forces(365). Proposed

uses for these peptides include drug delivery, scaffold sheaths for neural redevelopment, and, importantly, haemostasis.

NB: (Van der Waals forces are the residual attractive or repulsive forces between atomic groups or molecules that are not explained by covalent bonds or electrostatic forces. These forces are anisotropic i.e. they depend on the relative orientation of the molecules. They are also dependent on the size of the bodies. These forces can be seen in nature, with the van der Waal's forces between the filaments of hair on Gecko's feet and the surface upon which they are standing accounting for their adhesion to such a surface)(385-387).

Self-assembling peptides have been demonstrated to form 'gel in situ' in response to specific stimuli. Studies have demonstrated that it is possible to insert a bioactive drug into the peptide mix by gelation and form a scaffold. The porous nature of this scaffold can be modulated to affect the rate of drug delivery by altering peptide density(365, 388). These are generally formed into beta-pleated (β-pleated) sheets. The advantage to this method of drug delivery is that amalgamation into a protein sheet renders the drug less susceptible to metabolic processes within the body and allows it to be delivered unaltered to the site of action(387).

NB: A β-pleated sheet is comprised of chains of amino acids (8-10 amino acids long) known as strands. These are connected laterally by hydrogen bonds (generally 2-3 bonds per strand). Together, these form a twisted sheet of protein chains. These can be synthesised artificially and are analogous to the β-pleated sheets found in Alzheimer's disease, which exert a pathological effect by their insoluble nature and subsequent plaque formation(365).

Loo et al defined the ideal biological scaffold as meeting the following criteria(365)

"1. The basic building blocks should be derived from biological sources, namely, amino acids, lipids, nucleic acids, and sugars

2. Basic units should be amenable to design and modification at the single molecular level to achieve specific needs

3. The scaffolds should exhibit a controlled rate of material biodegradation

4. The materials should have no cytotoxicity

5. They promote cell-substrate interactions

6. The materials afford economically scale up and reproducible material production, purification, processing and long-term storage

7. The materials should be readily transportable

8. They should be chemically compatible with aqueous solutions and physiological conditions

9. They do not elicit immune responses and inflammation when used in human species

10. The materials should integrate with other materials and especially tissue in the body"

Medical Uses

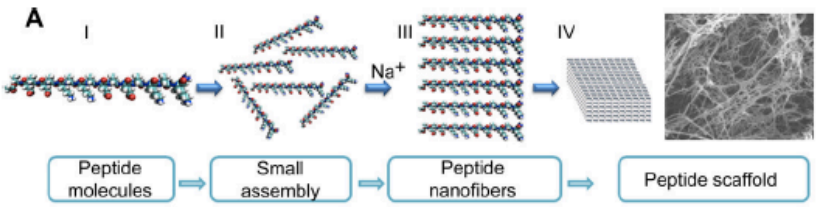
Self-assembling peptides have been described as haemostatic agents(389, 390), as adjuncts to ophthalmological surgeries (such as corneal endothelial stem cell transplantation) and as a self-assembling peptide to provide a scaffold or bridge for axonal regeneration in optic nerve reconnection(383). A peptide chain self-assembled into a β-pleated sheet named RADA16 has been used to deliver epidermal growth factor to increase the rate of wound healing and insulin-like growth factor, platelet-derived growth factor and stromal cell-derived growth factor-1 to post-infarction myocardium(365). These hydrogels allow the delivery of bioactive molecules to direct to areas that they are required whilst protecting

them from degradation in systemic circulation. Ellis-Behnke's group has also demonstrated the ability to use these nanofibre scaffolds to repair hamster brain lesions by allowing nerve fibres to reconnect post severance(391, 392). This is analogous to the role played by myelin sheaths in neural protection and redirection(392). Theories have also been advanced in the field of cancer medicine that nanostructures could change the extracellular matrix that facilitates cancer metastasis and ensure malignancy remains localised(393). When applied to drug delivery, nanobiotechnology may solve the problem of permeation through cell membranes. At less than 10nm, particles within the human body can be thought of as having "infinite permeability"(383). This might allow topical delivery of a therapeutic molecule if bound to these particles.

Nano-haemostats

'Nano-haemostat' refers to an amino acid chain forming a self-assembling peptide that is used as a scaffold for haemostasis, potentially trapping endogenous coagulation factors around the site of bleeding. There are multiple different types and formulations described but they common features are that they comprise a dissolvable L-amino acid chain that does not interfere with cellular pathways or promote or inhibit signaling and that is broken down via the body's natural peptidases and enzymes to constituent amino acids after its primary role has been performed(389). There are a number of studies demonstrating almost immediate haemostasis in small animal models without activation of the coagulation cascade. This has been demonstrated on mammalian brain, spinal cord, liver, kidney, femoral artery and skin.(379, 389, 390, 394). These solutions have been demonstrated to breakdown via natural enzymatic processes and are non-immunogenic and nontoxic(383). In

addition, the breakdown products are amino acids that may be used for subsequent tissue repair(383, 389).



Representation of stages of self-assembling peptide forming a scaffold(395)

The ideal haemostatic agent has previously been described as having 5 qualities; it must be safe, efficacious, easy to use, low cost, and able to undergo regulatory approval for human use(389). When applied specifically to the problem of bleeding in endoscopic surgery, it must be able to conform to irregular cavities, not interfere with or obstruct the surgical field, and not damage surrounding structures, either through thermal or chemical injury or through expansion and pressure injury to nerves or vessels(284, 396, 397).

Current methods of haemostasis used in carotid artery injury in the endoscopic surgical field (including muscle patches(117-121), Floseal, tisseal, thrombin with gelatin(223, 398), and haemostatic clips (anastoclip)(121, 122)) meet some of these requirements. Whilst these have been proven to be efficacious, they all have their limitations. There can be some delay in harvesting a muscle patch and it is partially dependent on platelet activation as part of its mechanism of action(121). Floseal and tisseal have been shown to wash out of the surgical field rapidly in the case of torrential haemorrhage(117-120). Anastoclip can be difficult to apply in situations with a tear in an artery at the lateral extremity of the field(122).

Nano-haemostat appears to form a scaffold around bleeding vessels independent of any coagulation or platelet pathway(394, 399, 400). Previous research in a rat model has shown that not only does it stop bleeding, but that this scaffold is then broken down into constitute amino acids(384). It also has the advantage of being a liquid and is thus more able to conform to irregular-shaped cavities. It does not appear to provoke an immune response. These peptide chains have been shown to work best as a haemostatic agent when comprised of amino acids of the same chirality. Alternating chirality gave bleeding times up to 8 times longer in a rat liver haemorrhage model(400).

Nano-haemostat is applied in liquid form when first mixed before gelating around the bleeding structure. Whilst it has been proven to be successful in a small animal model of vessel injury, this may not hold true in larger animal/human models where pressures may be many times that of smaller animals(332, 390, 398, 401). This pressure may wash the haemostat away from its site of action before it can take effect. The optimal delivery method and amount will need to be determined. Further, the liquid may need to be either impregnated in, or held on by a patty or similar. This may remove the advantage of having a clear liquid haemostat, at least in the immediate phase of application(402).

Safety studies will also be needed if nano-haemostat is to be used in the central nervous system. The build-up of abnormal proteins within the central nervous system is pathological and the basis for the development of diseases such as dementias and prion diseases. It would be incumbent upon designers of nano-haemostats to ensure that their solution is adequately broken down by the body's enzymatic processes and is not trapped in insoluble form within neurons or astrocyte.

There are thus many questions to answer before nano-haemostat technology can be utilized in skull base surgery.

- Which formula is best and why?
- What rate of flow can it stop?
- Does it need to be impregnated in a carrier?
- Does it preserve vessel lumen patency and thus allow distal brain perfusion?
- Does the scaffold last long enough for more permanent healing to occur?
- Do pseudo-aneurysms form after its use?
- Are there long-term effects on the brain – i.e. what happens to the breakdown products?
- Does any nano-haemostat remain on the vessel wall over time?
- Has re-epithelialisation of the vessel wall taken place?
- Is the vessel patent?
- Has there been pseudo-aneurysm formation?
- Do surrounding neurons/glial cells contain any evidence of abnormal protein inclusions?
- Is there evidence of distal embolic phenomena?

Review of studies of nano-haemostat

Several studies have looked at the role these self-assembling peptides may play in haemostasis(389, 390, 400, 403). A putative rational for these nano-peptide scaffolds is that they may provide a complex framework analogous to this mesh of platelets and leukocytes

to allow endogenous coagulation a more stable foundation. Evidence to support this hypothesis that nano-peptides in haemostasis provide more than a simple physical barrier comes from studies looking at peptide d-EAK16 by Luo et al(400).

Self-assembly of d-EAK16 was timed in a pure water solution and in a living animal model (rabbit liver). It took ~16 hours for d-EAK16 to self-assemble in water but ~20 seconds to self- assemble and control haemorrhage in bleeding(400). The authors conclude that ions, proteins, enzymes and "other factors" in blood work cooperatively to cause haemostasis. Their rationale is that normal haemostasis occurs when there is a high concentration of clotting factors held within a localised area whereas the same gross number of coagulation components would be ineffective if spread out over a larger area. A solution of d-EAK16 is thought to be triggered by Na+, Cu2+, K+, Mg+, Ca+, Fe3+ and Zn2+ to form a stable β pleated sheet in which endogenous coagulation factors become trapped and undergo activation in the usual fashion. This β pleated sheet however is not the final form the nano-peptide takes. Rather the sheets combine to form short and then long nanofibres which may then form 'tight junctions' impervious to liquids.

Wu et al performed a study to determine the haemostatic efficacy of RADA-16 in a rabbit model of bone bleeding using an ilium bone defect. 4mm bony defects were created which were filled with RADA-16, bone wax or saline. Whilst the RADA-16 and bone wax were comparable in terms of haemostatic efficacy, the RADA-16 permitted significant new osteogenic activity as opposed to the bone wax, which impaired new bone formation. The authors concluded that nanofibre hydrogel did not impair osteogenesis and allowed increased formation of new bone(346).

Ellis-Behnke and Colleagues performed several small animal experiments using RADA-16 1% weight/volume on brain, spinal cord, liver and femoral artery bleeds in rat models(389). The demonstrated that compared with iced saline, RADA-16 was capable of forming a gel that caused total bleeding cessation in under 15 seconds. They also demonstrated no evidence of neurofibrillary tangles in the brains of animals recovered out to 6 months post application of RADA-16(389).

AC5 is a proprietary, synthetic, nano-haemostat formulation(404). Currently in the initial phases of human trials, AC5 addresses the potential for haemostasis in context of anticoagulation(390). In a liver punch biopsy model in rats(390), AC5 achieved haemostasis to the same pre-determined degree in heparinized and non-heparinized rats with a 94% reduction in time to haemostasis compared to saline controls(390). A Full thickness 4mm punch biopsy to ventral liver was created to give a non-compressible wound. The core was then filled with 200 microliters of AC5-H or AC5-L or saline as control. Activated clotting time (ACT) and partial thromboplastin time (aPTT) were taken from animals. Animals were euthanized if time to haemostasis was >1500sec. Animals were not recovered post procedure. Histology showed that RBC surrounding the AC5 did not change shape(390).

Safety of nanopeptides in the central nervous system

RADA-16 is a self-assembling peptide of ionic hydrophilic and hydrophobic amino acids. It is 5 nanometers in length(384). It forms stable beta-pleated sheet structures which turn into hydrogels. Sang et al performed a study in which they divided 20 rats in 4 groups – (1) Intracerebral haemorrhage (ICH) without aspiration, (2) ICH with aspiration, (3) ICH with aspiration and saline and (4) ICH with aspiration and RADA-16 injection.

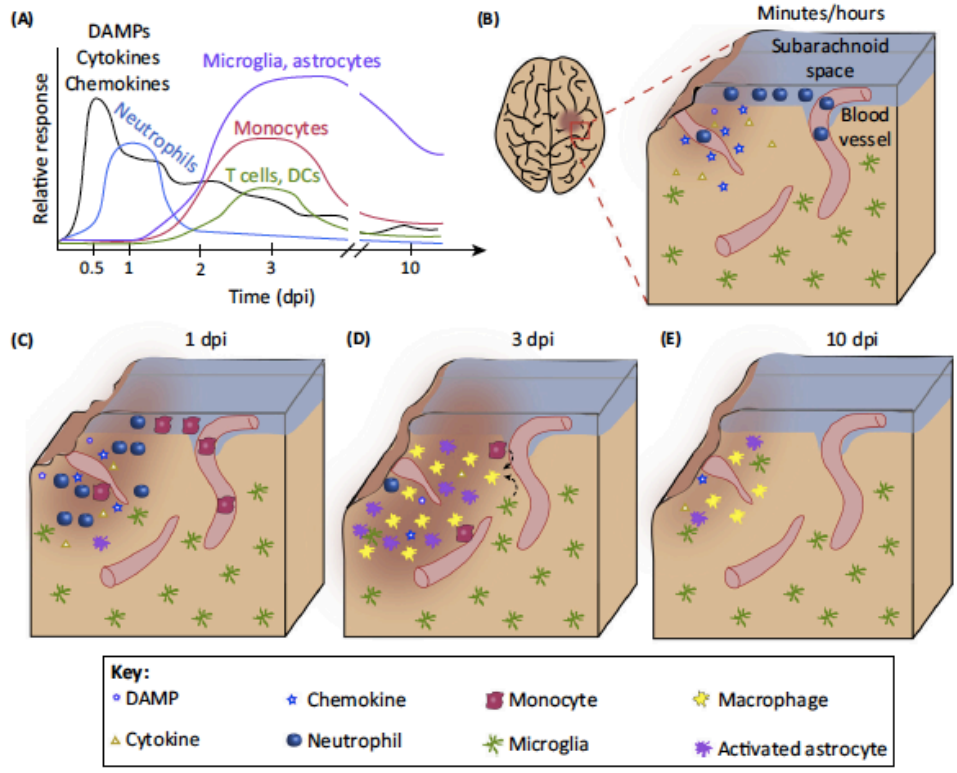
ICH was created via intrastriatal injection of collagenase. 210 mins later, the haematoma was aspirated and 20 microliters of saline or RADA-16 was injected into the ICH cavity. Differences in haematoma volume in aspiration vs saline vs RADA-16 group were not significant. Aspiration with injection of RADA-16 significantly reduced inflammatory cells in surrounding cortex on histological examination. There was a reduction in brain oedema, peri-haematoma apoptosis and inflammatory reaction in the RADA-16 group. The authors concluded that self-assembling peptide attenuated brain injury, enhanced functional recovery and reduced cavity volume(384). This reduction in secondary brain injury is thought to come about by decreasing the leakage of serum proteins into the surrounding parenchyma. Histology showed tight contact between self-assembling peptide and the cavity wall(384). More work is required to validate this concept in both the acute and chronic phases of brain inflammatory reactions. The longer-term effects of the breakdown of this nano-haemostat over months to years have not yet been studied.

Histology of brain response to ischaemia and foreign bodies

Histologically, ischaemic or toxic changes in the brain follow a predictable time course. With regard to neuronal reaction, 'red neurons' appear. These occur within 12-24 hours and are visual morphological markers of cell death. They include shrinkage of the cell body, pyknosis of the nucleus, disappearance of the nucleolus and loss of nissl substance. There is an eosinophilic appearance to the cell(405). Glial cells eventually undergo gliotic change, swell, develop pale cytoplasm and prominent nucleoli. Microglia are phagocytic cells within the brain which proliferate and form aggregates around damaged neurons and astrocytes. They may be accompanied by blood-derived macrophages in areas of toxic damage(405, 406).

Molecular mediators of responses to cell damage peak at different times post injury. Neutrophils are maximally seen at approximately 1 day post injury whilst monocytes, T-Cells and dendritic cells are found in maximal numbers at approximately 3 days post injury(407). The inflammatory reaction is generally full resolved by 10 days post injury and more chronic changes become apparent(408).

Histological examination allows identification of the acute inflammatory response that occurs as a reaction to foreign body exposure. It is essential to demonstrate that there is minimal inflammatory reaction to any haemostatic product that is to be placed within the central nervous system. The cortex is uniquely sensitive to damage and, given the limits of regenerative capacity and neural plasticity, any inflammation should be avoided.



Time course of cellular response to traumatic brain injury(407)

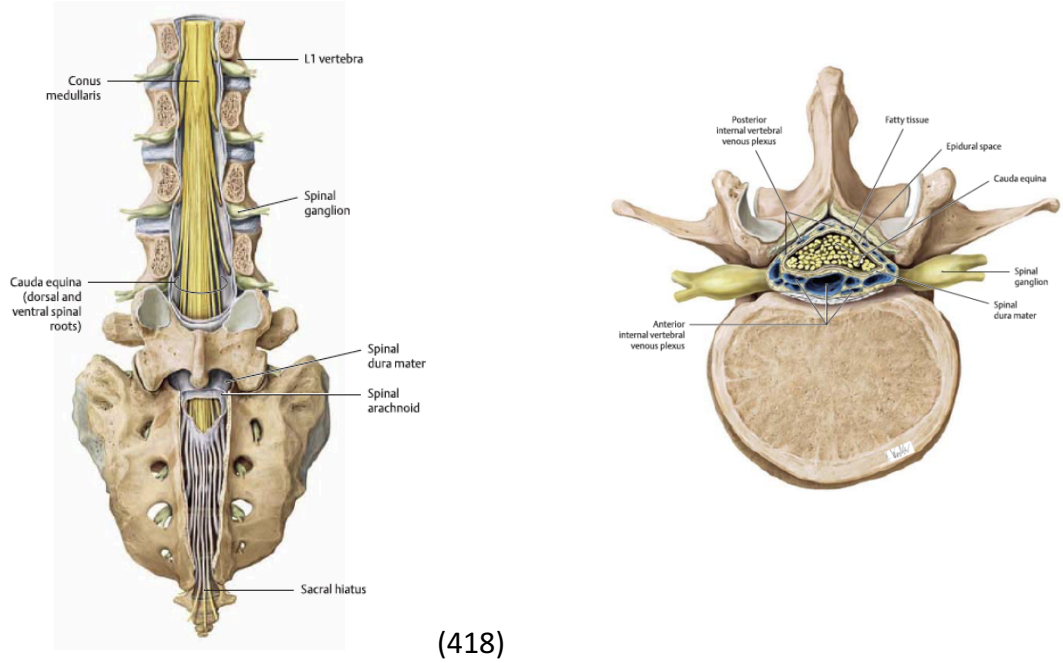
It appears that the inflammatory reaction within the CNS commences early in <6 hours and that there is a variation in the time course of peak numbers of inflammatory cell. These studies would appear to indicate that in order to determine the inflammatory response to foreign body haemostats, tissue should ideally be collected between 1 and 4 days post injury to see maximum inflammation.

Detrimental Effects of Bleeding Elsewhere in the Central Nervous System

Post-Laminectomy Adhesions

Laminectomy is a neurosurgical procedure commonly performed for spinal stenosis with claudicant leg pain, to decompress nerve roots impinged upon by bone, and to access the spinal cord to operate on intra and extradural pathology(409-411). This can be performed at any level with minor variations however the general workflow is thus: The patient is placed prone on blocks or on a Jackson spinal table after induction of general anaesthesia. After prepping and draping, a linear incision is made in the skin and dissection through adipose tissue to the lumbar fascia is made. The paraspinal multifidius and longissimus muscles are dissected off the spinous processes and held out to the width of the facet joints with the aid of retractors. Rongeurs and drill are used to remove the spinous processes and lamina. The ligamentum flavum is then exposed and removed, generally with a kerrison punch. This exposes the thecal sac. From here the operation varies depending on pathology. On closing, haemostasis is achieved in the field and the paraspinal muscles reapproximated with sutures. The lumbar fascia is closed and the extrafascial space obliterated with sutures. The skin and subcutaneous tissues are then closed. Patients are then recovered and, generally, ambulate as tolerated immediately. Published rates for reoperation and revision lumbar

laminectomy vary widely but range from 6-19%(412). This can be for a number of indications including early revision and washout for infection, progressive deformity and spondylosis, adjacent segment disease and recurrence of the pathology that prompted the initial laminectomy (tethered cord, malignancy etc)(409, 413-417).



(418)

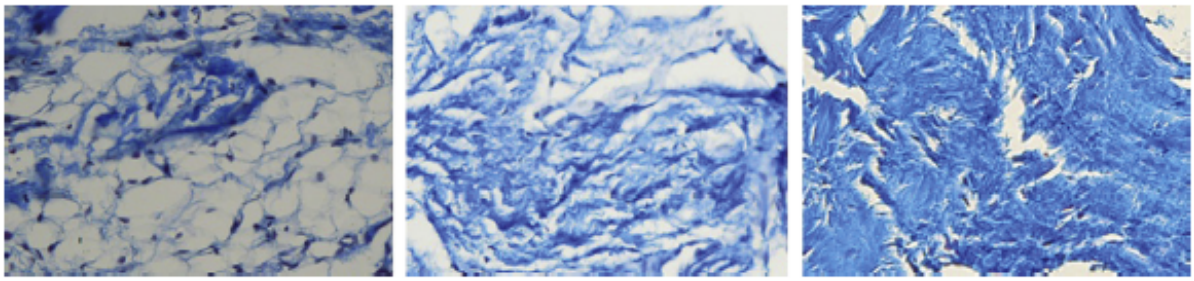
Posterior view of lumbar spine with spinous processes and lamina removed – section through pedicles (A) and axial section of lumbar spine with dorsal aspect most superior (B)(418)

Post-surgery adhesions and pain

There are a significant number of patients who develop back pain post laminectomy surgery(419-421). This is sometimes termed 'Failed back syndrome' (FBS) or Failed back surgery syndrome (FBSS). FBS is a chronic pain syndrome that occurs after spinal surgery. It may include leg or back pain, or a combination of both(413). Adhesions may play a role in this although the overall syndrome is thought to be complex and multifactorial with a number of biological, social, pathophysiological and legal factors at play(413). These adhesions prevent the spine, thecal sac and nerve roots from moving freely along with

biomechanical movement of the entire body. There is likely also a component of chronic biochemical inflammation and phospholipase A2 has been implicated in the development of this(422-426). The incidence of FBS ranges from 5-60% across case series(413, 427). It is difficult to quantify the exact cost of FBS or disabling pain post spine surgery however the resulting disability has been rated as higher than that of rheumatoid arthritis using the Oswestry disability index(428). The burden of disease is economic, social, psychological and physical. Current treatment strategies range from cognitive behavioral therapy to spinal cord stimulators(413, 429, 430). Part of the aetiology is thought to be due to the formation of adhesions post-operatively with consequent tension on nerve roots and pain generation(420, 431).

No matter what form of haemostatic technique (or combination of techniques) used, a post-operative haematoma forms over the thecal sac. This is then invaded by fibroblasts from adjacent muscle(420, 432, 433). These fibroblasts encourage the formation of adhesions. As patients mobilise, tension is placed upon nerve roots, facet joints and the surrounding tissues. This sensation is interpreted as pain(413). This adhesion work was first demonstrated in a canine model by LaRocca and McNab in 1974(434). They demonstrated the progression of immediate post-operative haematoma through stages of fibroblast invasion and subsequent formation of a 'laminectomy membrane', which consisted of a fibrous scar covering the surface of the thecal sac and extending some way out over the nerve roots. These nerve roots, dorsal ganglion and facet joints all have sensory innervation(418, 435, 436). Further studies have implicated platelet derived growth factor (PDGF) which may attract proliferation myofibroblasts to the area and possible defects in the fibrinolytic pathway(432).



Histology demonstrating mild, moderate, and severe fibrosis of the epidural sac in a rat model(437)

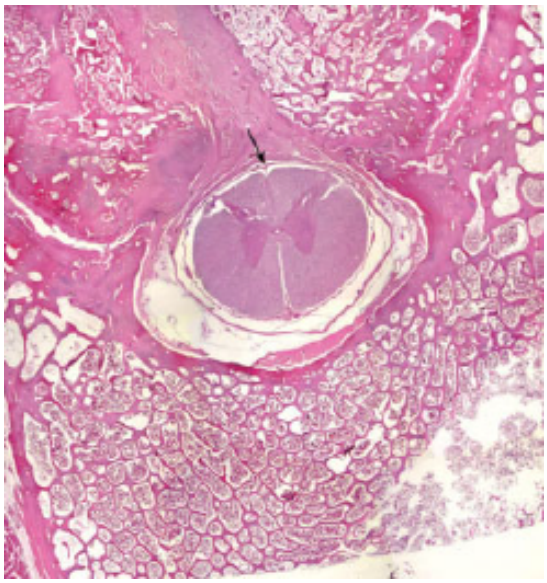


Photo of decalcified histological section through sheep laminectomy site showing dense adhesion of muscle to dura (arrow)(433).

Adhesions are also a concern in other areas of neurosurgery. Decompressive craniectomy for indications such as trauma, ischaemic and haemorrhagic stroke generally requires a wide dural opening(438-441). Some authors advocate using a pericranial graft at the time of operation whilst others use a synthetic graft to prevent periosteum and scalp flap from adhering to the brain and causing subsequent injury whilst raising the flap during cranioplasty(442). There is evidence that there is a higher infection rate with these synthetic materials(442). This is especially relevant in the setting of decompressive craniectomy as there is often gross tissue contamination of these wounds from the initial trauma. There is

also potentially an increased risk of the development of early (<48hrs) post-operative haematoma of up to 18%(442). Interestingly, postulated reasons for this include that the lack of adhesions between brain cortex and scalp flap leads to the development of a potential space for haematoma formation(442). The hunt for anti-adhesion agents has led to the development of a number of compounds and animal models(443-445). There are 5 potential ways in which this can be achieved(446).

1. Control the inflammatory reaction
2. Prevent clotting of inflammatory exudates that have already formed
3. Removal of fibrin deposit
4. Barriers to mechanically separate dura and clot
5. Inhibition of fibroblastic reaction

Adhesions are generally measured using the 'strip test' in which qualitative, blinded assessment is performed post mortem by measuring the resistance to separation of the tissue in question and its surrounding structures such as dura removal from paraspinal musculature and vertebra(433).

Score	Epidural Adhesion Formation
extent of adhesion	
0	no epidural scar
1	minimal, as much as 25% of the length of the dorsal dura involved in scar formation
2	moderate, from 26 to 75% of dorsal dura involved in scar formation
3	marked, >76% of the length of the dorsal dura involved in scar formation
density of adhesion	
0	no epidural scar
1	throughout length of adhesion, most connective tissue scant and poorly organized
2	scar more organized than Score 1, most of scar still composed of loosely arranged connective tissue stroma throughout its length
3	scar more organized than Score 2, throughout length of scar most connective tissue dense and organized connective tissue

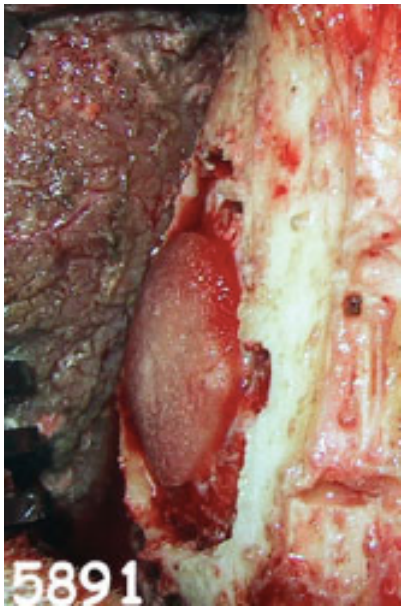
Qualitative scoring system to evaluate the extent of adhesion formation in the epidural space. (443)

Commercial Dural Sealants

There are a number of topical patches for dural repair currently commercially available. These include thrombin-based, cellulose sheets, fibrin gels, hydrogels and combinations of these(201, 223, 226, 230, 330, 333, 447, 448). There are also gels that act as physical barriers and dural substitutes that have been developed, a number of which are also commercially available(421, 449-451).

Duragen [Integra]

Duragen is a semi-synthetic collagen matrix sourced from bovine Achilles tendon(452). It is subsequently purified to minimise the risk of foreign body granulomatous reaction. It is provided in multiple patch sizes and used as a dural substitute when the dura is unable to be closed primarily. It is postulated to work as a dural repair matrix providing a scaffold for fibroblast infiltration and then being broken down at a rate comparable to endogenous tissue formation with decreased adhesion to pia and arachnoid(452, 453). Studies have demonstrated mixed results. Whilst studies of its use on spinal patients post tumour resection have demonstrated decreased incidence of spinal adhesions and CSF leaks(452-455), an Ovine study demonstrated increased adhesion to brain cortex when compared with bilayered collagen matrix or periosteum. In all studies, however, there was fibroblast invasion into the duragen within 1 month and complete integration after 6 months(456).



Duragen onlay in canine model of cranial dural repair(457)

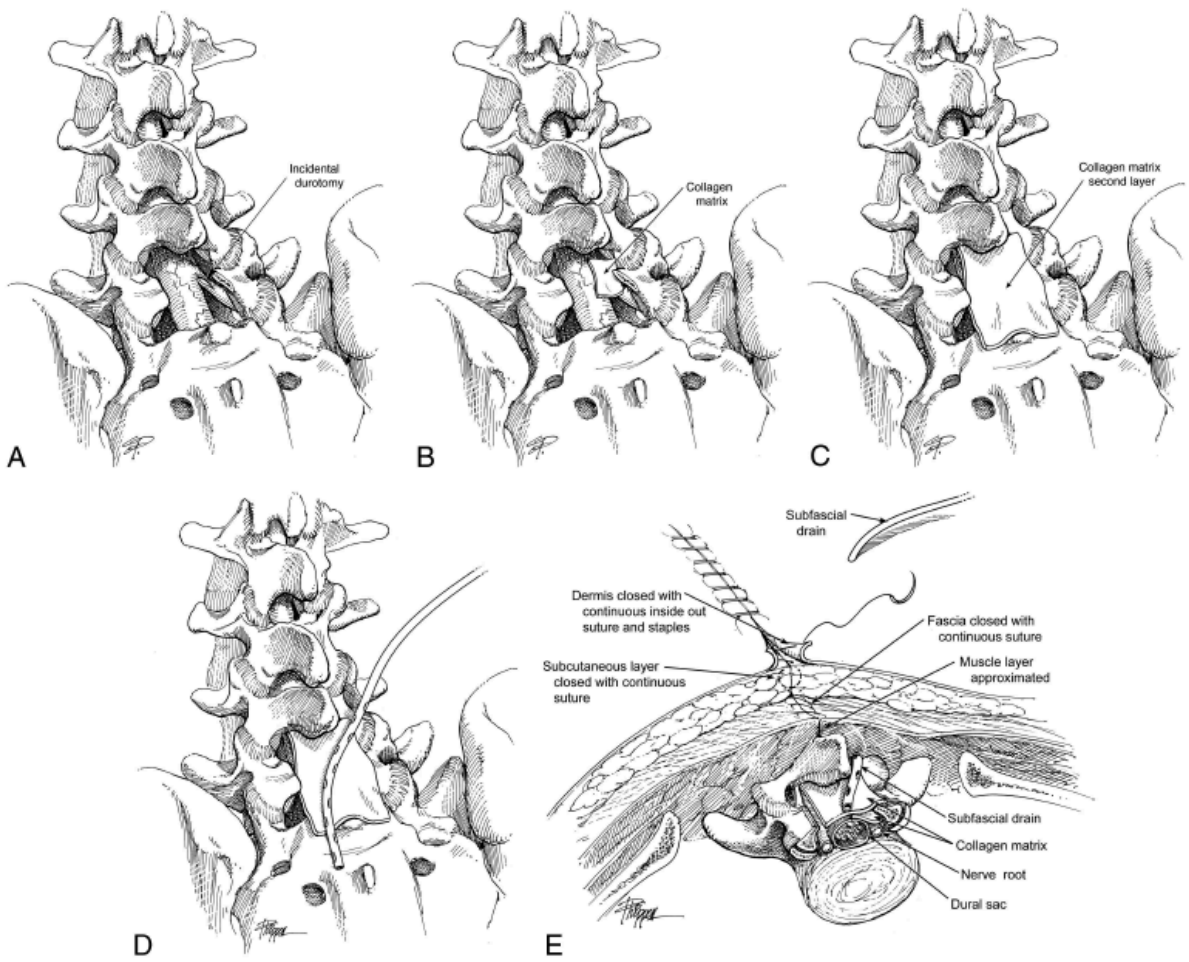
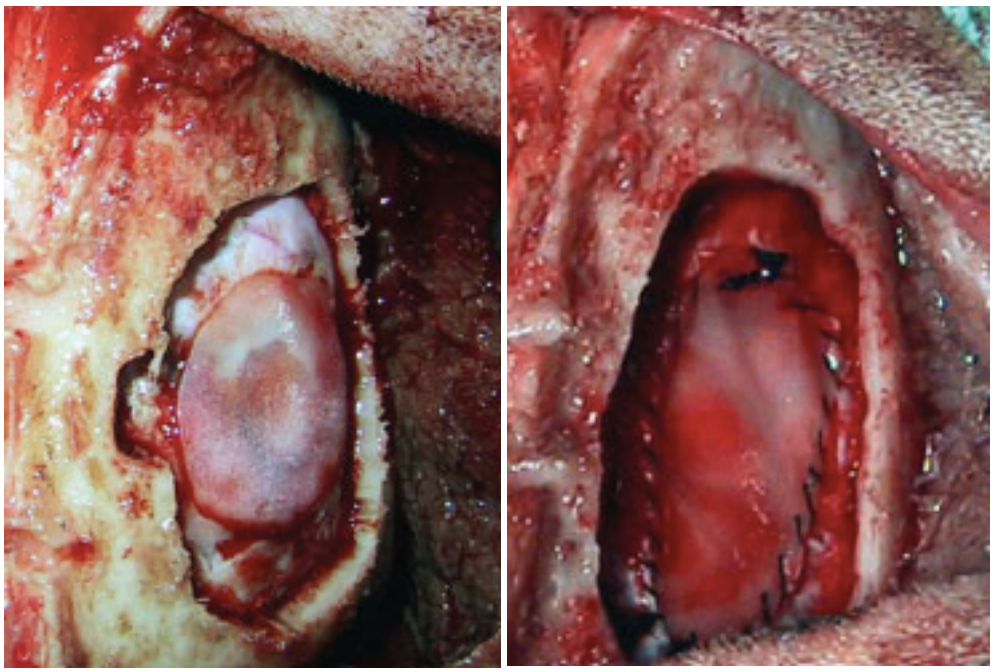


Diagram of duragen matrix placed over the dura exposed in a laminectomy(452)

Durepair [Medtronic]

Durepair [Medtronic] matrix consists of both Type I and Type III foetal bovine collagen that undergoes purification to remove the cellular components. The collagen fibres form a highly porous matrix with 10–100 micron pores. This matrix is a scaffold for the ingrowth of fibroblasts and blood vessels. It is used as a dural 'onlay' graft but is also able to be sutured(458, 459). Canine studies have demonstrated that durepair maintains its structural integrity as a barrier to CSF at 6 months and, at that time, histological examination shows infiltration of fibroblasts into the graft(457).



Durepair as onlay and sutured in a canine model of dura repair(457)

Duraseal [Medtronic]

Duraseal [Medtronic] is a polyethylene glycol-based gel that acts as a synthetic fibrosis inhibitor. It is composed of a polyethylene glycol (PEG) ester solution and a trilysine amine solution. When mixed together, the precursors cross link to form the hydrogel sealant(246,

315, 460). The hydrogel implant is absorbed in approximately 4 to 8 weeks and renally excreted. Prospective, randomized controlled studies of patients undergoing elective microdiscectomies have demonstrated reductions in pain scores at 30, 90 and 180 days in patients who were treated with duraseal versus controls(460).

Topical Steroids

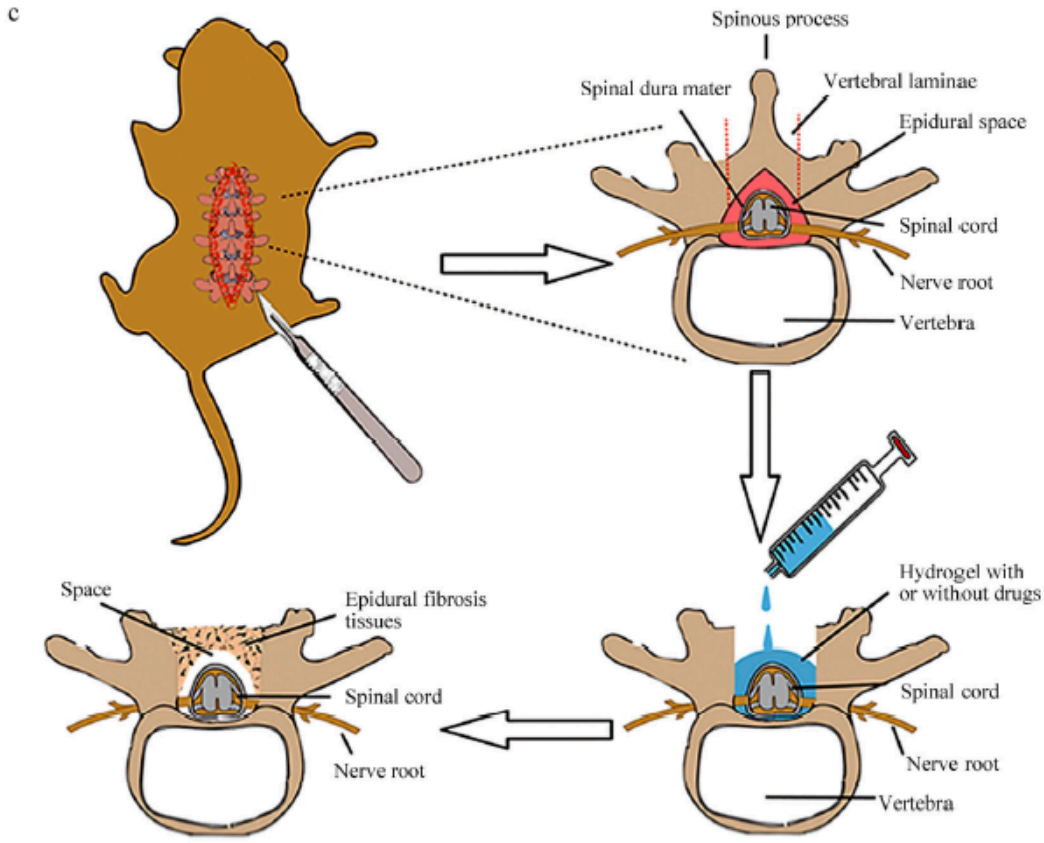
Steroid medications, such as budesonide and dexamethasone, target the nuclear membrane receptors to affect DNA transcription(461). Although they have a multitude of mechanisms of action that work synergistically, the overall effect is to reduce inflammation. They prevent leukocyte migration to areas of inflammation or injury(462). Budesonide is approved for inhalant use in chronic obstructive airways disease in Australia(463, 464). Budesonide:

- Controls the rate of protein synthesis.
- Depresses the migration of polymorphonuclear leukocytes and fibroblasts.
- Reverses capillary permeability and lysosomal stabilization at the cellular level to prevent or control inflammation.
- Has a potent glucocorticoid activity and weak mineralocorticoid activity.

Steroids in combination with hydrogels

Chen et al have developed an injectable hydrogel that they combined with dexamethasone for the prevention of epidural adhesions in rats(465). Their rationale was that by interposing a barrier between the thecal sac and the overlying soft tissue, scarring and adhesions would be reduced. The addition of anti-inflammatory steroid (dexamethasone) was postulated to

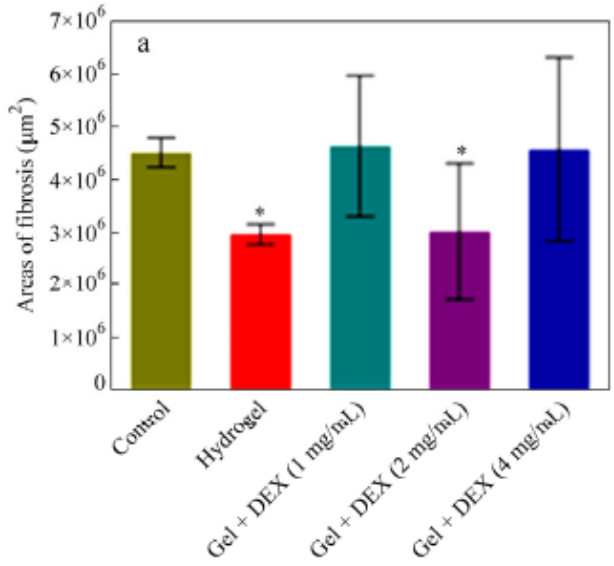
contribute further the adhesion reduction. Dexamethasone was suspended within hydrogel. This gel was formulated to form a non-flowing gel at body temperature. This is known as a 'thermal reversal' gel. Rats underwent a laminectomy and were randomised to five groups. (1) saline control; (2) placement of the hydrogel mixture; (3) 1mg/ml dexamethasone/hydrogel; (4) 2mg/ml dexamethasone/hydrogel; (5) 4mg/ml dexamethasone hydrogel. After 4 weeks, animals were sacrificed and the amount of adhesions quantified in 2 ways. A single rat in each group underwent re-exposure of the laminectomy site and the difficulty in re-exposure and inflammation in the surrounding tissues was scored. The remaining rats underwent histological examination and were scored for fibroblast and blood vessel density and extent of adhesions. This adhesion quantification used a scoring system adapted from He et al whereby 0=no scar tissue, 1=small fibrous bands, 2- continuous adherence in less than 2/3rds exposed area, 3-adherence greater than 2/3rds or extends to nerve roots(461).



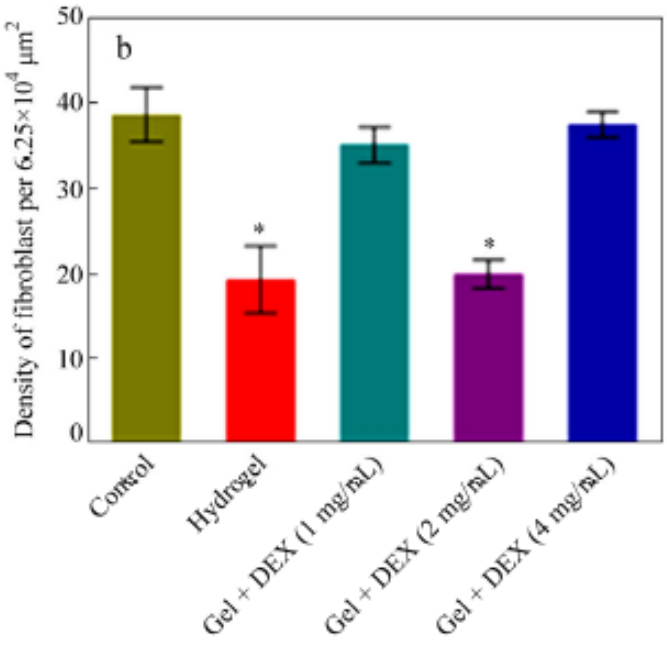
Representation of Chen et al's study demonstrating the laminectomy cuts, the placement of the hydrogel with dexamethasone and the epidural scar formation(465)

The study demonstrated a significant decrease in adhesions with the hydrogel barrier but that there was not a reliably significant decrease in adhesions with the addition of dexamethasone, nor was this dose dependant. This significant reduction in adhesions was only seen in the 2mg/ml dexamethasone concentration cohort. The authors attribute this to the possibility that the drug is not uniformly released from the hydrogel. The study did, however demonstrate that there was no evidence of haematotoxicity from the hydrogel or its breakdown products(465). This study would seem to at least partially confirm the theory that the migration of pro-inflammatory cells and direct contact of paraspinal tissue with the thecal sac results in adhesions and that a physical barrier to this may inhibit adhesion formation. It also provides some evidence for the use of anti-inflammatory medications in

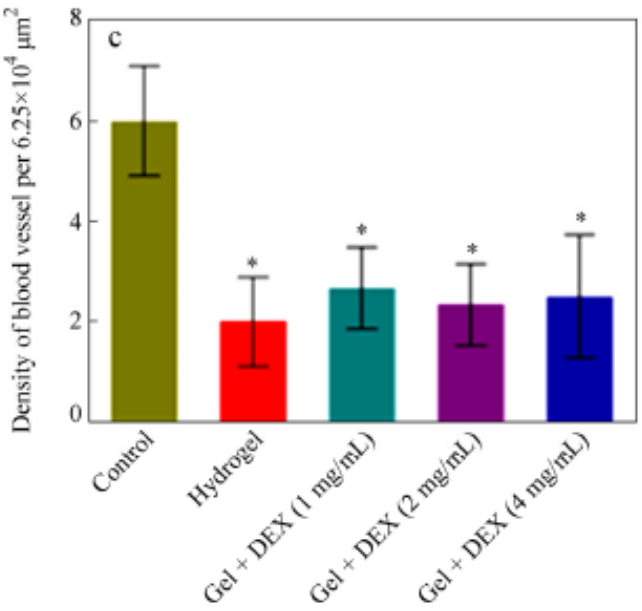
this barrier but there is conflicting evidence regarding dose and the issue of medication release from the barrier medium is one that requires further study. Ultimately this may be due to the mix of lipophilic steroid with a hydrophilic hydrogel.



Area of fibrosis divided by exposure type(465)



Fibroblast density divided by exposure type(465)



Blood vessel density divided by exposure type(465)

Deferiprone

Deferiprone is an iron chelator used in the treatment of thalassaemia major(466-468). It is an oral iron chelator that binds to iron in a 3:1 ratio. This binding acts to reduce total body iron in conditions such as thalassaemia where total iron is increased(469). Deferiprone complexed to iron is excreted renally. Iron is chelated from a number of sources within the body, including ferritin, transferrin, hepatocellular iron and abnormal intra-erythrocyte deposits(466). Deferiprone has also been shown to inhibit several cell lineages in vitro, especially myeloid precursors and may induce myelosuppression(466).

The theoretical framework for the use of deferiprone to prevent adhesions stems from its ability to inhibit free-radical formation. Hydroxyl radicals are liberated by free iron in vivo(470, 471). These hydroxyl radicals are toxic to tissues. Experimental induction of free radical damage to hepatocytes by exposure to hydrogen peroxide has been shown to be reduced by concurrent exposure of deferiprone 1mmol/L(472). It has also been shown to

prevent the oxidative damage from low density lipoprotein oxidation to blood vessels in rats and protect against reperfusion injury in rat hearts(473), possibly through the inhibition of free-radical formation, however human trials of these agents have no efficacy in the prevention or treatment of coronary heart disease(474, 475).

Kartikasari and colleagues demonstrated that monocyte adhesion to damaged endothelium is modulated by iron loading in these monocytes(470, 471). This iron is thought to upregulate chemokine receptors facilitating chemotactic protein-dependant trans-endothelial migration(470, 471). If the iron was chelated and the monocytes 'unloaded' of their iron, this migration and adhesion was blocked. Iron may therefore play a role in inflammation via modulating the movement of pro-inflammatory cells(471).

Deferiprone has also been trialled as an antibacterial agent in staphylococcus biofilms. Staphylococcus requires iron and sequesters it from its host(476). Exposure of staphylococcus biofilms to deferiprone results in a dose-dependent reduction of biofilms in vitro. This was enhanced by subsequent exposure to gallium protoporphyrin, a non-iron metalloporphyrin which acts as a haem analogue and is taken up by the staphylococcus in its search for iron. Once internalised, it interferes with cellular pathways in both the cytoplasm and the cellular membrane(476).

Given adhesion formation post-surgery is the result of inflammatory free-radical formation and the migration of pro-inflammatory cells in response to the chemotactic effect of these free-radicals, deferiprone may, when placed within a barrier gel substance, reduce adhesion formation.

There have been some initial attempts to demonstrate a positive effect of deferiprone on wound healing. Mohammadpour and colleagues showed improved cutaneous wound healing in rats with topical application of deferiprone at 3%, 6%, and 9%(477). The authors also used DPPH (2,2-diphenyl-1-picrylhydrazyl(478)) free radical assay, an antioxidant assay that determines the ability of a substance to give hydrogen to a free radical and thus neutralise it, and showed that deferiprone is able to act as an antioxidant, which may contribute to its wound healing effect(477).

Major Vessel Haemorrhage in Skull Base Surgery – A High Stress Situation

Impact of Stress on Surgical Performance

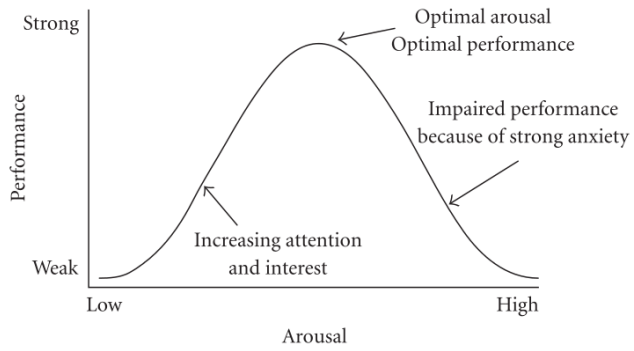
Patient outcome is dependent on a multitude of factors in surgery. The surgeon's technical skill is but one small part of this. The non-technical skills such as communication, planning, teamwork and decision making are increasingly recognised as playing a crucial role(479). When all goes well with surgery, it is relatively easy to maintain open lines of communication with all members of the surgical team – scrub and scout nurses, assistant, anaesthetist, orderlies and staff outside of theatre. It is when a potentially catastrophic event occurs that coordinated teamwork is vital and clear and concise communication so necessary(479). Unfortunately, this is often the time when stress and anxiety may hinder optimum performance(480, 481).

The aviation industry has long been a proponent of teamwork and communication training, along with fatigue management and critical incident reviews(482). The industry shares with

surgery the high stakes of a bad outcome and has many parallels. Human performance assessment and management in the world of aviation has much to teach surgeons about ways to train for adverse events. Critics of this approach argue that surgery is a different beast to aviation(483). We are, after all, dealing with the human body with all its frailties and variations, in comparison to the standardised mechanics of aeroplanes. There are, however, too many similarities when it comes to teamwork, resource management, critical incident reviews and learning from previous adverse events to ignore, and it is arguable that some of the greatest benefits to surgical practice within the operating room, such as 'team time outs" and the use of surgical checklists, have come about through the cross-pollination of ideas between the two areas(104, 105, 284, 480, 484-488).

The effect of anxiety on performance

The Yerkes-Dodson performance curve was developed in an attempt to explain the relationship between arousal and performance. Their original work showed that mice performed better when exposed to an intermediate-grade aversive stimulus rather than a high one when performing a complex task(489). The upward slope of the curve represents the positive effect of arousal on performance. The downward slope reflects the adverse effects of 'over-arousal' or stress on performance.



The Yerkes-Dodson performance curve plotting performance and its relationship to stress(489)

Studies looking at 'stress' as a concept vary widely in terms of both definition and methods of measurement. It is difficult to compare studies precisely because there is no standardised definition of this amorphous concept. Stokes and Kite define stress broadly and state their views on the difficulty of studying it as *"an agent, circumstance, situation, or variable that disturbs the 'normal' functioning of the individual…stress [is also] seen as an effect—that is the disturbed state itself…this bifurcation of meaning is arguably the most fundamental source of the confusion surrounding the stress concept(490).* Traditional research has assumed that external stimuli generate 'stress' in all subjects equally and researchers then manipulate these variables (temperature, noise etc) and observe the effect on the individual. This does not allow for individual differences in human responses and emotions. This is termed the 'stimulus-based' approach. An attempt to refine this has led to the 'response-based' approach, which defines stress as the pattern of responses that occur during exposure to a stimulus. These may be a mix of physiological and psychological(490). This response-based approach has been extended further to what is termed the "transactional approach" where the entire interaction between the individual and the environment is taken into account and stress becomes viewed as *"the result of a mismatch*

between individuals' perceptions of the demands of the task or situation and their perceptions of the resources for coping with them."(490).

Physiological markers of stress

Physiological markers of stress range from easily measurable markers of sympathetic nervous system activation, such as hypertension and tachycardia, to serum levels of catecholamines, such as noradrenaline and adrenaline, and hormones such as cortisol(485, 491). Evidence suggests that chronic stress may be reflected by a high cortisol level and that this may have adverse long-term outcomes on immunity and cardiovascular health(492-494). The half-life of cortisol and its secretion pathway preclude its use as a marker of immediate response to stress over seconds to minutes. Psychoneuroendocrinological studies have demonstrated the utility of salivary alpha-amylase as a marker of immediate sympathetic nervous system activation with a reliable correlation with plasma catecholamines(492, 495-501). This rises in response to acute stress(502, 503). Salivary alpha-amylase is comprised of 2 isoenzymes with a molecular weight of 57000kDa(495). It is synthesised primarily in the parotid gland and hydrolyses the linkages of starch to glucose and maltose(497). It initiates the digestion of starch in the mouth. Acinar cells are innervated by both the parasympathetic and sympathetic nervous system. The parasympathetic efferent pathway to the parotid gland is via the glossopharyngeal nerve and otic ganglion(53). The parasympathetic efferent pathway to the salivary and submandibular glands is via the facial nerve and the submandibular ganglion. The sympathetic post-ganglionic pathways are from the cervical ganglion(497, 498). Noradrenaline from sympathetic post ganglionic neurons binds to alpha and beta receptors on acinar cells. Alpha stimulation increases intracellular Ca+ and beta receptor stimulation

increases intracellular cAMP. Together these increase release of salivary secretions which are stored in membrane bound secretory granules(497). Murine models have demonstrated that sympathetic nervous system(SNS) stimulation to rat parotid results in a low flow rate/high protein salivary secretion. Parasympathetic nervous system (PSNS) stimulation results in the opposite effect(497). Thus mean concentrations of salivary alpha-amylase are higher when sympathetic nervous system stimulation is increased. Human studies have confirmed this using isoprenaline and cold water as SNS stimulants and propranolol for SNS blockade(497). Studies amongst humans have demonstrated a positive correlation between salivary alpha-amylase levels and traffic noise exposure, video games, skydiving and tests of mental arithmetic(497, 500). It is a generally accepted biomarker for acute stress in test subjects in the psychoneuroendocrinological literature(504). Salivary alpha-amylase would appear to hold promise as a biological marker. It reflects trends in sympathetic nervous system activation over the short term. It is easily collectable with a salivary swab and is stable in transit. It should be analysed within 4 hours or frozen for intervals of >4 hours between collection and ELISA testing.

Surgical simulations and stress

From the point of view of surgical simulation and training, the most relevant description of stress is probably that described by McGrath in 1976. Stress is "*the interaction between three elements: perceived demand, perceived ability to cope, and the perception of the importance of being able to cope with the demand*"(505). Surgical simulation and training is therefore able to be focused on improving people's perceived ability to cope through repeated exposure to an environment where the consequences of task failure are minimized compared with surgery on humans.

'*When performed well, surgery should be boring*' is a phrase many of us have heard early in surgical training. It is a function of the fact that surgery deals with the changing and diverse array of pathology in the human body that makes it impossible to state with absolute certainty what will happen in any particular case. Experience and exposure allow for not only more precise operating but also allow the surgeon to more accurately predict risks and complications and take action to minimise the chances of adverse events(506).

Simulation in surgery is a valuable tool. It has been incorporated into many training programs and varies widely from laparoscopic and animal training models to videotaped sessions with actors and other members of the surgical team to develop communication and team work skills(119, 488, 507-509). It also provides surgeons with the ability to experience situations that they may never encounter in their entire careers such as cerebral gunshot wounds – analogous to a pilot simulating multiple engine failures just after aircraft rotation – allowing them to develop strategies to safely master the event(273).

Simulation models

As has previously been described, there is a risk of arterial injury in endoscopic endonasal surgery which, depending on the approach, is in the order of 0.5%-9%. Valentine and Wormald have developed a sheep model of carotid artery injury(117). Merino sheep are anaesthetised and undergo a midline neck dissection. The carotid is identified in the neck and a length approximately 20cm is freed. A functional endoscopic sinus surgery (FESS) training device is applied to the carotid and surgeons can drill the posterior plastic wall to expose the carotid itself. The carotid is then sharply incised to create a model of haemorrhage. Surgeons are taught to manipulate the endoscopic camera and keep their

visual and working fields free, enabling them to control the haemorrhage. From the point of view of surgical training, this is a valuable model that allows surgeons to be exposed to a relatively rare and potentially fatal event and equip them with the skills required to deal with this safely. There are a number of studies that demonstrate that skills learned in simulation training successfully transfer across into the operative setting(510-517).

It is difficult to quantify the stress response that occurs in surgeons when faced with a novel or challenging situation. Contemporaneous questionnaires are obviously tempered somewhat by the surgeon filling it out after having dealt with the situation and being aware of the outcome. They are also somewhat subjective. This is not entirely inappropriate as a tool for research as the stress response has a psychological component to it however it is subject to biases and a relative and subjective grading system(484). The ideal method would be to capture the subjective psychological and objective physiological responses and then correlate them with both the time course of the stressful situation and with each other(480, 518).

The use of independent observer or rater analysis of communication skills is a useful adjunct to the assessment of stress on performance(519). Multiple studies have video-taped surgeons in training as part of simulation exercises. These have the dual purpose of allowing surgeons to watch themselves and pick up any deficits in communication or non-surgical technical skills that may occur whilst also allowing independent surgeons and psychologists to rate communication skills outside of the scenario. Stress can have a negative impact upon communication, especially at the higher levels which, given successful surgery relies upon

the actions of a team (anaesthetist, surgeon, nurses, and orderlies) may result in negative outcomes for patients(484, 520, 521)

Chapter 2

Nano-haemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery.

Jukes A, Murphy J, Vreugde S, Psaltis A, Wormald P. Nano-hemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery. Journal of Neurological Surgery Part B: Skull Base. 2016;e-first(Dec).

Statement of Authorship

Title of Paper	Nano-hemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	Jukes A, Murphy J, Vreugde S, Psaltis A, Wormald P. Nano-hemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery. Journal of Neurological Surgery Part B: Skull Base. 2016;e-first(Dec).

Principal Author

Name of Principal Author (Candidate)	Jukes, Alistair
Contribution to the Paper	Study design, Ethics application, Peptide preparation, animal operating, tissue harvest, electron microscopy, data analysis, manuscript writing and preparation
Overall percentage (%)	90
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 1/1/18

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Murphy, Jae
Contribution to the Paper	Peptide preparation, animal operating, tissue harvest
Signature	Date: 1/1/18

Name of Co-Author	Vreugde, Sarah
Contribution to the Paper	Peptide preparation, animal operating, tissue harvest
Signature	Date: 1/1/18

Name of Co-Author	Psaltis, Alkis		
Contribution to the Paper	Study design, manuscript review		
Signature		Date	1/12/17

Name of Co-Author	Wormald, Peter-John		
Contribution to the Paper	Study design, manuscript review		
Signature		Date	1/1/18

Nano-hemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery

Alistair Jukes[1,2] Jae Murphy[1,2] Sarah Vreugde[1,2] Alkis Psaltis[1,2] P. J. Wormald[1,2]

[1] Department of Otolaryngology, Head and Neck Surgery, Queen Elizabeth Hospital, Adelaide, Australia
[2] Department of Neurosurgery, Royal Adelaide Hospital, Adelaide, Australia

Address for correspondence Alistair Jukes, MBBS, Department of Otolaryngology, Head and Neck Surgery, Queen Elizabeth Hospital, Woodville Rd Woodville South, South Australia 5011, Australia (e-mail: alistair.jukes@adelaide.edu.au; alistairjukes@me.com).

J Neurol Surg B

Abstract

Nano-hemostats are synthetic amino acid chains that self-assemble into a scaffold under certain conditions. These have been shown to be effective in stopping bleeding in small animal models of hemorrhage. Proposed mechanisms for their effect are that they form a mesh analogous to the fibrin plug in native hemostasis and that they may potentiate both platelet activation and the coagulation cascade. These may potentially become valuable adjuncts to endoscopic skull base surgery where there is the potential for both major vessel injury and smaller perforator injury to eloquent areas where bipolar cautery may not be suitable. We present a summary of the clinical studies to date and a small pilot study of nano-hemostat in an endoscopic sheep model of major vessel hemorrhage to determine its efficacy in stopping bleeding in this potentially catastrophic complication.

Keywords

- endoscopic transsphenoidal surgery
- hemorrhage control
- nano-medicine
- pituitary surgery
- internal carotid artery injury

Introduction

"Nano-technology" or "nano-engineering" involves working with structures that are less than 100 nm in size. The challenges that this poses are immense and involve controlling entities on an atomic scale.[1] Given that many disease processes involve damage to cell structures, the ability to administer a substance that either provides a scaffold for ordered cellular regeneration or delivers growth factors that stimulate such recovery is attractive, especially if this scaffold were composed of materials that were able to be broken down without causing long-term damage once their function had expired.[1,2]

The Use of Self-Assembling Peptides in Medical Applications

The medical applications of nanotechnology have revolutionized our options for drug delivery, in vivo medical imaging, and biotechnological techniques.[3] Typically these applications utilize amino acid chains that are amenable to self-assembly—that is, forming secondary structures according to their amino acid sequence under certain external conditions.[1,4] They are, as Loo and colleagues described in 2012, "versatile building blocks for fabricating supramolecular architectures."[4] The development of these has come about through increased understanding of the protein chains found in naturally occurring substances. Peptide self-assembly involves a complex interaction of forces, including hydrogen bonding, ionic forces, hydrophobic forces, van der Waals interactions, and electrostatic forces.[4] Proposed uses for these peptides include drug delivery, scaffold sheaths for neural redevelopment, and, importantly for surgeons, hemostasis.

Multiple different types of self-assembling peptides and their formulations have been described. The feature common to all is that they comprise a dissolvable L-amino acid chain that does not interfere with cellular pathways or promote or inhibit signaling and that is broken down via the body's

received
June 5, 2016
accepted
October 25, 2016

DOI http://dx.doi.org/10.1055/s-0036-1597277.
ISSN 2193-6331.

natural peptidases and enzymes to constituent amino acids after its primary role has been performed.[5] These peptides have been used as hemostatic agents,[5,6] as an adjunct to ophthalmologic surgeries (e.g., corneal endothelial stem cell transplantation) and, intriguingly, as a self-assembling peptide to provide a scaffold or bridge for axonal regeneration in optic nerve reconnection.[1] Theories have also been advanced in the field of oncology that nanostructures could change the extracellular matrix that facilitates cancer metastasis and ensure malignancy remains localized.[7] A peptide chain self-assembled into a β-pleated sheet named RADA-16 has been used to deliver epidermal growth factor, insulin-like growth factor, platelet-derived growth factor, and stromal cell-derived growth factor-1 to postinfarction myocardium to increase the rate of wound healing.[4] The advantage to this method of drug delivery is that amalgamation into a protein sheet renders the drug less susceptible to metabolic processes within the body and allows it to be delivered unaltered to the site of action.[8] Ellis-Behnke and colleagues have also demonstrated the ability to use these nano-fiber scaffolds to repair hamster brain lesions by allowing nerve fibers to reconnect postseverance.[9,10] This is analogous to the role played by myelin sheaths in neural protection and redirection.[10]

Self-Assembling Peptides as Nano-hemostats

Coagulation and subsequent hemostasis depends on multiple factors. These include the endothelial cells of blood vessels, platelets, leukocytes, the coagulation cascade, and the milieu of temperature and blood pH. There are also multiple anticoagulant forces and inhibitors. The precipitating event for nonpathologic coagulation initiation is vessel wall damage. This causes activation of endothelial cells and subsequent platelet activation. Platelets roll along the damaged cells and, using von Willebrand factor adhesion to bind to newly exposed subendothelial cells, aggregate in platelet clumps. Post this event, a mesh of platelets and trapped leukocytes form a scaffold for development of a fibrin clot as part of the coagulation pathway.[11–13]

Several studies have examined the role that these self-assembling peptides may play in hemostasis.[5,6,14] There are a number demonstrating almost immediate hemostasis in small animal models without activation of the coagulation cascade. This is thought to occur as the nano-hemostat scaffold acts in an analogous fashion to fibrin clot, forming a barrier that both holds platelets in situ and provides an activation stimulus, leading to further aggregation. This has been demonstrated on the mammalian brain, spinal cord, liver, kidney, femoral artery, and skin.[5,6,15,16] The peptide solutions have been shown to breakdown via natural enzymatic processes and are nonimmunogenic and nontoxic.[1] In addition, the breakdown products are amino acids that may be used for subsequent tissue repair.[1,5]

Nano-hemostat is a particularly attractive concept in skull base surgery, especially when performed endoscopically. Surgeons are constrained by relatively narrow access corridors that are in close proximity to major vascular structures such as the internal carotid artery, anterior cerebral arteries, and basilar artery and venous structures such as the cavernous sinus.[17,18] Injury to these structures carries the potential for devastating and potentially fatal bleeding.[17–24] Much work has been performed to determine the safest way to halt bleeding in this environment and the safest way to train surgeons to operate in this area.[25–29] In this situation, the ideal hemostatic agent should be able to conform to irregular cavities, to not obstruct the surgical field, and to not damage surrounding structures, either through thermal or chemical injury or through expansion and pressure injury to nerves or vessels.[30–32] Current methods of hemostasis used in majorvessel injury to the skull base include muscle patches,[25–28,33] Floseal (Baxter Healthcare, Hayward, California, United States), Surgiflo with thrombin (Ethicon, Somerville, New Jersey, United Stated), Tisseel (Baxter Healthcare),[11,34] and hemostatic clips.[33,35] While these are efficacious to varying degrees, they all have their limitations. There can be some delay in harvesting a muscle patch, and it is partially dependent on platelet activation as part of its mechanism of action.[33] Floseal and Tisseel have been shown to wash out of the surgical field rapidly in the case of torrential hemorrhage.[25–28] Hemostatic clips can be difficult to apply in situations with a tear in an artery at the lateral extremity of the surgical field.[35]

Nano-hemostats in Animal Models

d-EAK16

Evidence to support the hypothesis that nano-peptides in hemostasis provide more than a simple physical barrier comes from studies looking at peptide d-EAK16 by Luo et al.[14] Self-assembly of d-EAK16 was timed in a pure water solution and in a living animal model (rabbit liver). It took approximately 16 hours for d-EAK16 to self-assemble in water but approximately 20 seconds to assemble in whole blood. The time to hemostasis in this d-EAK16 group was significantly lower when compared with controls (no treatment) (20 vs. 80–120 seconds). The authors conclude that ions, proteins, enzymes, and "other factors" in blood work cooperatively to cause hemostasis. Their rationale is that normal hemostasis occurs when there is a high concentration of clotting factors held within a localized area whereas the same gross number of coagulation components would be ineffective if spread out over a larger area. d-EAK16 is thought to be triggered by sodium, magnesium, potassium, calcium, iron, and zinc within the circulating plasma to form a stable β-pleated sheet in which platelets and subsequently endogenous coagulation factors become trapped and undergo activation in the usual fashion. This β-pleated sheets then combine to form first short and then long nano-fibers that may then form "tight junctions" impervious to liquids. These peptide chains have been shown to work best as a hemostatic agent when comprised of amino acids of the same chirality. Alternating chirality gave bleeding times up to eight times longer in a rat liver hemorrhage model.[14]

AC5

AC5 is a proprietary, synthetic nano-hemostat formulation developed by Ellis-Behnke and colleagues, which is currently

in preclinical trials.[6] It addresses the potential for hemostasis in the context of anticoagulation.[6] In a noncompressible, full-thickness 4-mm liver punch biopsy model in rats, AC5 achieved hemostasis equivalently in heparinized and non-heparinized rats with a 94% reduction in time to hemostasis compared with saline controls.[6]

RADA-16

RADA-16 is a self-assembling peptide of ionic hydrophilic and hydrophobic amino acids. It is 5 nm in length and forms stable β-pleated sheet structures that turn into hydrogels.[2] Sang et al performed a study in which they divided 20 rats in four groups: intracerebral hemorrhage (ICH) without aspiration, ICH with aspiration, ICH with aspiration and saline, and ICH with aspiration and RADA-16 injection. ICH was created via intrastriatal injection of collagenase. After 210 minutes, the hematoma was aspirated and 20 µL of saline or RADA-16 was injected into the ICH cavity. Differences in hematoma volume in aspiration versus saline versus RADA-16 group were not significant. Aspiration significantly reduced inflammatory cells in surrounding cortex on histologic examination. The authors concluded that self-assembling peptide attenuated brain injury; enhanced functional recovery; and reduced cavity volume with a reduction in brain edema, peri-hematoma apoptosis, and inflammatory reaction.[2] This reduction in secondary brain injury is thought to come about by decreasing the leakage of serum proteins into the surrounding parenchyma. Histology showed tight contact between RADA-16 and the cavity wall.[2] More work is required to validate this concept in both the acute and chronic phases of brain inflammatory reactions.

Possible Limitations

While nano-hemostats have been proven to be successful in a small animal model of vessel injury, this may not hold true in larger animal/human models where pressures may be many times that of smaller animals.[6,34,36,37] This pressure may wash the hemostat away from its site of action before it can take effect. Further, the liquid may need to be either impregnated in or held on by a patty or similar. This may remove the advantage of having a clear liquid hemostat—at least in the immediate phase of application.[38] While this has not been seen in nano-hemostats to date, a buildup of abnormal proteins within the central nervous system is the basis of conditions such as dementias and prion diseases. It would be incumbent upon designers of nano-hemostats to ensure that their solution is adequately broken down by the body's enzymatic processes and is not trapped in insoluble form within neurons or astrocytes.

Pilot Study of Nano-hemostat on an Animal Model of Major Vessel Injury Relevant to Skull Base Surgery

Aim

The aim is to determine the feasibility of a nano-hemostat solution in stopping bleeding from major arterial and venous sources and its ease of use in an endoscopic setting.

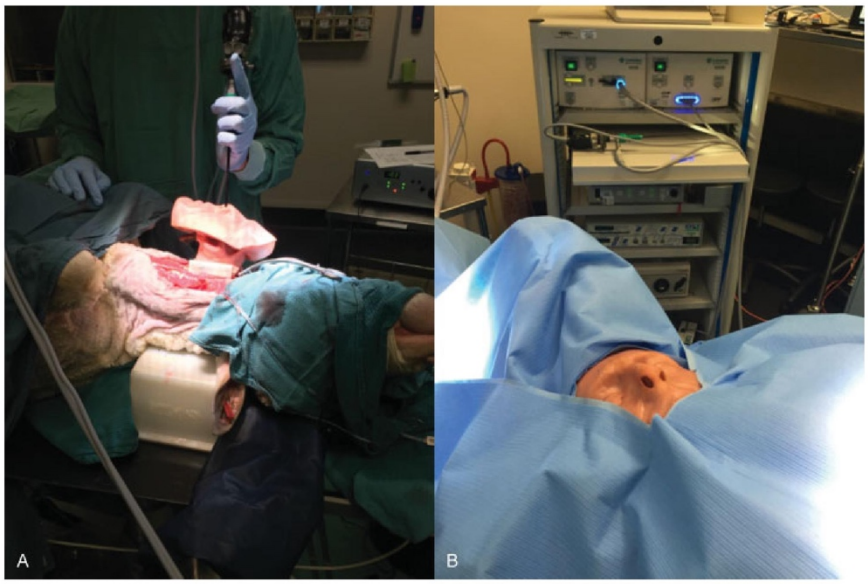


Fig. 1 Endoscopic trainer model **(A)** placed on sheep's carotid artery and **(B)** draped and ready for use.

The peptide chosen was RADA-16 (Arg - Ala - Asp - Ala - Arg - Ala - Asp - Ala - Arg - Ala - Asp - Ala - Arg - Ala - Asp - Ala - NH_2), which is known for its propensity to self-assemble and has been used as a nano-hemostat in small animal models.[5,8,16,39] This was sourced from China Peptides as a 70% pure powder.

Method

Ethics approval was obtained from the Animal Ethics Committee of the South Australian Health and Medical Research Institute. Two merino sheep were anaesthetized and placed supine. A midline neck dissection was performed, and left carotid artery was cannulated for an arterial line and the left internal jugular cannulated for a central venous catheter. The right carotid artery and right internal jugular vein were dissected free and a segment of at least 8 cm was cleared of surrounding fascia. An endoscopic training model was placed around the internal jugular. These silicon-based models re-create the sinonasal cavity with the vessel running through a groove at the base of the model. The sheep is then draped and provides a realistic representation of endoscopic surgery (►**Fig. 1A, B**).

RADA-16 nano-hemostat was mixed with saline in a 10% mixture forming a gel at room temperature. This was drawn up into a 10-mL syringe and a blunt cannula attached. Using a 19-gauge needle, a hole was made in the internal jugular and blood allowed to escape for 20 seconds (►**Fig. 2A**). RADA-16 was then applied to the jugular injury at the rate of 1 mL/s (►**Fig. 2B**). While it slowed the bleeding and provided a semitranslucent membrane over the injury, blood could still be seen escaping from the hole in the jugular vein underneath this for a period of 15 seconds before hemostasis occurred. A proximal injury was subsequently made in the same animals as an untreated control that continued to bleed for 5 minutes until RADA-16 was applied and hemostasis occurred at approximately 15 seconds. The nano-hemostat did not appear to set in a solid fashion but remained as a jelly-like substance when observed for 20 minutes.

The endoscopic trainer was then applied to the carotid artery of the same sheep. A curved aneurysm clip was placed half way across the carotid artery and a 4-mm linear incision was made in the wall of carotid artery (►**Fig. 2C**). The aneurysm clip was removed and the injury allowed to bleed for 3 seconds (►**Fig. 2D**). A sucker was used to control the field, and an identical mix of 10 mL of RADA nano-hemostat was immediately applied to the injury. There was no discernable decrease in the bleeding rate. RADA-16 appeared to be washed away by the pressure of the bleeding (systolic range:

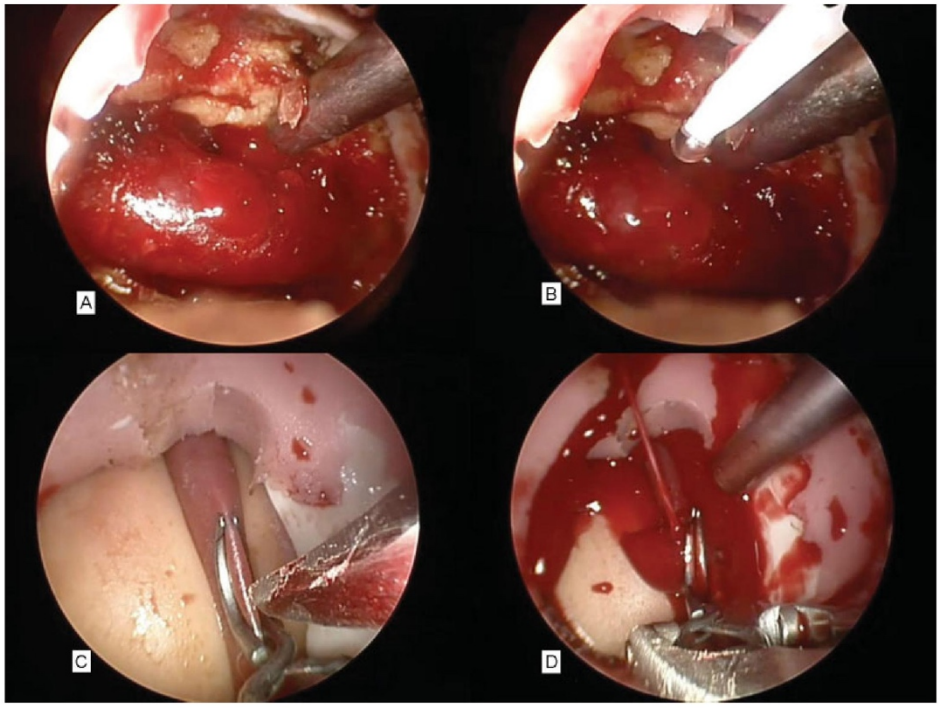


Fig. 2 **(A)** Bleeding from the jugular vein. **(B)** RADA-16 being applied to the jugular vein. **(C)** Aneurysm clip across carotid artery and scalpel making linear incision. **(D)** Arterial hemorrhage from carotid injury.

70–90 mm Hg). We were unable to control the bleeding and after a period of 90 seconds were forced to use a crushed muscle patch to stop the bleeding that worked to good effect as previously reported.[25–28] This was repeated in a second sheep with identical results. Sheep were then humanely killed. The jugular and carotid were dissected out and placed in formalin and scanning electron microscopy (SEM) fixative. A small amount of muscle was also covered in nano-hemostat postmortem and sent for histology and SEM. SEM was performed postpreparation and coating of the samples in platinum. The nano-hemostat could be seen coating the muscle patch and forming a "glue" or matrix with long scaffolds or fibers with a width of 30 to 70 nm. Erythrocytes could be seen trapped within this matrix (►**Fig. 3**).

Discussion

This pilot study demonstrates some of the inherent limitations of gels or liquids in high-flow, high-pressure bleeding. While the bleeding from the jugular vein injury could be stopped with this nano-hemostat mixture, this is a comparatively low-flow, low-pressure injury. The carotid artery injury was unable to be controlled with the nano-hemostat, and eventual control was achieved with the conventional option of crushed muscle patch.

There are several questions raised by this small pilot study that may warrant further investigation. The literature is not clear regarding the percentage strength of solution used in previous nano-hemostat studies utilizing RADA-16.[2,5,15,40,41] While these have stopped bleeding in mouse models, this may simply be a reflection of the lower pressure and lower volume of flows in these injuries. The ideal percentage is not yet clear. Our 10% solution provided a solution viscous enough to adhere to the side of the plastic syringe. This was chosen because we were interested in its ability to be applied in an endoscopic scenario, and it appeared to provide a balance between applicability and viscosity.

This pilot study appears to add weight to previously published studies that have described success in bleeding control of small vessels in small animal models using nano-hemostat.[5,15,40,41] Certainly it appeared to have some success controlling a small jugular vein puncture. There may be future avenues worth exploring regarding the use of nano-hemostat in areas where visualization of the bleeding point would be useful such as a venous ooze from the lateral borders of the sphenoid corridor of a pituitary resection or in endoscopic resection of a skull base tumor. Current teaching involves packing these areas and moving the dissection to another region rather than laboriously obtaining control and drying the field with bipolar as this

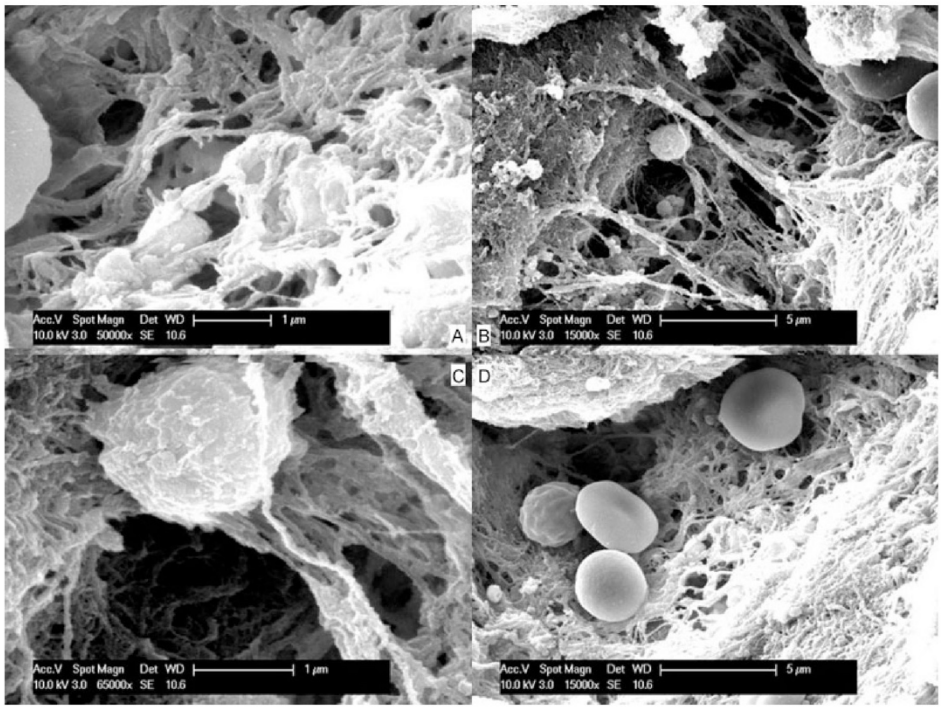


Fig. 3 **(A)** Scanning electron microscope view of RADA-16 scaffold. **(B–D)** Scanning electron microscope views of erythrocytes and platelets trapped in scaffold of fibrin and RADA-16.

would add inordinate amounts of time to what is already a long operation. It would be advantageous to have a semitranslucent hemostat that would allow the surgeon to see the bleeding point underneath the hemostat rather than having to remove the pack and potentially remove the fibrin/platelet plug/clot in doing so, causing further bleeding.

This pilot study appears to show that nano-hemostat is unlikely to adequately control a high-flow, high-pressure bleed such as that from a carotid artery injury. SEM shows the nano-hemostat forming a matrix in which circulating erythrocytes and platelets are trapped (in much the same way as a fibrin plug is formed), but this only formed in the postmortem samples where there was obviously no pressure washing the nano-hemostat away. For this to work in vivo, the nana-hemostat would need to be held in situ for a prolonged period, possibly as part of a patch or pad. It would also be useful to test this nano-hemostat in a recovery model to determine whether pseudoaneurysms form and whether the scaffold holds for sufficient time for re-endothelialization to occur. Further research is needed to address the optimum formulation and indications for use of this potentially revolutionary new hemostatic agent.

Acknowledgments

This research was funded by the NeuroSurgical Research Foundation, Adelaide. The authors would like to thank Lynnette Waterhouse for assistance devising the scanning electron microscopy protocol to view the nano-hemostat.

References

1 Ellis-Behnke R, Jonas JB. Redefining tissue engineering for nanomedicine in ophthalmology. Acta Ophthalmol 2011;89(2): e108–e114
2 Sang LY, Liang YX, Li Y, et al. A self-assembling nanomaterial reduces acute brain injury and enhances functional recovery in a rat model of intracerebral hemorrhage. Nanomedicine (Lond) 2015;11(3):611–620
3 Hirst AR, Escuder B, Miravet JF, Smith DK. High-tech applications of self-assembling supramolecular nanostructured gel-phase materials: from regenerative medicine to electronic devices. Angew Chem Int Ed Engl 2008;47(42):8002–8018
4 Loo Y, Zhang S, Hauser CA. From short peptides to nanofibers to macromolecular assemblies in biomedicine. Biotechnol Adv 2012; 30(3):593–603
5 Ellis-Behnke R. At the nanoscale: nanohemostat, a new class of hemostatic agent. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2011;3(1):70–78
6 Csukas D, Urbanics R, Moritz A, Ellis-Behnke R. AC5 Surgical Hemostat™ as an effective hemostatic agent in an anticoagulated rat liver punch biopsy model. Nanomedicine (Lond) 2015;11(8): 2025–2031
7 Ling PM, Cheung SW, Tay DK, Ellis-Behnke RG. Using self-assembled nanomaterials to inhibit the formation of metastatic cancer stem cell colonies in vitro. Cell Transplant 2011;20(1):127–131
8 Khadka DB, Haynie DT. Protein- and peptide-based electrospun nanofibers in medical biomaterials. Nanomedicine (Lond) 2012; 8(8):1242–1262
9 Cho Y, Borgens RB. Polymer and nano-technology applications for repair and reconstruction of the central nervous system. Exp Neurol 2012;233(1):126–144
10 GhoshMitra S, Diercks DR, Mills NC, Hynds DL, Ghosh S. Role of engineered nanocarriers for axon regeneration and guidance: current status and future trends. Adv Drug Deliv Rev 2012; 64(1):110–125
11 Yao HH, Hong MK, Drummond KJ. Haemostasis in neurosurgery: what is the evidence for gelatin-thrombin matrix sealant? J Clin Neurosci 2013;20(3):349–356
12 Geczy CL. Cellular mechanisms for the activation of blood coagulation. Int Rev Cytol 1994;152:49–108
13 Tynngård N, Lindahl TL, Ramström S. Assays of different aspects of haemostasis—what do they measure? Thromb J 2015;13:8
14 Luo Z, Wang S, Zhang S. Fabrication of self-assembling D-form peptide nanofiber scaffold d-EAK16 for rapid hemostasis. Biomaterials 2011;32(8):2013–2020
15 Huri E, Beyazit Y, Mammadov R, et al. Generation of chimeric "ABS nanohemostat" complex and comparing its histomorphological in vivo effects to the traditional ankaferd hemostat in controlled experimental partial nephrectomy model. Int J Biomater 2013; 2013:949460
16 Song H, Zhang L, Zhao X. Hemostatic efficacy of biological self-assembling peptide nanofibers in a rat kidney model. Macromol Biosci 2010;10(1):33–39
17 AlQahtani A, Castelnuovo P, Nicolai P, Prevedello DM, Locatelli D, Carrau RL. Injury of the internal carotid artery during endoscopic skull base surgery: prevention and management protocol. Otolaryngol Clin North Am 2016;49(1):237–252
18 Biswas D, Daudia A, Jones NS, McConachie NS. Profuse epistaxis following sphenoid surgery: a ruptured carotid artery pseudoaneurysm and its management. J Laryngol Otol 2009;123(6): 692–694
19 Berker M, Aghayev K, Saatci I, Palaoğlu S, Onerci M. Overview of vascular complications of pituitary surgery with special emphasis on unexpected abnormality. Pituitary 2010;13(2):160–167
20 Davies A, Dale O, Renowden S. Spontaneous rupture of an intracavernous internal carotid artery aneurysm presenting with massive epistaxis. J Laryngol Otol 2011;125(10):1070–1072
21 Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. Neurosurgery 2013;73, (2 Suppl Operative) ons261–ons269, discussion ons269–ons270
22 Gardner PA, Vaz-Guimaraes F, Jankowitz B, et al. Endoscopic endonasal clipping of intracranial aneurysms: surgical technique and results. World Neurosurg 2015;84(5):1380–1393
23 Griauzde J, Gemmete JJ, Pandey AS, McKean EL, Sullivan SE, Chaudhary N. Emergency reconstructive endovascular management of intraoperative complications involving the internal carotid artery from trans-sphenoidal surgery. J Neurointerv Surg 2015; 7(1):67–71
24 Inamasu J, Guiot BH. Iatrogenic carotid artery injury in neurosurgery. Neurosurg Rev 2005;28(4):239–247, discussion 248
25 Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. Int Forum Allergy Rhinol 2011;1(2): 118–122
26 Valentine R, Wormald PJ. A vascular catastrophe during endonasal surgery: an endoscopic sheep model. Skull Base 2011;21(2): 109–114
27 Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. Laryngoscope 2011;121(3): 562–566
28 Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. Otolaryngol Clin North Am 2011;44(5):1059–1079
29 Solares CA, Ong YK, Carrau RL, et al. Prevention and management of vascular injuries in endoscopic surgery of the sinonasal tract and skull base. Otolaryngol Clin North Am 2010;43(4): 817–825

30 Arnaud F, Parreño-Sadalan D, Tomori T, et al. Comparison of 10 hemostatic dressings in a groin transection model in swine. J Trauma 2009;67(4):848–855
31 Kessler CM, Ortel TL. Recent developments in topical thrombins. Thromb Haemost 2009;102(1):15–24
32 Tomizawa Y. Clinical benefits and risk analysis of topical hemostats: a review. J Artif Organs 2005;8(3):137–142
33 Padhye V, Valentine R, Paramasivan S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. Int Forum Allergy Rhinol 2014;4(8):651–657
34 Dyer SR, Bathula S, Durvasula P, et al. Intraoperative use of FloSeal with adenotonsillectomy to prevent adverse postoperative outcomes in pediatric patients. Otolaryngol Head Neck Surg 2013; 149(2):312–317
35 Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. Int Forum Allergy Rhinol 2015;5(3):253–257
36 Ahmed EM. Hydrogel: preparation, characterization, and applications: a review. J Adv Res 2015;6(2):105–121
37 Gazzeri R, Galarza M, Fiore C, Callovini G, Alfieri A. Use of tissue-glue-coated collagen sponge (TachoSil) to repair minor cerebral dural venous sinus lacerations: technical note. Neurosurgery 2015;11(Suppl 2):32–36, discussion 36
38 Ellis-Behnke RG, Tay DKC, Liang YX, Zhang S, Schneider GE, So KF. Crystal clear surgery with self-assembling molecules that act as a bio barrier in the brain and intestine (Abstract). Nanomedicine 2005;1(3):269–270
39 Paradís-Bas M, Tulla-Puche J, Zompra AA, Albericio F. RADA-16: a tough peptide—strategies for synthesis and purification. Eur J Org Chem 2013;26:5871–5878
40 Ellis-Behnke RG, Liang YX, Tay DK, et al. Nano hemostat solution: immediate hemostasis at the nanoscale. Nanomedicine (Lond) 2006;2(4):207–215
41 Heller M, Wei C. Self-assembly peptide prevents blood loss. Nanomedicine (Lond) 2006;2(4):216

Nano-haemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery.

Jukes A, Murphy J, Vreugde S, Psaltis A, Wormald P.

Nano-hemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery. Journal of Neurological Surgery Part B: Skull Base. 2016;e-first(Dec).

Introduction:

'Nano-technology' or 'nano-engineering' involve structures that are <100nm in size. The challenges that this poses are immense and involve controlling structures on an atomic scale(383). Given many disease processes involve damage to cell structures, the ability to administer a substance that either provides a scaffold for ordered cellular regeneration or delivers growth factors that stimulate such recovery is attractive, especially if this scaffold were comprised of materials that were able to be broken down without causing long term damage once their function had expired(383, 384).

The use of self-assembling peptides in medical applications:

The medical applications of nanotechnology have revolutionized our options for drug delivery, in vivo medical imaging and biotechnological techniques(522). Typically, these applications utilize amino acid chains that are amenable to self-assembly – that is, forming secondary structures according to their amino acid sequence under certain external conditions(365, 383). They are, as Loo and colleagues described in 2012, "*versatile building blocks for fabricating supramolecular architectures*"(365). The development of these has come about through increased understanding of the protein chains found in naturally occurring substances. Peptide self-assembly involves a complex interaction of forces including hydrogen bonding, ionic forces, hydrophobic forces, van der Waals interactions,

and electrostatic forces(365). Proposed uses for these peptides include drug delivery, scaffold sheaths for neural redevelopment, and, importantly for surgeons, haemostasis. Multiple different types of self-assembling peptides and their formulations have been described. The feature common to all is that they comprise a dissolvable L-amino acid chain that does not interfere with cellular pathways or promote or inhibit signaling and that is broken down via the body's natural peptidases and enzymes to constituent amino acids after its primary role has been performed(389). These peptides have been used as haemostatic agents(389, 390), as an adjunct to ophthalmological surgeries (such as corneal endothelial stem cell transplantation) and, intriguingly, as a self-assembling peptide to provide a scaffold or bridge for axonal regeneration in optic nerve reconnection(383). Theories have also been advanced in the field of oncology that nanostructures could change the extracellular matrix that facilitates cancer metastasis and ensure malignancy remains localised(393). A peptide chain self-assembled into a β-pleated sheet named RADA16 has been used to deliver epidermal growth factor, insulin-like growth factor, platelet-derived growth factor and stromal cell-derived growth factor-1 to post infarction myocardium to increase the rate of wound healing (365). The advantage to this method of drug delivery is that amalgamation into a protein sheet renders the drug less susceptible to metabolic processes within the body and allows it to be delivered unaltered to the site of action(387). Ellis-Behnke and colleagues have also demonstrated the ability to use these nanofibre scaffolds to repair hamster brain lesions by allowing nerve fibres to reconnect post severance(391, 392). This is analogous to the role played by myelin sheaths in neural protection and redirection(392).

Self-Assembling peptides as Nano-haemostats:

Coagulation and subsequent haemostasis depends on multiple factors. These include the endothelial cells of blood vessels, platelets, leukocytes, the coagulation cascade, and the milieu of temperature and blood pH. There are also multiple anticoagulant forces and inhibitors. The precipitating event for non-pathological coagulation initiation is vessel wall damage. This causes activation of endothelial cells and subsequent platelet activation. Platelets roll along the damaged cells and, using von Willebrand factor adhesion to bind to newly exposed subendothelial cells, aggregate in platelet clumps. Post this event, a mesh of platelets and trapped leukocytes form a scaffold for development of a fibrin clot as part of the coagulation pathway(196, 223, 523).

Several studies have examined the role that these self-assembling peptides may play in haemostasis(389, 390, 400). There are a number demonstrating almost immediate haemostasis in small animal models without activation of the coagulation cascade. This is thought to occur as the nano-hamostat scaffold acts in an analogous fashion to fibrin clot, forming a barrier that both holds platelets in situ, and provides an activation stimulus, leading to further aggregation. This has been demonstrated on mammalian brain, spinal cord, liver, kidney, femoral artery and skin.(379, 389, 390, 394). The peptide solutions have been shown to breakdown via natural enzymatic processes and are non-immunogenic and nontoxic(383). In addition, the breakdown products are amino acids that may be used for subsequent tissue repair(383, 389).

Nano-haemostat is a particularly attractive concept in skull base surgery, especially when performed endoscopically. Surgeons are constrained by relatively narrow access corridors

that are in close proximity to major vascular structures such as the internal carotid artery, anterior cerebral arteries and basilar artery and venous structures such as the cavernous sinus(138, 524). Injury to these structures carries the potential for devastating and potentially fatal bleeding (4, 7, 123, 125, 138, 168, 524, 525). Much work has been performed to determine the safest way to halt bleeding in this environment and the safest way to train surgeons to operate in this area(65, 117-120). In this situation, the ideal haemostatic agent should be able to conform to irregular cavities, to not obstruct the surgical field, and to not damage surrounding structures, either through thermal or chemical injury or through expansion and pressure injury to nerves or vessels(284, 396, 397). Current methods of haemostasis used in major vessel injury in the skull base include muscle patches(117-121), Floseal[Baxter], Surgiflow with thrombin[Ethicon], Tisseal[Baxter](223, 398), and haemostatic clips (121, 122). Whilst these are efficacious to varying degrees, they all have their limitations. There can be some delay in harvesting a muscle patch and it is partially dependent on platelet activation as part of its mechanism of action(121). Floseal and Tisseal have been shown to wash out of the surgical field rapidly in the case of torrential haemorrhage(117-120). Haemostatic clips can be difficult to apply in situations with a tear in an artery at the lateral extremity of the surgical field(122).

Nano-haemostats in animal models

d-EAK16

Evidence to support the hypothesis that nano-peptides in haemostasis provide more than a simple physical barrier comes from studies looking at peptide d-EAK16 by Luo et al(400). Self assembly of d-EAK16 was timed in a pure water solution and in a living animal model (rabbit liver). It took ~16 hours for d-EAK16 to self-assemble in water but ~20 seconds to

assemble in whole blood. The time to haemostasis in this d-EAK16 group was significantly lower when compared with controls (no treatment) (20 seconds vs. 80-120 seconds). The authors conclude that ions, proteins, enzymes and "*other factors*" in blood work cooperatively to cause haemostasis. Their rationale is that normal haemostasis occurs when there is a high concentration of clotting factors held within a localised area whereas the same gross number of coagulation components would be ineffective if spread out over a larger area. d-EAK16 is thought to be triggered by sodium, magnesium, potassium, calcium, iron and zinc within the circulating plasma to form a stable β-pleated sheet in which platelets and subsequently endogenous coagulation factors become trapped and undergo activation in the usual fashion. These β-pleated sheets then combine to form first short and then long nanofibres which may then form 'tight junctions' impervious to liquids. These peptide chains have been shown to work best as a haemostatic agent when comprised of amino acids of the same chirality. Alternating chirality gave bleeding times up to 8 times longer in a rat liver haemorrhage model(400).

AC5

AC5 is a proprietary, synthetic nano-haemostat formulation developed by Ellis-Behnke and colleagues which is currently in pre-clinical trials(390). AC5 addresses the potential for haemostasis in the context of anticoagulation(390). In a non-compressible, full thickness 4mm liver punch biopsy model in rats, AC5 achieved haemostasis equivalently in heparinized and non-heparinized rats with a 94% reduction in time to haemostasis compared to saline controls(390).

RADA-16

RADA-16 is a self-assembling peptide of ionic hydrophilic and hydrophobic amino acids. It is 5 nanometers in length and forms stable β-pleated sheet structures which turn into hydrogels(384). Sang et al performed a study in which they divided 20 rats in 4 groups – Intracerebral haemorrhage (ICH) without aspiration, ICH with aspiration, ICH with aspiration and saline and ICH with aspiration and RADA-16 injection. ICH was created via intra-striatal injection of collagenase. 210 minutes later, the haematoma was aspirated and 20 microliters of saline or RADA-16 was injected into the ICH cavity. Differences in haematoma volume in aspiration vs saline vs RADA-16 group were not significant. Aspiration significantly reduced inflammatory cells in surrounding cortex on histological examination. The authors concluded that self assembling peptide attenuated brain injury, enhanced functional recovery and reduced cavity volume with a reduction in brain oedema, peri-haematoma apoptosis and inflammatory reaction(384). This reduction in secondary brain injury is thought to come about by decreasing the leakage of serum proteins into the surrounding parenchyma. Histology showed tight contact between RADA-16 and the cavity wall(384). More work is required to validate this concept in both the acute and chronic phases of brain inflammatory reactions.

Further research

There are many questions to answer before nano-haemostat technology can be utilized in human surgery routinely, namely; Which formula is best and why? What rate of flow can it stop? Does it need to be impregnated in a carrier? Does it preserve vessel lumen patency and thus allow distal brain perfusion? Does the scaffold last long enough for more permanent healing to occur? Do pseudo-aneurysms form after its use? Are there long term effects on native tissues and what happens to the breakdown products? Is there pseudo-

aneurysm formation? Do surrounding neurons/glial cells contain any evidence of abnormal protein inclusions? And finally, is there evidence of distal embolic phenomena?

Possible Limitations

Whilst nano-haemostats have been proven to be successful in a small animal model of vessel injury, this may not hold true in larger animal/human models where pressures may be many times that of smaller animals(332, 390, 398, 401). This pressure may wash the haemostat away from its site of action before it can take effect. Further, the liquid may need to be either impregnated in, or held on by a patty or similar. This may remove the advantage of having a clear liquid haemostat, at least in the immediate phase of application(402). Whilst this has not been seen in nano-haemostats to date, a build up of abnormal proteins within the central nervous system is the basis of conditions such as dementias and prion diseases. It would be incumbent upon designers of nano-haemostats to ensure that their solution is adequately broken down by the body's enzymatic processes and is not trapped in insoluble form within neurons or astrocytes.

Pilot study of nano-haemostat on an animal model of major vessel injury relevant to skull base surgery

Aim: To determine the feasibility of a nano-haemostat solution in stopping bleeding from major arterial and venous sources and its ease of use in an endoscopic setting.

The peptide chosen was RADA-16 (Arg - Ala - Asp - Ala - Arg - Ala - Asp - Ala - Arg - Ala - Asp - Ala - Arg - Ala - Asp - Ala -NH_2), which is known for its propensity to self-assemble and has been used as a

nano-haemostat in small animal models(387, 389, 394, 526). This was sourced from China Peptides as a 70% pure powder.

Method: Ethics approval was obtained from the Animal Ethics Committee of the South Australian Health and Medical Research Institute. Two merino sheep were anaesthetised and placed supine. A midline neck dissection was performed and left carotid artery cannulated for an arterial line and the left internal jugular cannulated for a central venous catheter. The right carotid artery and right internal jugular vein were dissected free and a segment of at least 8cm was cleared of surrounding fascia. An endoscopic training model was placed around the internal jugular (Figure. 1). These silicon-based models recreate the sinonasal cavity with the vessel running through a groove at the base of the model. The sheep is then draped and provides a realistic representation of endoscopic surgery.

RADA-16 nano-haemostat was mixed with saline in a 10% mixture forming a gel at room temperature. This was drawn up into a 10ml syringe and a blunt cannula attached. Using a 19-gauge needle, a hole was made in the internal jugular and blood allowed to escape for 20 seconds (Figure. 2A). RADA-16 was then applied to the jugular injury at the rate of 1ml per second (Figure. 2B). Whilst it slowed the bleeding and provided a semi-translucent membrane over the injury, blood could still be seen escaping from the hole in the jugular vein underneath this for a period of 15 seconds before haemostasis occurred. A proximal injury was subsequently made in the same animals as an untreated control which continued to bleed for 5 minutes until RADA-16 was applied and haemostasis occurred at ~15 seconds. The nano-haemostat did not appear to set in a solid fashion but remained as a jelly-like substance when observed for 20 minutes.

The endoscopic trainer was then applied to the carotid artery of the same sheep. A curved aneurysm clip was placed half way across the carotid artery and a 4mm linear incision was made in the wall of carotid artery (Figure. 2C). The aneurysm clip was removed and the injury allowed to bleed for 3 seconds (Figure. 2D). A sucker was used to control the field and an identical mix of 10ml of RADA nano-haemostat was immediately applied to the injury. There was no discernable decrease in the bleeding rate. RADA-16 appeared to be washed away by the pressure of the bleeding (Systolic range 70-90mmHg). We were unable to control the bleeding and after a period of 90 seconds were forced to use a crushed muscle patch to stop the bleeding which worked to good effect as previously reported(117-120). This was repeated in a second sheep with identical results. Sheep were then humanely killed. The jugular and carotid were dissected out and placed in formalin and scanning electron microscopy (SEM) fixative. A small amount of muscle was also covered in nano-haemostat post mortem and sent for histology and SEM. SEM was performed post preparation and coating of the samples in platinum. The nano-haemostat could be seen coating the muscle patch and forming a 'glue' or matrix with long scaffolds or fibres with a width of 30-70 nanometres. Erythrocytes could be seen trapped within this matrix (figure. 3).

Discussion: This pilot study demonstrates some of the inherent limitations of gels or liquids in high flow, high pressure bleeding. Whilst the bleeding from the jugular vein injury could be stopped with this nano-haemostat mixture, this is a comparatively low flow, low pressure injury. The carotid artery injury was unable to be controlled with the nano-haemostat and eventual control was achieved with the conventional option of crushed muscle patch.

There are a number of questions raised by this small pilot study that may warrant further investigation. The literature is not clear regarding the percentage strength of solution used in previous nano-haemostat studies utilising RADA-16(379, 384, 389, 527, 528). Whilst these have stopped bleeding in mouse models, this may simply be a reflection of the lower pressure and lower volume of flows in these injuries. The ideal percentage is not yet clear. Our 10% solution provided a solution viscous enough to adhere to the side of the plastic syringe. This was chosen because we were interested in its ability to be applied in an endoscopic scenario and it appeared to provide a balance between applicability and viscosity.

This pilot study appears to add weight to previously published studies that have described success in bleeding control of small vessels in small animal models using nano-haemostat(379, 389, 527, 528). Certainly, it appeared to have some success controlling a small jugular vein puncture. There may be future avenues worth exploring with regards to the use of nano-haemostat in areas where visualisation of the bleeding point would be useful such as a venous ooze from the lateral borders of the sphenoid corridor of a pituitary resection or in endoscopic resection of a skull base tumour. Current teaching involves packing these areas and moving the dissection to another region rather than laboriously obtaining control and drying the field with bipolar as this would add inordinate amounts of time to what is already a long operation. It would be advantageous to have a semi-translucent haemostat that would allow the surgeon to see the bleeding point underneath the haemostat rather than having to remove the pack and potentially remove the fibrin/platelet plug/clot in doing so, causing further bleeding.

This pilot study appears to show that nano-haemostat is unlikely to adequately control a high-flow, high-pressure bleed such as that from a carotid artery injury. SEM shows the nano-haemostat forming a matrix in which circulating erythrocytes and platelets are trapped (in much the same way as a fibrin plug is formed) but this only formed in the post mortem samples where there was obviously no pressure washing the nano-haemostat away. In order for this to work in vivo, the nana-haemostat would need to be held in situ for a prolonged period, possibly as part of a patch or pad. Further research is needed to address the questions posed in the earlier part of this paper regarding the optimum formulation and indications for use of this potentially revolutionary new haemostatic agent.

Acknowledgements: This research was funded by the NeuroSurgical Research Foundation, Adelaide. The authors would like to thank Lynnette Waterhouse for assistance devising the Scanning electron microscopy protocol to view the nano-haemostat.

Chapter 3

An Endoscopic Trial of Fibrin/Thrombin patches, Fibrin/Thrombin glue and Beta-Chitin patches for major vessel bleeding

Alistair Jukes, Jae Murphy, Sathish Paramasivan, Alkis J Psaltis, Lyall Hanton, Marina Roxburgh, Simon Moratti, PJ Wormald

Statement of Authorship

Title of Paper	An Endoscopic Trial of Fibrin/Thrombin patches, Fibrin/Thrombin glue and Beta-Chitin patches for major vessel bleeding
Publication Status	☐ Published ☐ Accepted for Publication ☒ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Alistair Jukes, Jae Murphy, Sathish Paramasivan, Alkis J Psaltis, Lyall Hanton, Marina Roxburgh, Simon Moratti, PJ Wormald

Principal Author

Name of Principal Author (Candidate)	Jukes, Alistair		
Contribution to the Paper	Study design, Ethics application, study conduct and data collection, animal operating and recovery, tissue harvest, data analysis, manuscript writing and preparation		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	2/12/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Murphy, Jae		
Contribution to the Paper	Study conduct and data collection, animal operating and recovery, tissue harvest		
Signature		Date	2/12/17

Name of Co-Author	Paramasivan, Sathish		
Contribution to the Paper	Study conduct and data collection, animal operating and recovery, tissue harvest		
Signature		Date	4/12/17

Name of Co-Author	Psaltis, Alkis		
Contribution to the Paper	Study design and manuscript review		
Signature		Date	2/12/17

Name of Co-Author	Hanton, Lyell		
Contribution to the Paper	Patch design and testing, manuscript contribution		
Signature		Date	6/12/17

Name of Co-Author	Roxburgh, Marina		
Contribution to the Paper	Patch design and testing, manuscript contribution		
Signature		Date	10/12/17

Name of Co-Author	Moretti, Simon		
Contribution to the Paper	Patch design and testing, manuscript contribution		
Signature		Date	7/12/17

Name of Co-Author	Wormald, Peter-John		
Contribution to the Paper	Study design and manuscript review		
Signature		Date	2/12/17

An Endoscopic Trial of Fibrin/Thrombin patches, Fibrin/Thrombin glue and Beta-Chitin patches for major vessel bleeding

Alistair Jukes, Jae Murphy, Sathish Paramasivan, Alkis J Psaltis, Lyall Hanton, Marina Roxburgh, Simon Moratti, PJ Wormald

Abstract

Background: As indications and approaches in endoscopic skull base surgery expand, the potential for major vessel haemorrhage in areas that are relatively constrained from an anatomical perspective increases. These expanded endonasal approaches can carry risks of cavernous carotid injury of up to 5-9%. This study looks at methods to expand the surgeon's armamentarium when dealing with these potentially catastrophic bleeds. This study aims to demonstrate efficacy, ease of application and safety of a fibrin/thrombin patch - Tachosil [Baxter, Deerfield, IL], fibrin/thrombin glue - Evicel [Ethicon, Sommerville, NJ]) combined with oxidized cellulose - Surgicel snow [Ethicon, Sommerville, NJ], and a squid-derived Beta-Chitin patch [Department of Chemistry, University of Otago, Dunedin, New Zealand] in the acute management of major vessel haemorrhage in endoscopic skull base surgery using a sheep model of carotid artery bleeding and compare these with muscle patch and anastoclip controls.

Methods: 18 sheep underwent neck dissection and placement of an endoscopic trainer over the carotid artery. Standardised incisions were made in the artery and the experimental patches used to control the haemorrhage with a 2-surgeon endoscopic technique. Haemodynamic changes, time to haemostasis and volume loss were measured. Animals were recovered for 3 months and underwent Magnetic resonance angiography (MRA) to

determine incidence of pseudoaneurysm formation. Histology and electron microscopy of the site of injury was then performed.

Results: Mean time to haemostasis was 124 seconds (95% CI 70.9-177) in the Tachosil group, 79.8 seconds in the Evicel/Snow group (95% CI 69-90.6) and 67 seconds (95% CI 52.3-81.8) in the Beta-Chitin group. Mean blood loss was 281ml (95% CI 106-456ml) in the Tachosil group, 150ml (95% CI 105-196ml) in the Evicel/Snow group and 121ml (95%CI 97-146ml) in the Beta-Chitin group.

Conclusions: Tachosil, Evicel/Snow and Beta-Chitin are potentially valuable adjuncts to the control of major vessel haemorrhage in endoscopic surgery. They are able to be introduced and manipulated trans-nasally with endoscopic instruments and have a lower rate of pseudoaneurysm formation than muscle patch. They also have the advantage of being immediately available and do not require harvesting by a second surgeon.

Introduction

The extended, endonasal approach to the skull base continues to gain popularity and increasing numbers of pathologies are now removed through this approach. As more surgeons become proficient at these techniques and extend their skills, it is important to acknowledge that there is a real risk of potentially catastrophic major arterial haemorrhage(125, 128, 529). This is typically from the cavernous segment of the carotid artery but, as approaches to deeper and more complex pathologies are developed, the risk to the anterior communicating artery complex and the basilar regions increases. Large, pooled case series put this risk at between 1-9% in extended endonasal approaches(117-120). This bleeding is often difficult to control as anatomic restrictions often preclude the application of surgical clips and other devices. Currently, the gold standard for treatment of these injuries is the application of crushed autologous muscle patches. Whilst this treatment is able to control the bleeding, it has a high incidence of pseudoaneurysm development over the post-operative period. Some estimates range as high as 66% but most pooled series put this in the region of 20-40%(121-123, 125, 167, 524). Muscle patches also take time and a second surgeon to harvest and significant blood loss can occur whilst gaining definitive haemostasis. Our research group has developed an ovine endoscopic carotid artery injury model which is used for both research and surgical training(117). Several techniques have been trialled on this model including the muscle patch and direct vessel closure via the anastoclip device (LeMitre, Burlington, USA)(117, 119, 122). In an effort to reduce the time to haemostasis, blood volume loss, and the incidence of pseudoaneurysm formation, we trialled three commercially available products (2 in combination) and a Beta-Chitin patch that we have formulated from squid pen on the endoscopic injury model.

Materials and Method

Ethics approval for this study was granted from the South Australian Health & Medical Research Institute Animal Ethics Committee and the University of Adelaide Animal Ethics Research Committee.

Animal Model

18 merino sheep underwent a general anaesthetic using intravenous ketamine and diazepam for induction and intubated. Isoflurane was given for maintenance of anaesthesia. and the animals placed supine with neck extended. Intramuscular antibiotics were given. A midline neck dissection was performed and an arterial line and 12-french central line placed in the left common carotid and internal jugular vein respectively. The right common carotid was dissected free from surrounding strap muscles and adventitia and encased in a modified SIMONT endoscopic trainer (Pro-delphus, Sao Paulo, Brazil) following the procedure established by Valentine and Wormald and utilized in studies by Padhye and Wormald subsequently(117, 121, 122, 128, 140). A curved aneurysm clip was placed half way across the common carotid endoscopically to isolate a segment of the vessel. A scalpel was then used to make a standardised 4mm linear incision in the carotid artery. Warmed intravenous saline (Baxter, Sydney, Australia) was infused through the central line. The aneurysm clip was then removed and the vessel allowed to bleed for 5 seconds. Haemorrhage control was then achieved by endoscopic application of the haemostats being tested. A 2 surgeon, 4 hand technique was used. Once bleeding had been controlled, the area was observed for ten minutes to ensure no rebleeding. The endoscopic training model was removed, the central and arterial lines removed, and the neck incision sutured closed in a single absorbable layer. Sheep were then given IM analgesia, recovered and observed with

monitoring of vital signs, feeding and behaviour in line with local animal ethics protocol. Sheep were kept alive for 3-months post operatively and then underwent MRA of the neck vessels to determine if a pseudoaneurysm had formed. Sheep were then humanely euthanised and the right sided carotid removed en-bloc with surrounding strap muscles. In 2 sheep from each group, this was placed in electron microscopy fixative for 7 days and in 4 sheep from each group, this underwent 7 days of formalin fixation. The specimens were then prepared for formal histology and electron microscopy.

Haemostats

18 Merino Sheep were used in this study. These were divided into 3 groups. 6 sheep underwent application of Tachosil (Baxter, Deerfield, IL), 6 sheep underwent application of Evicel/Surgicel snow (Ethicon, Sommerville, NJ) and 6 underwent application of Beta-Chitin patch (Department of Chemistry, University of Otago, Dunedin, New Zealand). Samples were made from squid pens (Nototodarus sloanii from Sealord, NZ). Squid pens were cut into small pieces, washed with ethanol to dry, and ground to a fine powder in a coffee grinder. The powder (7.50 g) was sieved through a sieve with a 0.420 mm mesh size, and stirred in NaOH (1 M, 200 mL) for 3 days. The solid was filtered off, washed with H_2O until the pH was neutral, washed with EtOH, and air dried on the funnel to give a sticky solid which eventually (30 min) dried to a white solid (2.53 g, 33%). A portion (1.00 g) was then sonicated with acetic acid (1% v/v, 200 mL) with a probe sonicator (UP100H Heilscher) for 30 min, alternating the site which the probe sat in, to give an opaque thick solution with no separate liquid. To determine the concentration of chitin fibres in suspension a portion of the liquid (11.6 g) was filtered on a Büchner flask, the collected solid was dried at 45°C O/N. This gave a solid film of chitin fibres (56 mg). To make a bulk solution containing 20% w/w

PEG (based on chitin content), melted PEG (1K, 168 mg) was dissolved in a portion of the remaining solution (139.2 g). The backing films were made by filtering the PEG/Chitin solution (11.6 g) using a Büchner flask of desired width (4 cm). Once flat, the sample was further dried under a weight in the oven at 45°C O/N. The foams are made on top of the backing films by dampening a backing film to adhere to the bottom of a beaker, then pouring PEG/chitin solution (11.6 g) on top. The sample was then frozen at -4°C O/N, and lyophilised giving the desired film/foam (thickness ~0.7 cm, width 4 cm).

Outcome Measures

Parameters measured included blood pressure, heart rate, blood loss volume, and time to haemostasis. All endoscopic cases were video-recorded for timekeeping and record purposes. The outcome measures were measured using identical protocols to those published by Padhye et al as we used their muscle patch and anastoclip animals as historical controls(122). “Primary hemostasis” was said to be gained if haemostasis was achieved during the operation and up to 1 hour post-operation with MAP > 55 mmHg. “Time to hemostasis” was taken in seconds from the time the aneurysm clip was removed to the time all instruments were removed from the nasal cavity in the absence of bleeding. “Blood loss” was measured in milliliters (mL) from suction canisters and calculated from weight of soiled drapes in grams (g). A “secondary bleed” was deemed to have occurred if bleeding was noted from the operative site in the form of neck hematoma or rapid exsanguination from the time the neck wound had been closed until the 3-month end point. “Pseudoaneurysm” was deemed to have occurred if features noted on MRA scan or rupture of pseudoaneurysm occurred resulting in sheep death.

Results

Statistical analysis was performed using SPSS. One-way ANOVA was performed utilising Kruskal-Wallis test with uncorrected Dunn's test to perform multiple comparisons of the haemostatic agents relative to each other.

Short term outcomes: Haemorrhage control was achieved in all 18 sheep. Mean time to haemostasis was 124 seconds (95% CI 70.9-177) in the Tachosil group, 79.8 seconds in the Evicel/Snow group (95% CI 69-90.6) and 67 seconds (95% CI 52.3-81.8) in the Beta-Chitin group. This was compared with historical anastoclip and muscle patch controls from our group using identical methodology. Mean time to haemostasis for Anastoclip was 249.1 seconds (95%CI 112.9-385.3) and mean for muscle patch was 850.3 seconds (95%CI 593.1-1108). Overall effect of haemostatic agent was significant (P=0.0001). There was a significantly reduced time to haemostasis between Evicel vs. Anastoclip (P=0.007), Evicel vs. muscle (P=0.001), Tachosil vs. muscle (P=0.027), Chitin vs. Tachosil (P=0.004), Chitin vs. Anastoclip (P=0.0003), and Chitin vs. Muscle (P=0.0001). The differences between Tachosil and Evicel (P=0.264), Tachosil and anastoclip (P=0.142), Anastoclip and muscle (P=0.232), and Chitin and Evicel (P=0.384) were not significant.

	Mean	SD	95% CI lower	95% CI upper
Muscle	850.3	103.6	593.1	1108
Anastoclip	249.1	177.2	112.9	385.3
Tachosil	124	50.5	70.9	177
Evicel	79.8	10.3	69	90.6
Chitin	67	14	52.3	81.6

Table 1: Mean times to haemostasis

	Summary	P Value
Muscle vs. Anastoclip	ns	0.2324
Muscle vs. Tachosil	*	0.027
Muscle vs. Evicel	**	0.0018
Muscle vs. Chitin	***	0.0001
Anastoclip vs. Tachosil	ns	0.1452
Anastoclip vs. Evicel	**	0.0074
Anastoclip vs. Chitin	***	0.0003
Tachosil vs. Evicel	ns	0.2643
Tachosil vs. Chitin	*	0.047
Evicel vs. Chitin	ns	0.3842

Table 2: Comparison of significance in mean times to haemostasis between products

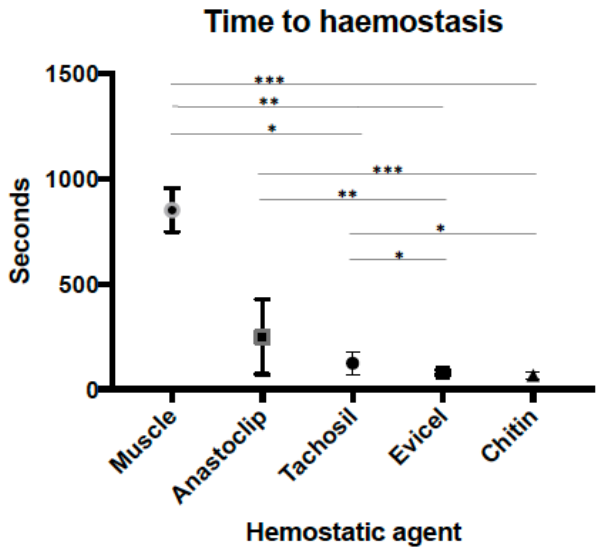


Figure 1: Time to haemostasis

Mean blood loss was 281ml (95% CI 106-456ml) in the Tachosil group, 150ml (95% CI 105-196ml) in the Evicel/Snow group and 121ml (95%CI 97-146ml) in the Beta-Chitin group. Mean Blood loss was 146ml (95%CI 17-298ml) for anastoclip and 928 (95%CI 406-1449ml) for muscle patch. The overall effect of haemostatic agent on volume of blood loss was

significant (P=0.005). The difference in mean blood loss was significantly different between muscle vs. anastoclip (P=0.0008), Muscle vs. Evicel (P=0.031), Muscle vs. Chitin (P=0.006) and Anastoclip vs Tachosil (P=0.010). The differences between Muscle vs. Tachosil (P=0.212), Anastoclip vs. Evicel (P=0.181), Anastoclip vs Chitin (P=0.561), Tachosil vs. Evicel (0.264), Tachosil vs. Chitin (P=0.071) and Evicel vs. Chitin (P=0.490) were not significant.

	Mean	SD	95% CI lower	95% CI upper
Muscle	928	209.8	406.7	1449
Anastoclip	146.2	197.7	17.4	298.2
Tachosil	281.7	166.5	106.9	456.4
Evicel	150.8	43.1	105.5	196.1
Chitin	121.7	23.1	97.36	146

Table 3: Mean blood volume loss in milliliters

	Summary	P Value
Muscle vs. Anastoclip	***	0.0008
Muscle vs. Tachosil	ns	0.2129
Muscle vs. Evicel	*	0.0311
Muscle vs. Chitin	**	0.0066
Anastoclip vs. Tachosil	*	0.0105
Anastoclip vs. Evicel	ns	0.1817
Anastoclip vs. Chitin	ns	0.5613
Tachosil vs. Evicel	ns	0.2647
Tachosil vs. Chitin	ns	0.0712

Table 4: Comparison of significance in milliliters of blood loss between products

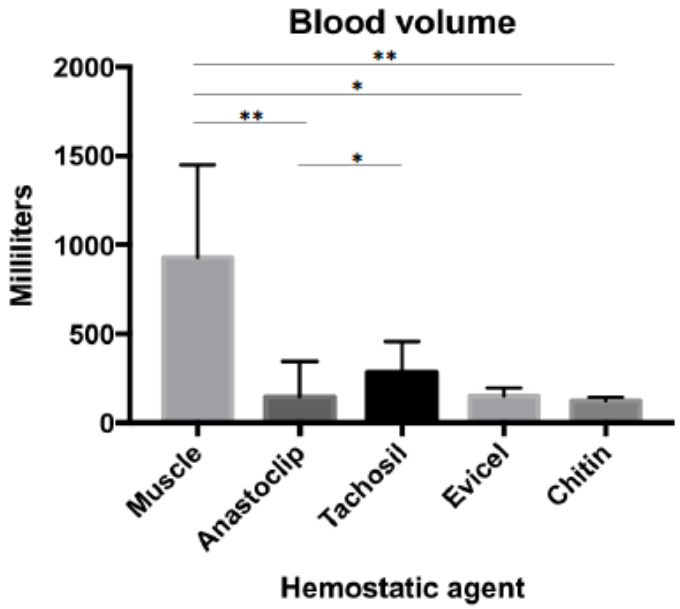


Figure 2: Mean blood volume loss (mls)

Haemodynamic parameters did not vary significantly between the groups with mean arterial pressure (MAP) of 67.7mmHg at the beginning of the procedure in the Tachosil group, 69.8 in the Evicel/Snow group and 70 mmHg in the beta Chitin group. MAP at the end of the procedure was 64.3mmHg in the Tachosil group, 62.5mmHg in the Evicel/Snow group, 66 mmHg in the beta Chitin group. The change in MAP was not significant for any group.

Long-term outcomes: 1 sheep in the Tachosil group died of acute neck swelling on post-operative day 11 and autopsy demonstrated a large clot compressing the neck vessels. The carotid artery was removed and a pseudoaneurysm was discovered on histological examination with a defect in the muscular layer of the artery with disorganized collagen fibres overlying this and a fresh blood clot (figure). Two sheep in the Beta-Chitin group underwent humane killing on day 11 and day 13 post-operatively for infection not responsive to antibiotics. They underwent formal angiography prior to this which

demonstrated no significant abnormalities of the carotid. The remainder of the sheep survived to 3 months. MRA of the neck vessels did not demonstrate the development of pseudoaneurysm in any of these sheep. In the historical controls, 1 sheep in the anastoclip group had an unruptured pseudoaneurysm and 1 of the sheep in the muscle patch group suffered a ruptured pseudoaneurysm. Histological examination of the carotid arteries did not demonstrate any adverse inflammatory response to any of the patches used.

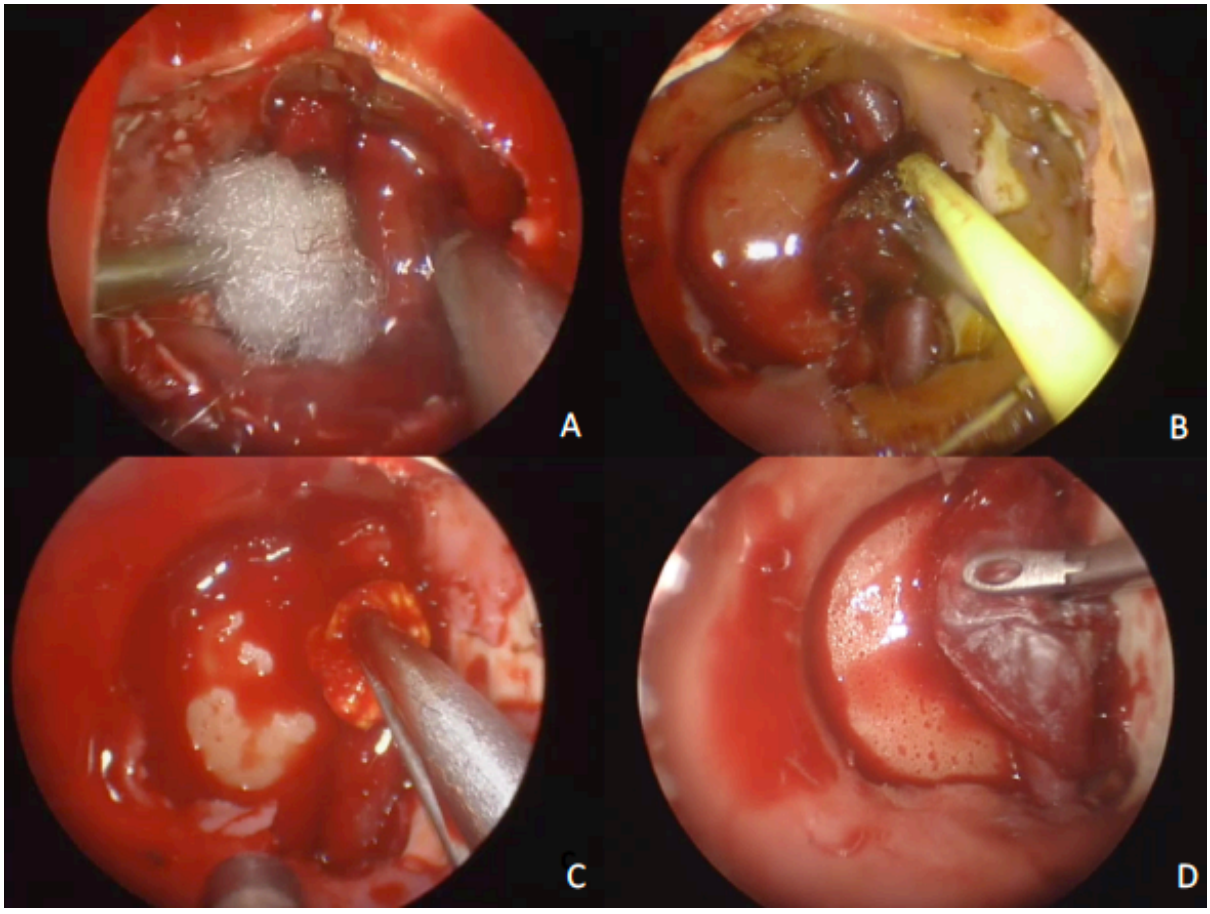


Figure 3: Endoscopic views of application of different haemostatic agents - (A) Surgicel Snow; (B) Evicel glue; (C) Tachosil; (D) Chitin

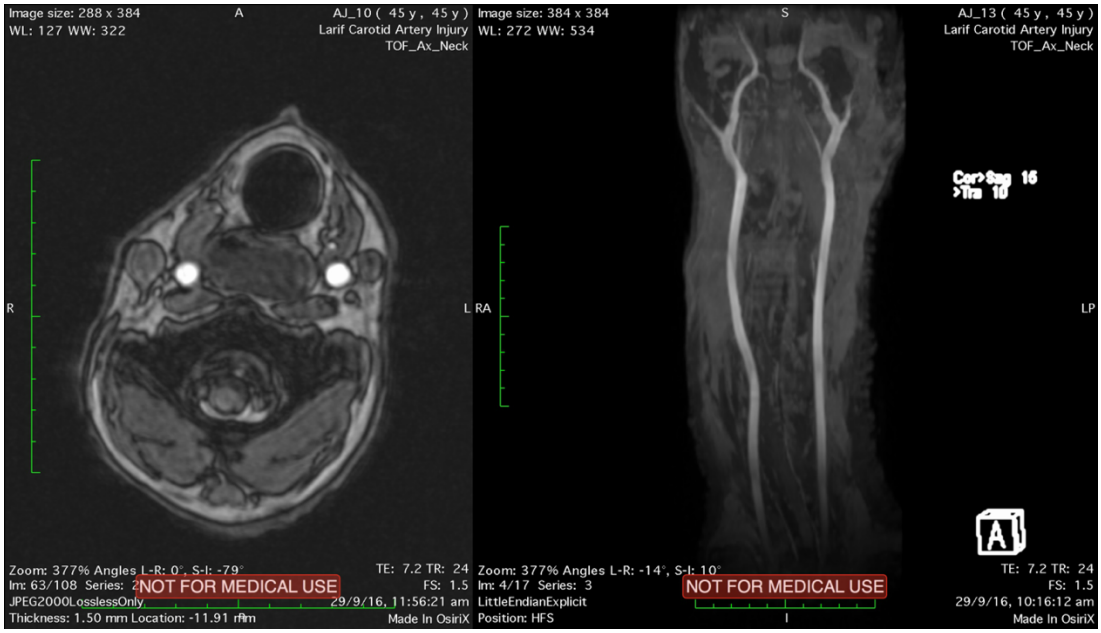


Figure 4: Axial (left) and Coronal (right) magnetic resonance angiograms at 3 months post injury

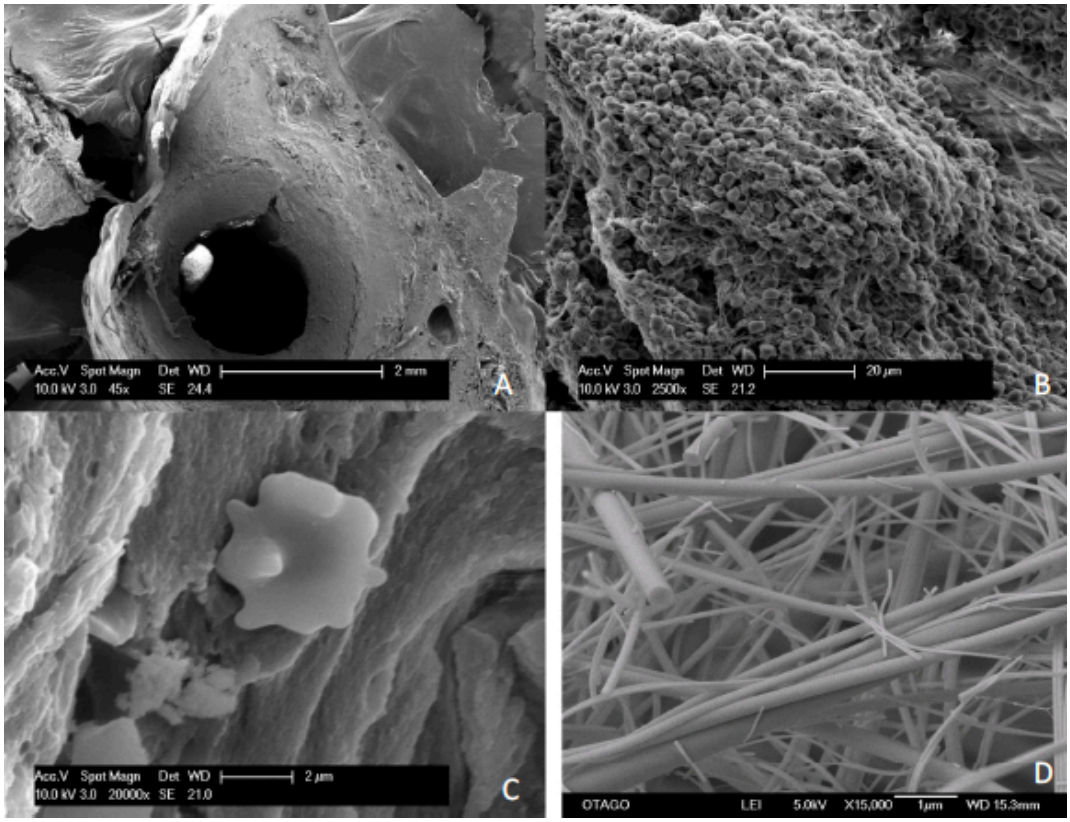


Figure 5: Electron microscopy images of (A) carotid artery injury with tachosil patch overlying the artery wall defect; (B) Fibrin clot with erythrocytes and plates enmeshed; (C) Ovine platelet undergoing activation and projecting podocytes; (D) Chitin fibres

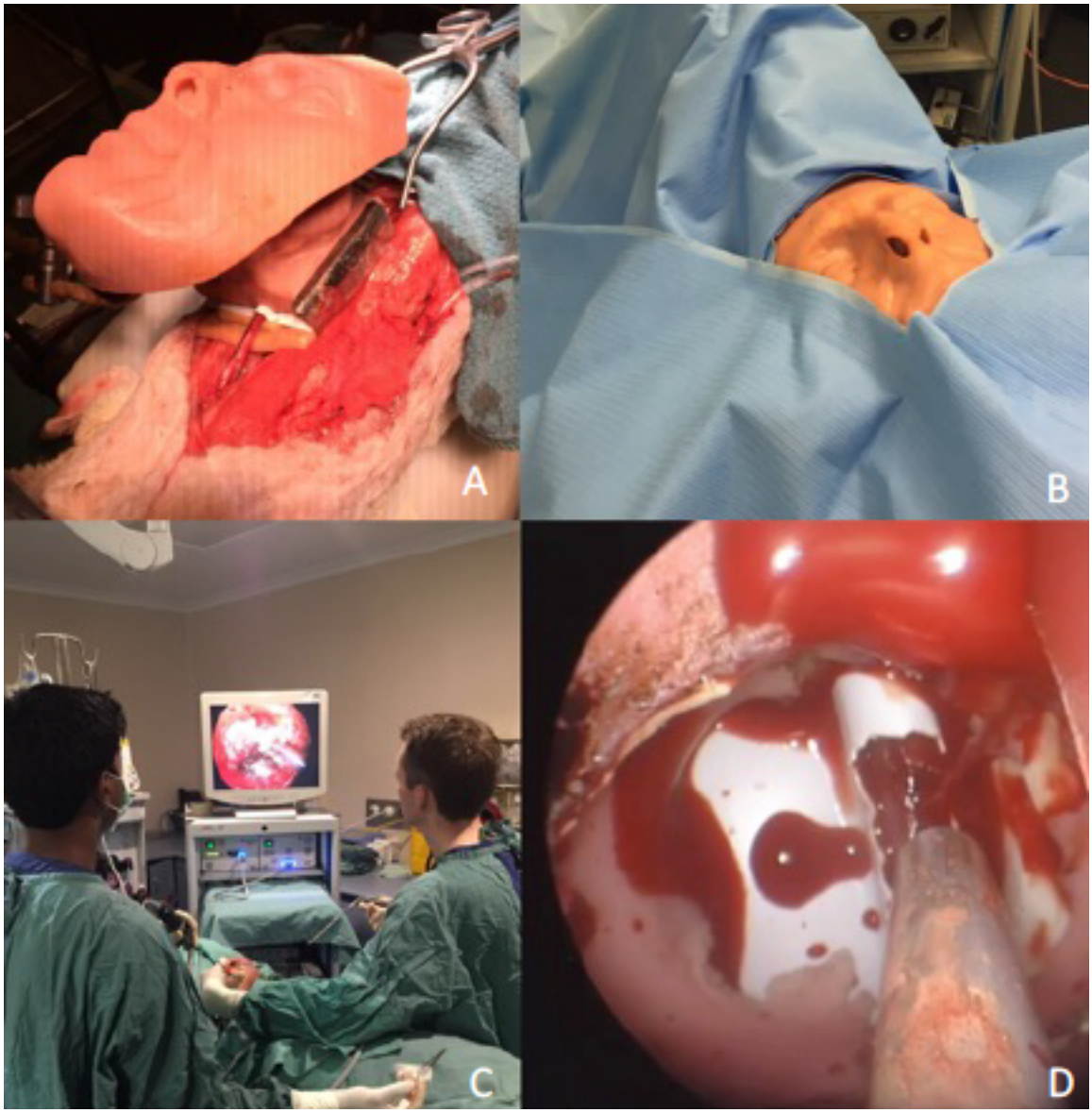


Figure 6: The sheep model demonstrating (A) Trainer on carotid artery; (B) Draped; (C) Haemorrhage control; (D) Arterial jet from injury (endoscopic view)

Discussion

Definitive hemostasis was achieved in all cases in this study. There was a significant improvement in time to haemostasis and blood loss for all three haemostat types compared to muscle patch controls. Blood loss was significantly reduced in all groups when compared with muscle patch controls. We elected to trial haemostatic agents that work on different parts of the coagulation and platelet activation pathways.

Tachosil [Baxter] is a topical sealant patch (9.5x4.8cm) that consists of human fibrinogen (3.6 to 7.4 mg (5.5 mg) per cm^2 and human thrombin (1.3 to 2.7 Units (2.0 U) per cm^2)(291) coated onto an equine-derived collagen sponge. It is thought to work by activation of fibrinogen into fibrin monomers with subsequent polymerization. The fibrin polymers and platelet activation lead to subsequent activation of the coagulation cascade with further adherence to the wound surface via further thrombin mediated fibrin polymerization and conglutination of the patch's collagen matrix and the wound surface, forming a tight seal(242, 243, 247, 248, 293). Tachosil is indicated for use in cardiac and hepatic surgery as an adjunct to haemostasis where it is placed as a sheet upon the bleeding surface(291). The literature is replete with case reports and small series of novel use. Several cardiac case studies report its use to seal ventricular bleeding post infarction and rupture and at least one case of its use to seal a high pressure ruptured coronary artery(296-299). It has also been trialed to prevent the development of pericardial adhesions post cardiac surgery(300, 301), been used in swine liver and spleen injury models(299, 302-304), gynaecological surgery for uterine wrapping and myomectomy bleeding(305, 306), in renal artery aneurysm surgery(307, 308) and post nephrectomy surgery(309, 310). A large case series at the University of Helsinki retrospectively analysed the use of Tachosil in 100 neurosurgical patients as both a haemostat and as a dural sealant(293). The authors describe its use in a number of settings from stopping small arteriole bleeding to reinforcing suture lines as a dural sealant. The most applicable from a haemostatic perspective was its use to repair the superior sagittal sinus in vertex and parafalcine meningioma surgery. The authors describe laying the Tachosil in strips over dural defects when resecting meningiomas with a patent superior sagittal sinus. There were no instances of post-operative haematoma or evidence

of thrombosis of the sinus as a result of its use(293). Whilst the incidence of the development of antibodies to the human fibrin and thrombin is less than 1%, a paper reporting the use of Tachosil in a hepatic haemorrhage model reported that the incidence of development of antibodies to equine collagen was 26%(293, 313). This did not result in any known clinical harm. It does not appear to carry an increased risk of systemic or localized thromboembolic complications(234).

Evicel [Ethicon] consists of 55-85 mg/ml fibrinogen and 800-1200 IU/ml human thrombin in frozen solution provided in separate vials which are mixed in a sterile applicator. It is dripped or sprayed onto the wound. In a prospective randomised controlled trial, Evicel was compared with manual compression only in patients undergoing end to side femoral or upper extremity arterial anastomoses(245). Evicel was significantly more effective at achieving haemostasis than manual compression alone at the 4-minute mark (85% vs 35%)(245). Evicel has also been trialled in endoscopic endonasal surgery using post-operative mucosal bleeding rates as an outcome measure(266-268). Post-operative bleeding occurred in 0-5% of patients in the Evicel groups compared with 23-37% of patients in the nasal packing alone groups(268).

Beta-Chitin is a form of Poly-N-acetyl Glucosamine (pGlcNAc). This is a carbohydrate polymer derived from animal, fungal and algal sources. In its natural form, it is a major component of crustacean exoskeletons, squid pens, the wings of insects, marine algae, or fungal mycelial mats(279, 348-350). It consists of polymers in a crystalline form arranged in either parallel lines (where they may be termed β-chitin) or antiparallel lines (where they may be termed α-chitin). They are able to be formed into nano-fibres measuring 60-100

micrometers in length and 20-80 nanometres in diameter. In-vitro studies have shown that Chitin is able to increase red blood cell aggregation and endothelial-dependant vasoconstriction and also to activate platelets(200, 349, 355, 356, 367, 530, 531). The Beta-Chitin form of pGlcNAc is more effective in tests of haemostasis than the Alpha-Chitin form of pGlcNAc (356, 365). Thatte and colleagues performed in vitro studies on alpha and beta forms of chitin and demonstrated that contact with Beta-Chitin caused total and irreversible activation of platelets(366). The authors propose a three-step activation mechanism(366). (1) - pGlcNAc binds to immobilised plasma proteins such as fibrinogen and/or platelets directly bind to pGlcNAc. (2) - Integrin-mediated platelet activation activated the intrinsic coagulation pathway via Hageman activation factor (Factor XII). This generates thrombin and forms a stable clot. This clot is further stabilised by the tendency for platelets to aggregate on pGlcNAc matrices and generate vasospastic substances such as thromboxane and serotonin. (3) - Clot retraction via platelet mediators and local vasospasm accelerates wound healing. Valeri et al used thromboelastography to demonstrate platelet activation upon exposure to chitin with an increase in the platelet production of thromboxane A2, an increase in platelet Factor X and Annexin V and increased platelet microparticles, all suggesting increased platelet activation(280). Fischer et al performed a series of experiments to determine the mechanical events that lead to platelet activation when exposed to algae-derived pGlcNAc and concluded that GP1b, a platelet protein that ordinarily binds to von Willebrand's factor, binds strongly to the pGlcNAc fibres to prevent platelet shearing and allow further binding to fibrin(194). Follow up work by the same group using thromboelastography demonstrated that beta pGlcNAc (marine algae source) accelerated fibrin polymerisation to the extent that it negated the effect of Glycoprotein IIbIIa inhibition(192, 280, 354). Jegatheeswaran and colleagues performed in vivo

experiments to determine the effect of pGlcNAc- derived chitosan on haemostasis in an ovine hepatic injury(367). They performed partial liver injuries and subsequently hepatectomies on pigs and demonstrated a non-significant decrease in time to haemostasis when compared with manual pressure (control) with a median time of 2 minutes less in the chitosan group(367). Horio et al performed a similar experiment on liver injury in rats, comparing hydrogel-mixed chitosan sponges to fibrin/thrombin patches (TachoComb)(368). They demonstrated no difference in haemostatic efficacy between these groups in non-heparinised rats however the chitosan sponges had a significantly higher haemostatic efficacy in the heparinised rats, with all rats in this group achieving haemostasis within 5 minutes and all rats in the control group exsanguinating(368). Chitosan is thought to increase granulation around wound sites. As it is broken down, it depolymerises and releases N-acetyl-D-glucosamine which stimulates fibroblasts and increases hyaluronic acid production at the wound site(355, 361, 370). This allows increased delivery of oxygen to surrounding tissues and increased neutrophil activation(200). Whilst chitin is derived from shellfish, there has not been a reported case of allergic reaction to purified chitin/chitosan in either humans or animals(362, 373, 381).

Conclusion

This trial has shown the efficacy of ready-to-apply patches for hemorrhage control in cases of carotid injury. These patches add to the skull base surgeon's armamentarium when dealing with potentially catastrophic arterial bleeding. They appear to be safe, efficacious and provide long term haemostasis out to three months with no significantly increased risk of pseudoaneurysm formation over muscle patch or anastoclip.

References

1. Vaz-Guimaraes F, Su SY, Fernandez-Miranda JC, Wang EW, Snyderman CH, Gardner PA. Hemostasis in Endoscopic Endonasal Skull Base Surgery. J Neurol Surg B Skull Base. 2015;76(4):296-302.
2. Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. Neurosurgery. 2013;73(2 Suppl Operative):ons261-9; discussion ons9-70.
3. Padhye VV, R; Wormald, PJ. Management of Carotid Artery Injury in Endonasal Surgery. Int Arch Otorhinolaryngol 2014;18(S02):S173-S8.
4. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. Int Forum Allergy Rhinol. 2011;1(2):118-22.
5. Valentine R, Wormald PJ. A Vascular Catastrophe during Endonasal Surgery: An Endoscopic Sheep Model. Skull Base. 2011;21(2):109-14.
6. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. Laryngoscope. 2011;121(3):562-6.
7. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. Otolaryngol Clin North Am. 2011;44(5):1059-79.
8. Berker M, Aghayev K, Saatci I, Palaoglu S, Onerci M. Overview of vascular complications of pituitary surgery with special emphasis on unexpected abnormality. Pituitary. 2010;13(2):160-7.
9. Biswas D, Daudia A, Jones NS, McConachie NS. Profuse epistaxis following sphenoid surgery: a ruptured carotid artery pseudoaneurysm and its management. J Laryngol Otol. 2009;123(6):692-4.
10. Fu M, Patel T, Baehring JM, Bulsara KR. Cavernous carotid pseudoaneurysm following transsphenoidal surgery. J Neuroimaging. 2013;23(3):319-25.
11. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. Int Forum Allergy Rhinol. 2015;5(3):253-7.
12. Padhye V, Valentine R, Paramasivan S, Jardeleza C, Bassiouni A, Vreugde S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. Int Forum Allergy Rhinol. 2014;4(8):651-7.
13. Padhye V, Naidoo Y, Alexander H, Floreani S, Robinson S, Santoreneos S, et al. Endoscopic endonasal resection of anterior skull base meningiomas. Otolaryngol Head Neck Surg. 2012;147(3):575-82.
14. Matonick JP, Hammond J. Hemostatic efficacy of EVARREST, Fibrin Sealant Patch vs. TachoSil(R) in a heparinized swine spleen incision model. J Invest Surg. 2014;27(6):360-5.
15. Bivalacqua TJ, Guzzo TJ, Schaeffer EM, Gebska MA, Champion HC, Burnett AL, et al. Application of Evicel to cavernous nerves of the rat does not influence erectile function in vivo. Urology. 2008;72(5):1169-73.
16. Budde S, Noll Y, Zieglschmid V, Schroeder C, Koch A, Windhagen H. Determination of the efficacy of EVICEL on blood loss in orthopaedic surgery after total knee replacement: study protocol for a randomised controlled trial. Trials. 2015;16:299.
17. Hickerson WL, Nur I, Meidler R. A comparison of the mechanical, kinetic, and biochemical properties of fibrin clots formed with two different fibrin sealants. Blood Coagul Fibrinolysis. 2011;22(1):19-23.

18. Yang MB, Melia M, Lambert SR, Chiang MF, Simpson JL, Buffenn AN. Fibrin glue for closure of conjunctival incision in strabismus surgery: a report by the american academy of ophthalmology. Ophthalmology. 2013;120(9):1935-41.
19. Kivelev J, Gohre F, Niemela M, Hernesniemi J. Experiences with TachoSil(R) in microneurosurgery. Acta Neurochir (Wien). 2015;157(8):1353-7; discussion 7.
20. Alizadeh Ghavidel A, Mirmesdagh Y, Samiei N, Gholampour Dehaki M. Haemostatic Role of TachoSil Surgical Patch in Cardiac Surgery. J Cardiovasc Thorac Res. 2014;6(2):91-5.
21. Siddiqui S, Keita L, Veerasingham D. Successful management of penetrating cardiac injury using TachoSil®. Injury Extra. 2012;43(12):142-3.
22. Celiento M, Scioti G, Pratali S, Bortolotti U. Repair of coronary artery perforation following angioplasty using TachoSil patches. Interact Cardiovasc Thorac Surg. 2010;10(2):328-30.
23. Agger P, Langhoff J, Smerup MH, Hasenkam JM. Comparison between TachoComb and TachoSil for surgical hemostasis in arterial bleeding: an animal experimental study. J Trauma. 2010;68(4):838-42.
24. Hajj-Chahine J, Jayle C, Tomasi J, Corbi P. TachoSil to prevent postoperative pericardial adhesions. Ann Thorac Surg. 2014;97(1):378-9.
25. Kuschel TJ, Gruszka A, Hermanns-Sachweh B, Elyakoubi J, Sachweh JS, Vazquez-Jimenez JF, et al. Prevention of postoperative pericardial adhesions with TachoSil. Ann Thorac Surg. 2013;95(1):183-8.
26. Antonelli A, Minervini A, Mari A, Bertolo R, Bianchi G, Lapini A, et al. TriMatch comparison of the efficacy of FloSeal versus TachoSil versus no hemostatic agents for partial nephrectomy: results from a large multicenter dataset. Int J Urol. 2015;22(1):47-52.
27. Grottke O, Braunschweig T, Daheim N, Coburn M, Grieb G, Rossaint R, et al. Effect of TachoSil in a coagulopathic pig model with blunt liver injuries. J Surg Res. 2011;171(1):234-9.
28. Dregelid EB, Pedersen G. Treatment of major vein injury with the hemostatic fleece TachoSil by interposing a peritoneal patch to avoid vein thrombosis: A feasibility study in pigs. J Emerg Trauma Shock. 2011;4(1):77-81.
29. Santulli P, Marcellin L, Touboul C, Ballester M, Darai E, Rouzier R. Experience with TachoSil in obstetric and gynecologic surgery. Int J Gynaecol Obstet. 2011;113(2):112-5.
30. Raga F, Sanz-Cortes M, Bonilla F, Casan EM, Bonilla-Musoles F. Reducing blood loss at myomectomy with use of a gelatin-thrombin matrix hemostatic sealant. Fertil Steril. 2009;92(1):356-60.
31. De Rosa P, Valeriani G, Barbato G, Cerbone V, Ciccone M, Russo E, et al. Postexplant residual cavity hemostasis with a TachoSil patch. Transplant Proc. 2011;43(4):1069-71.
32. De Rosa P, Russo E, Altieri R, Cerbone V, Papale PA, Santopietro A, et al. Saccular aneurysm of graft renal artery: case report. Transplant Proc. 2011;43(4):1213-4.
33. Sagalowsky AI. Editorial Comment on: Efficacy and safety of TachoSil as haemostatic treatment versus standard suturing in kidney tumour resection: a randomised prospective study. Eur Urol. 2007;52(4):1162-3.
34. Siemer S, Lahme S, Altziebler S, Machtens S, Strohmaier W, Wechsel HW, et al. Efficacy and safety of TachoSil as haemostatic treatment versus standard suturing in kidney tumour resection: a randomised prospective study. Eur Urol. 2007;52(4):1156-63.
35. Maplethorpe C. STN125351/172 TachoSil (Fibrin Sealant Patch) (Takeda Pharma A/S) as an adjunct to hemostasis for adult and pediatric hepatic resection surgery: FDA; 2014 [Available from:

http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/UCM458010.pdf.
36. Birth M, Figueras J, Bernardini S, Troen T, Gunther K, Mirza D, et al. Collagen fleece-bound fibrin sealant is not associated with an increased risk of thromboembolic events or major bleeding after its use for haemostasis in surgery: a prospective multicentre surveillance study. Patient Saf Surg. 2009;3(1):13.
37. Chalmers RT, Darling Iii RC, Wingard JT, Chetter I, Cutler B, Kern JA, et al. Randomized clinical trial of tranexamic acid-free fibrin sealant during vascular surgical procedures. Br J Surg. 2010;97(12):1784-9.
38. Vaiman M. Effectiveness of second-generation fibrin glue in endonasal operations. Otolaryngology - Head and Neck Surgery. 2002;126(4):388-91.
39. Vaiman M. Effect of Modern Fibrin Glue on Bleeding after Tonsillectomy and Adenoidectomy. Ann otot Rhinol Larvngol 2003;112:410-4.
40. Vaiman M. Use of Fibrin Glue as a hemostatic in Endoscopic sinus surgery. Ann otot Rhinol Larvngol. 2005;114:237-41.
41. Pusateri AE, Modrow HE, Harris RA, Holcomb JB, Hess JR, Mosebar RH, et al. Advanced hemostatic dressing development program: animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in Swine. J Trauma. 2003;55(3):518-26.
42. Jewelewicz DD, Cohn SM, Crookes BA, Proctor KG. Modified rapid deployment hemostat bandage reduces blood loss and mortality in coagulopathic pigs with severe liver injury. J Trauma. 2003;55(2):275-80; discussion 80-1.
43. Okamoto Y, Yano R, Miyatake K, Tomohiro I, Shigemasa Y, Minami S. Effects of chitin and chitosan on blood coagulation. Carbohydrate Polymers. 2003;53(3):337-42.
44. Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science. 2006;31(7):603-32.
45. Agrawal P, Soni S, Mittal G, Bhatnagar A. Role of polymeric biomaterials as wound healing agents. Int J Low Extrem Wounds. 2014;13(3):180-90.
46. Jegatheeswaran S, Bhanot U, Siriwardena AK. In vivo evaluation of the chitosan-based haemostatic agent Omni-stat(R) in porcine liver resection and in liver injury. Eur Surg Res. 2012;49(2):73-9.
47. Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H. Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv. 2011;29(3):322-37.
48. Kadokawa J-i, Takegawa A, Mine S, Prasad K. Preparation of chitin nanowhiskers using an ionic liquid and their composite materials with poly(vinyl alcohol). Carbohydrate Polymers. 2011;84(4):1408-12.
49. Fischer TH, Bode AP, Demcheva M, Vournakis JN. Hemostatic properties of glucosamine-based materials. J Biomed Mater Res A. 2007;80(1):167-74.
50. Whang HS, Kirsch W, Zhu YH, Yang CZ, Hudson SM. Hemostatic Agents Derived from Chitin and Chitosan. Journal of Macromolecular Science, Part C: Polymer Reviews. 2005;45(4):309-23.
51. Loo Y, Zhang S, Hauser CA. From short peptides to nanofibers to macromolecular assemblies in biomedicine. Biotechnol Adv. 2012;30(3):593-603.
52. Thatte HS, Zagarins S, Khuri SF, Fischer TH. Mechanisms of Poly-N-Acetyl Glucosamine Polymer???Mediated Hemostasis: Platelet Interactions. The Journal of Trauma: Injury, Infection, and Critical Care. 2004;57(Supplement):S13-S21.

53. Valeri CR, Srey R, Tilahun D, Ragno G. In Vitro Effects of Poly-N-Acetyl Glucosamine on the Activation of Platelets in Platelet-Rich Plasma with and without Red Blood Cells. The Journal of Trauma: Injury, Infection, and Critical Care. 2004;57(Supplement):S22-S5.
54. Fischer TH, Thatte HS, Nichols TC, Bender-Neal DE, Bellinger AD, Vournakis JN. Synergistic platelet integrin signaling and factor XII activation in poly-N-acetyl glucosamine fiber-mediated hemostasis. Biomaterials. 2005;26(27):5433-43.
55. Fischer TH, Hays WE, Valeri CR. Poly-N-acetyl glucosamine fibers accelerate hemostasis in patients treated with antiplatelet drugs. J Trauma. 2011;71(2 Suppl 1):S176-82.
56. White JG. Platelets. 3rd ed. Minnesota Elsevier; 2013.
57. Horio T, Ishihara M, Fujita M, Kishimoto S, Kanatani Y, Ishizuka T, et al. Effect of photocrosslinkable chitosan hydrogel and its sponges to stop bleeding in a rat liver injury model. Artif Organs. 2010;34(4):342-7.
58. Jayakumar R, Prabaharan M, Nair SV, Tamura H. Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol Adv. 2010;28(1):142-50.
59. Reys LL, Silva SS, Oliveira JM, Caridade SG, Mano JF, Silva TH, et al. Revealing the potential of squid chitosan-based structures for biomedical applications. Biomed Mater. 2013;8(4):045002.
60. Muzzarelli RA. Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. Mar Drugs. 2010;8(2):292-312.
61. Muzzarelli RAA. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydrate Polymers. 2009;76(2):167-82.
62. Turner P, Ng I, Kemp A, Campbell D. Seafood allergy in children: a descriptive study. Ann Allergy Asthma Immunol. 2011;106(6):494-501.

Chapter 4

Flow cytometry study to quantify platelet activation by crushed and non-crushed muscle supernatant and fibrin/thrombin patches

Platelet activation by crushed and uncrushed muscle: a flow cytometry analysis

Jukes, Alistair; Miljkovic, Dijana; Wormald, P. J.; Psaltis, Alkis J.
International Forum of Allergy & Rhinology, September 2017, Vol.7(9), pp.916-919 [Peer Reviewed Journal]

Statement of Authorship

Title of Paper	Platelet activation by crushed and uncrushed muscle: a flow cytometry analysis
Publication Status	[x] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	Jukes, Alistair ; Miljkovic, Dijana ; Wormald, P. J. ; Psaltis, Alkis J. International Forum of Allergy & Rhinology, September 2017, Vol.7(9), pp.916-919 [Peer Reviewed Journal]

Principal Author

Name of Principal Author (Candidate)	Jukes, Alistair		
Contribution to the Paper	Study design, Ethics application, study conduct and data collection, data analysis, manuscript writing and preparation		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	1/12/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Miljkovic, Dijana		
Contribution to the Paper	Study design, Study conduct and data collection, manuscript writing and preparation		
Signature		Date	4/12/17

Name of Co-Author	Psaltis, Alkis		
Contribution to the Paper	Study design, manuscript editing		
Signature		Date	2/12/17

Name of Co-Author	Wormald, Peter-John		
Contribution to the Paper	Study design and manuscript editing		
Signature		Date	1/12/17

Platelet activation by crushed and uncrushed muscle: a flow cytometry analysis

Alistair Jukes, MBBS(Hons) B.LibStud[1,2,3], Dijana Miljkovic, BMedPharmBtech PhD[1,3], P.J. Wormald, MD FRACS[1,3] and Alkis J. Psaltis, MD PhD FRACS[1,3]

Background: Crushed autologous muscle is used in skull base surgery in the acute phase of major arterial hemorrhage to stop bleeding. The mechanism of this is not yet clear, but is thought to involve the formation of a platelet plug, which seals the vessel wall defect but still allows ongoing blood flow to the brain.

Methods: In this study we use flow cytometry to replicate the in-vivo actions of crushed muscle on platelets in whole blood. We compare the ratio of activation of platelets exposed to crushed and uncrushed muscle supernatant in control patients and in patients on antiplatelet agents.

Results: Crushed muscle activated platelets to a higher degree than uncrushed muscle: 5.18-fold greater in control blood ($p = 0.002$); 6.53-fold greater in aspirin-exposed blood ($p < 0.0001$); and 9.4-fold greater in clopidogrel-exposed blood ($p < 0.0001$).

Conclusion: Crushed muscle caused a consistently increased ratio of platelet activation when compared with uncrushed muscle across all groups, adding to the evidence that at least part of its clinical effect is the result of platelet activation.

Key Words:
anterior skull base; endoscopic skull base surgery; endoscopic minimally invasive surgery of the skull base; endoscopy; hemorrhagic disorders

***How to Cite this Article*:**
Jukes A, Miljkovic D, Wormald PJ, Psaltis AJ. Platelet activation by crushed and uncrushed muscle: a flow cytometry analysis. *Int Forum Allergy Rhinol*. 2017;XX:1-4.

Crushed autologous muscle is a known adjunct to major arterial hemorrhage control in a number of surgical specialties. It is currently the "gold standard" in controlling cavernous-segment carotid artery bleeding during endoscopic skull base surgery. In this setting the constrained anatomy of the skull base precludes direct vessel closure with sutures or other devices. The incidence of bleeding ranges from 0.16% to 1.1% in uncomplicated pituitary surgery to 5% to 9% in extended, endonasal approaches to the skull base.[1,2] Previously published research from our department has demonstrated that applying crushed muscle patches to carotid artery injuries results in decreased blood volume loss and faster time to hemostasis when compared with chitosan patches and surgical clips in preclinical models.[3–7] There are a number of case reports of the use of crushed muscle patches in potentially life-threatening carotid injury.[8–11] The mechanism for this hemostatic effect is unknown. It is thought that crushed muscle releases factors that induce platelet activation and aggregation, which is then is assisted by the formation of a fibrin scaffold where procoagulant factors are trapped and subsequently activated.[12] This forms a "platelet plug" over the artery wall defect. We hypothesize that this is due to release of factors from crushed vessel walls and collagen within the muscle patch. In an effort to demonstrate this effect in vitro, Rajiv and colleagues used platelet aggregrometry to demonstrate significantly increased amounts of platelet aggregation with increasing concentrations of muscle supernatant (ranging from 0.1 to 0.8 mg/mL).[12] Flow cytometry provides a better picture of in-vivo platelet activity as it analyses whole blood. In view of the findings by Rajiv et al, we hypothesized that crushed muscle would activate a higher percentage of platelets than uncrushed muscle.

[1]*Department of Otolaryngology, Head and Neck Surgery, Queen Elizabeth Hospital, Woodville South, Adelaide, SA, Australia;* [2]*Department of Neurosurgery, Royal Adelaide Hospital, Adelaide, SA, Australia;* [3]*Department of Medicine, University of Adelaide, Queen Elizabeth Hospital, Woodville South, Adelaide, SA, Australia*

Correspondence to: Alistair K. Jukes, MBBS(Hons) B.LibStud, Department of Otolaryngology, Head and Neck Surgery, Level 3, Queen Elizabeth Hospital, 28 Woodville Road, Woodville South, 5011, Australia; e-mail: alistair.jukes@adelaide.edu.au

Potential conflict of interest: P.J.W.: Medtronic ENT, royalties for instruments designed; Neilmed, consultant.

Received: 4 March 2017; Revised: 11 May 2017; Accepted: 23 May 2017
DOI: 10.1002/alr.21977
View this article online at wileyonlinelibrary.com.

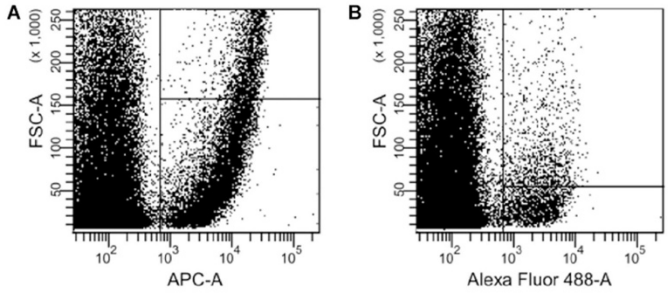


FIGURE 1. Platelets identified by CD42 APC (A) and activated platelets by PAC-1 Alexa Fluor 488 (B).

Methods

Patient sample collection

This study was approved by the human research ethics committee of the Queen Elizabeth Hospital, Adelaide, Australia. All patients provided informed written consent prior to enrollment. Blood specimens were prospectively collected from 30 patients. Patients were divided into those not taking antiplatelet medications for at least 3 months (controls) and those on antiplatelet medications, clopidogrel or aspirin (blood was collected from patients already taking aspirin or clopidogrel who had not undergone a diagnosed ischemic event in the previous 3 months). After discarding the first 3 mL of blood, samples were collected in ethylene-diamine tetraacetic acid (EDTA) tubes and then prepared within 5 minutes of collection.

Crushed muscle preparation

Fresh human muscle was obtained from the viable proximal region of below-knee amputation specimens at the Queen Elizabeth Hospital. The muscle tissue was immediately divided into 1-g blocks and either crushed immediately between 2 kidney dishes for 5 seconds or left whole and placed in 1 mL of phosphate-buffered saline (PBS). Both samples were then agitated for 5 minutes and the macroscopic muscle component removed. Supernatant was stored at −80°C until use.

Flow cytometry

Fifty microliters of muscle supernatant, adenosine diphosphate (ADP) or PBS, was incubated at room temperature for 5 minutes with 0.45 mL of blood per sample. Samples were then stained for 15 minutes at room temperature with CD62 (APC, Clone AK-4) to define unactivated platelets and PAC-1 (Alexa Fluor 488 Clone PAC-1) to determine activated platelets (Becton Dickinson Biosciences, San Jose, CA). Gates were based on fluorescence minus 1 (FMO) controls (Fig. 1).

We sought to identify whether exposure to crushed and uncrushed muscle supernatant results in direct platelet activation by using flow cytometry to examine whole blood exposed to each of these. To determine the potential inhibitory effect of commonly prescribed antiplatelet agents on this interaction, we also exposed blood from separate groups of patients taking clopidogrel (a thienopyridine-class ADP receptor antagonist) and aspirin (a cyclooxygenase-1 [COX-1] inhibitor) to the crushed and uncrushed supernatants. The ratio of platelets activated in each group along with the total percentage of platelets activated when crushed was compared with those uncrushed. Ratios of the mean percentage activation in each blood group between each hemostatic agent were calculated according to the geometric mean with lower and upper limits representing the 95% confidence interval (CI).

Results

Platelet activation

Crushed muscle activated platelets to a higher degree than uncrushed muscle: 5.18-fold greater in control blood ($p = 0.002$); 6.53-fold greater in aspirin-exposed blood ($p < 0.0001$); and 9.4-fold greater in clopidogrel-exposed blood ($p < 0.0001$) (Table 1).

Total percentage activation findings show that rushed muscle caused a mean platelet activation of 6.63% (95% CI, 0.55-12.71) in control blood, 31.38% (95% CI, 14.22-48.54) in aspirin-exposed blood, and 26.08% (95% CI, 16.02-36.14) in clopidogrel-exposed blood (Fig. 2 and Table 2).

Uncrushed muscle caused a mean platelet activation of 0.99% (95% CI, 0.44-2.42) in control blood, 5.84%

TABLE 1. Ratios of mean percentage activation in each blood group between each hemostatic agent

Group	Agent 1	Agent 2	Mean ratio (95% CI)	*p* value
Aspirin	Crushed	Uncrushed	6.53 (3.00 to 14.22)	<0.0001
Clopidogrel	Crushed	Uncrushed	9.40 (4.30 to 20.53)	<0.0001
Normal	Crushed	Uncrushed	5.18 (1.90 to 14.16)	0.002

CI = confidence interval.

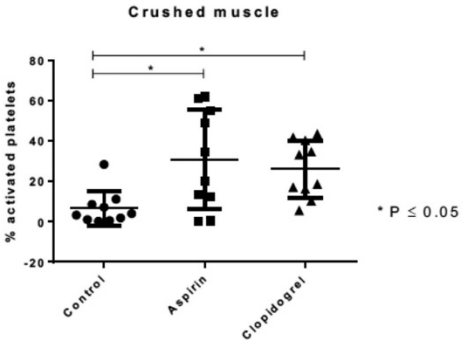


FIGURE 2. Percentage of activated platelets divided by blood cohort.

TABLE 2. Mean percentage platelet activation divided by hemostatic agent

Group	Presentation	Mean (95% CI)	Median (95% CI)
Aspirin	ADP	45.46 (16.80 to 74.12)	54.50 (7.4 to 68.0)
Aspirin	Crushed	31.38 (14.22 to 48.54)	27.40 (0.2 to 62.0)
Aspirin	Unactivated	15.94 (4.80 to 27.08)	11.90 (0.3 to 36.8)
Aspirin	Uncrushed	5.84 (0.31 to 11.37)	1.90 (0.4 to 23.4)
Clopidogrel	ADP	38.68 (14.03 to 63.33)	46.60 (5.2 to 54.6)
Clopidogrel	Crushed	26.08 (16.02 to 36.14)	25.85 (5.5 to 43.5)
Clopidogrel	Unactivated	10.01 (2.25 to 17.77)	7.55 (0.0 to 28.4)
Clopidogrel	Uncrushed	4.67 (0.40 to 8.94)	2.45 (0.1 to 19.5)
Normal	ADP	20.08 (9.21 to 30.95)	22.00 (1.1 to 52.3)
Normal	Crushed	6.63 (0.55 to 12.71)	3.65 (0.2 to 28.4)
Normal	Unactivated	0.78 (0.40 to 1.16)	0.75 (0.0 to 1.9)
Normal	Uncrushed	0.99 (−0.44 to 2.42)	0.20 (0.1 to 6.5)

ADP = adenosine diphosphate; CI = confidence interval.

(95% CI, 0.31-11.37) in aspirin-exposed blood, and 4.67% (95% CI, 0.40-8.94) in clopidogrel blood. ADP was used as a positive control. ADP exposure resulted in a mean platelet activation of 20.08% (95% CI, 9.21-30.95) in control blood, 45.46% (95% CI, 16.80-74.12) in aspirin-exposed blood, and 36.68% (95% CI, 14.03-63.33) in clopidogrel-exposed blood. "Unactivated" exposure, where blood was not exposed to any hemostatic agent but just labeled with antibodies, resulted in a mean platelet activation of 0.78% (95% CI, 0.40-1.16) in control blood, 15.94% (95% CI, 4.80-27.08) in aspirin-exposed blood, and 10.01% (95% CI, 2.25-17.77) in clopidogrel-exposed blood (Fig. 3).

Discussion

In this study we found that crushed muscle effectively activated platelets within whole blood at a ratio of between 5.18- and 9.40-fold greater than uncrushed muscle. Crushed muscle activated platelets to a higher degree than uncrushed muscle in both controls and patients taking platelet-inhibiting medications. This indicates a consistent effect even in the face of platelet activation pathway inhibition at 2 different points. There are multiple methods and phases of platelet activation but the most prominent is exposure of platelets to collagen, which is found almost everywhere in the body apart from the inner wall of the normal vascular endothelium. Platelets adhere to exposed collagen and extracellular membrane proteins on damaged endothelium despite extensive "shear stress" caused by rapidly flowing blood.[13,14] The platelets do this via a number of mechanisms, the most prominent being glycoprotein Ib-IX (GPIb-IX) binding to von Willebrand factor (vWF) and glycoprotein IIbIIIa (GPIIbIIIa) binding to fibrin and fibrinogen.[15] Crushing muscle likely results in the release of large amounts of prothrombotic factors, such as collagen, to platelets in the vicinity of the arterial injury, allowing subsequent activation. This effect appears to be reflected in surgical practice where crushed muscle (generally vastus or rectus muscle) has proven to be lifesaving in a number of instances in which autologous muscle was harvested emergently, crushed, and applied to the bleeding point.[2,6,16,17]

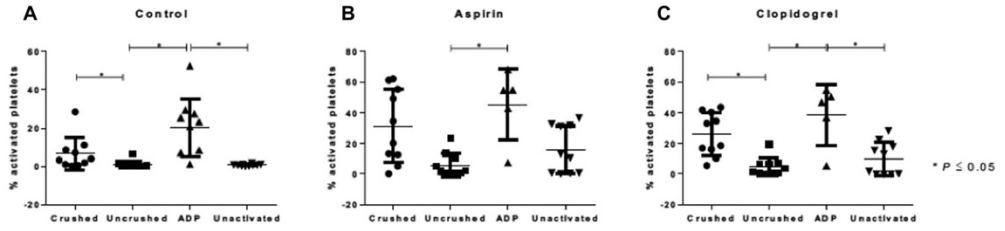


FIGURE 3. Percentage of activated platelets (PAC1+) out of total platelets (CD42+), in (A) control patients, (B) patients on aspirin, and (C) patients on clopidogrel in crushed, uncrushed, and ADP-activated and -unactivated patient samples. Data expressed as median with interquartile range. $p \leq 0.05$, Kruskal-Wallis test.

Our group has developed a preclinical ovine model of endoscopic carotid artery hemorrhage control for surgeons. In this model, the carotid artery of an anesthetized Merino sheep is dissected free of surrounding adventitia and a modified Sinus Model Otorhino Neuro Trainer (SIMONT; Pro Delphus, Pernambuco, Brazil) is placed over this artery. An incision is made in the artery, and surgeons, who are trained to control the bleeding vessel, harvest muscle from the neck or thigh, crush it, and apply it to the bleeding artery. We have found that crushing the muscle provides a significantly reduced time to bleeding control, lower total blood volume loss, and less hemodynamic deterioration in the animal when compared with noncrushed muscle.[4,7,16,18,19]

Although the ratios of activation between crushed and uncrushed muscle remained somewhat consistent between groups in our study, the absolute value of the percentage of platelets activated was increased in the aspirin and clopidogrel cohorts. We included these commonly prescribed antiplatelet agents as there are instances where operations may need to be performed without the usual medical cessation period (such as in the case of pituitary apoplexy with visual impairment).[20–26]

It should be reiterated that platelet transfusions do not entirely reverse or negate the effect of antiplatelet agents.[27,28] Blood was collected from patients already taking aspirin or clopidogrel and who had not undergone a diagnosed ischemic event in the previous 3 months. Patients were not placed on these medications solely for the purpose of our study. It is possible that these patients may have had more reactive platelets, predisposing them to a thromboembolic event, and therefore already had a higher circulating percentage of activated platelets. This may have accounted for the higher absolute levels of platelet activation seen in our study. We therefore chose to report both absolute values and ratios. Our results may be reflective of real-world practice, as patients often have multiple medical comorbidities when they come to surgery.

Conclusion

Exposing whole blood to crushed muscle supernatant caused platelet activation at a ratio of between 5.18- and 9.40-fold greater than uncrushed muscle supernatant, indicating the release of platelet-activating factors by this crushing. We hypothesize that there are both intracellular and extracellular factors at play. The extracellular proteins exposed to whole blood may be insufficient to activate platelets and the exposure to intracellular components that occurs when cells are crushed increases platelet activation. These components, likely collagen fibers and cytoplasmic proteins, are usually shielded from circulating blood, so their exposure renders platelets more active and thrombogenic. The higher absolute percentage of platelets activated in the clopidogrel and aspirin groups may be reflective of an innate propensity of these patients to arterial thrombosis; however, it also suggests that the crushed muscle patch may still provide some in-vivo platelet-activating effect in the face of what is, ostensibly, significant platelet inhibition. It would be of benefit to surgeons and patients alike if a component of crushed muscle could be isolated that is efficacious in activating platelets. Further research is required to determine the precise components released by crushed muscle that cause platelet activation and whether these may be isolated or synthesized for clinical use.

References

1. Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. *Neurosurgery*. 2013;73:261.
2. AlQahtani A, Castelnuovo P, Nicolai P, Prevedello DM, Locatelli D, Carrau RL. Injury of the internal carotid artery during endoscopic skull base surgery: prevention and management protocol. *Otolaryngol Clin North Am*. 2016;49:237–252.
3. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. *Int Forum Allergy Rhinol*. 2011;1:118–122.
4. Valentine R, Wormald PJ. A vascular catastrophe during endonasal surgery: an endoscopic sheep model. *Skull Base*. 2011;21:109–114.
5. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. *Laryngoscope*. 2011;121:562–566.
6. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. *Otolaryngol Clin North Am*. 2011;44:1059–1079.
7. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. *Int Forum Allergy Rhinol*. 2015;5:253–257.
8. Weidenbecher M, Huk WJ, Iro H. Internal carotid artery injury during functional endoscopic sinus surgery and its management. *Eur Arch Otorhinolaryngol*. 2005;262:640–645.
9. Koitschev A, Simon C, Lowenheim H, Naegele T, Ernemann U. Management and outcome after internal carotid artery laceration during surgery of the paranasal sinuses. *Acta Otolaryngol*. 2006;126:730–738.
10. Gardner PA, Tormenti MJ; Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. *Neurosurgery*. 2013;73:261–269.
11. Fu M, Patel T, Baehring JM, Bulsara KR. Cavernous carotid pseudoaneurysm following transsphenoidal surgery. *J Neuroimaging*. 2013;23:319–325.
12. Rajiv S, Rodgers S, Bassiouni A, Vreugde S, Wormald PJ. Role of crushed skeletal muscle extract in hemostasis. *Int Forum Allergy Rhinol*. 2015;5:431–434.
13. Perzborn E, Heitmeier S, Laux V. Effects of Rivaroxaban on platelet activation and platelet-coagulation pathway interaction: in vitro and in vivo studies. *J Cardiovasc Pharmacol Ther*. 2015;20:554–562.
14. Evangelista V, Smyth SS. *Interactions between platelets, leukocytes and the endothelium*. 2013:295–312.
15. Kamath S, Blann AD, Lip GY. Platelet activation: assessment and quantification. *Eur Heart J*. 2001;22:1561–1571.
16. Padhye V, Valentine R, Paramasivan S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. *Int Forum Allergy Rhinol*. 2014;4:651–657.
17. Chin OY, Ghosh R, Fang CH, Baredes S, Liu JK, Eloy JA. Internal carotid artery injury in endoscopic endonasal surgery: a systematic review. *Laryngoscope*. 2016;126:582–590.
18. Padhye V, Naidoo Y, Alexander H, et al. Endoscopic endonasal resection of anterior skull base meningiomas. *Otolaryngol Head Neck Surg*. 2012;147:575–582.
19. Padhye VV, R, Wormald, PJ. Management of carotid artery injury in endonasal surgery. *Int Arch Otorhinolaryngol*. 2014;18(suppl):S173–S178.
20. Berker M, Aghayev K, Saatci I, Palaoglu S, Onerci M. Overview of vascular complications of pituitary surgery with special emphasis on unexpected abnormality. *Pituitary*. 2010;13:160–167.
21. Chuang CC, Chen YL, Pai PC. A giant intracavernous carotid artery aneurysm embedded in pituitary macroadenoma presenting with pituitary apoplexy. *Cerebrovasc Dis*. 2006;21:142–144.
22. Fyrmpas G, Constantinidis J, Foroglou N, Selviaridis P. Pituitary apoplexy following endoscopic sinus surgery. *J Laryngol Otol*. 2010;124:677–679.
23. Harrold C, Randeva H. Conservative versus surgical decompression for pituitary. *Apoplexy*. 2014:13–17.
24. Razak AA, Horridge M, Connolly DJ, et al. Comparison of endoscopic and microscopic trans-sphenoidal pituitary surgery: early results in a single centre. *Br J Neurosurg*. 2013;27:40–43.
25. Takeda N, Fujita K, Katayama S, Akutu N, Hayashi S, Kohmura E. Effect of transsphenoidal surgery on decreased visual acuity caused by pituitary apoplexy. *Pituitary*. 2010;13:154–159.
26. Zhang X, Zhang W, Fu LA, et al. Hemorrhagic pituitary macroadenoma: characteristics, endoscopic endonasal transsphenoidal surgery, and outcomes. *Ann Surg Oncol*. 2011;18:246–252.
27. Kimpton G, Dabbous B, Leach P. New oral anticoagulant and antiplatelet agents for neurosurgeons. *Br J Neurosurg*. 2015;29:614–621.
28. Herbstreit FP. Spinal anaesthesia despite combined clopidogrel and aspirin therapy in a patient awaiting lung transplantation: effects of platelet transfusion on clotting tests. *Anaesthesia*. 2005;60:85–87.

Platelet activation by crushed and uncrushed muscle. A flow cytometry analysis

Alistair Jukes[1,2,3], Dijana Miljkovic[1,3], PJ Wormald[1,3], Alkis J. Psaltis[1,3]

1. Department of Otolaryngology, Head and Neck Surgery. Queen Elizabeth Hospital, Woodville South, Adelaide, Australia
2. Department of Neurosurgery, Royal Adelaide Hospital, Adelaide, Australia
3. Department of Medicine - The University of Adelaide, Queen Elizabeth Hospital, Woodville South, Adelaide, Australia

Short title: Platelet activation by crushed muscle

Keywords:

Endoscopic minimally invasive surgery of the skull base

Endoscopic Skull Base Surgery

Haemorrhagic Disorders

Anterior skull base

Endoscopy

Corresponding author:
Dr Alistair K Jukes
Department of Otolaryngology, Head and Neck Surgery
Level 3
Queen Elizabeth Hospital, 28 Woodville Rd, Woodville South, 5011, Australia
Phone +61 08 82227158
Fax +61 08 82227419
alistair.jukes@adelaide.edu.au

Disclosures of potential conflicts of interest: PJ Wormald receives royalties from Medtronic ENT for instruments designed and is a consultant for Neilmed.

There are no external funding sources to declare

Abstract:

Background: Crushed autologous muscle is used in skull base surgery in the acute phase of major arterial hemorrhage to stop bleeding. The mechanism of this is not yet clear but is thought to involve the formation of a platelet plug, that seals the vessel wall defect but still allows ongoing blood flow to the brain.

Methods: This study uses flow cytometry to attempt to replicate the in vivo actions of crushed muscle on platelets in whole blood. It compares the ratio of activation of platelets exposed to crushed and uncrushed muscle supernatant in control patients and those on antiplatelet agents.

Results: Crushed muscle activated platelets to a higher degree than uncrushed muscle. This ratio was 5.18 times greater in control blood ($P=0.002$), 6.53 times greater in aspirin-exposed blood ($P<0.0001$) and 9.4 times greater in clopidogrel exposed blood ($P<0.0001$).

Conclusion: Crushed muscle caused a consistently increased ratio of platelet activation when compared with uncrushed muscle across all groups, adding to the evidence that at least part of its clinical effect is the result of platelet activation.

Introduction:

Crushed autologous muscle is a known adjunct to major arterial hemorrhage control in a number of surgical specialties. It is currently the gold standard in controlling cavernous-segment carotid artery bleeding during endoscopic skull base surgery. Here the constrained anatomy of the skull base precludes direct vessel closure with sutures or other devices. The incidence of bleeding ranges from 0.16-1.1% in uncomplicated pituitary surgery to 5-9% in extended, endonasal approaches to the skull base(125, 138). Previously published research from our department demonstrates that applying crushed muscle patches to carotid artery

injuries results in decreased blood volume loss and faster time-to-hemostasis when compared with chitosan patches and surgical clips in pre-clinical models(117-120, 122). There are a number of case reports of its use in potentially life-threatening carotid injury(130, 142, 167, 532). The mechanism for this hemostatic effect is unknown. It is thought that crushed muscle releases factors that induce platelet activation and aggregation, which is then is assisted by the formation of a fibrin scaffold in which pro-coagulant factors are trapped and subsequently activated(145). This then forms a 'platelet plug' over the artery wall defect. We hypothesise that this is due to release of factors from crushed vessel walls and collagen within the muscle patch. In an effort to demonstrate this effect in vitro, Rajiv and colleagues performed a study using platelet aggregrometry which demonstrated significantly increased amounts of platelet aggregation with increasing concentrations of muscle supernatant (ranging from 0.1mg/ml to 0.8mg/ml)(145). Flow cytometry provides a better picture of in vivo platelet activity as it analyses whole blood. Given the findings of Rajiv et al, we hypothesise that crushed muscle should activate a higher percentage or platelets than uncrushed muscle.

Methods:

Patient Sample Collection

This study was approved by the Human Research Ethics Committee of the Queen Elizabeth Hospital, Adelaide, Australia. All patients provided informed written consent prior to enrollment. Blood specimens were prospectively collected from 30 patients. Patients were divided into those not taking antiplatelet medications for at least three months(controls) and those on antiplatelet medications; clopidogrel or aspirin (Blood was collected from patients already taking aspirin or clopidogrel who had not undergone a diagnosed ischaemic

event in the previous 3 months). After discarding the first 3ml of blood, samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and then prepared within 5 minutes of collection.

Crushed Muscle Preparation

Fresh human muscle was obtained from the viable proximal region of below knee amputation specimens at the Queen Elizabeth Hospital. This was immediately divided into 1g blocks and either crushed immediately between two kidney dishes for five seconds or left whole and placed in 1ml phosphate buffered saline (PBS). Both samples were then agitated for 5 minutes, then the macroscopic muscle component removed. Supernatant was stored at -80*C until use.

Flow Cytometry

50μl of muscle supernatant, adenosine diphosphate (ADP) or PBS was incubated at room temperature for 5 minutes with 0.45ml of blood per sample. Samples were then stained for 15 minutes at room temperature with CD62 (APC, clone AK-4) to define unactivated platelets and PAC-1 (AF488 clone PAC-1) to determine activated platelets. (Becton Dickinson Biosciences, San Jose, CA, USA). Gates were based on Fluorescence minus one (FMO) Controls. (Fig. 1).

We sought to identify whether exposure to crushed and uncrushed muscle supernatant results in direct platelet activation, using flow cytometry to examine whole blood exposed to each of these. In order to determine the potential inhibitory effect of commonly prescribed anti-platelet agents on this interaction, we also exposed blood from separate groups of patients taking clopidogrel (a thienopyridine-class Adenosine Diphosphate (ADP)

receptor antagonist) and aspirin (a Cyclooxygenase-1 (COX-1) inhibitor) to the crushed and uncrushed supernatants. The ratio of platelets activated in each group along with the total percentage of platelets activated when crushed was compared to uncrushed**.** Ratios of the mean percentage activation in each blood group between each hemostatic agent were calculated according to the geometric mean with lower and upper limits representing the 95% confidence interval (95%CI).

Results:

Platelet Activation:

Crushed muscle activated platelets to a higher degree than uncrushed muscle at a ratio of 5.18 times greater in control blood (P=0.002), 6.53 times greater in aspirin-exposed blood (P<0.0001) and 9.4 times greater in clopidogrel exposed blood (P<0.0001) (Table 1)

Total Percentage activation: Crushed muscle caused a mean platelet activation of 6.63% (95% CI 0.55-12.71) in control blood, 31.38% (95% CI 14.22-48.54) in aspirin-exposed blood and 26.08% (95% CI 16.02-36.14) in clopidogrel-exposed blood (Figure 2) (Table 2).

Uncrushed muscle caused a mean platelet activation of 0.99% (95% CI-0.44-2.42) in control blood, 5.84% (95% CI 0.31-11.37) in aspirin-exposed blood and 4.67% (95% CI 0.40-8.94) in clopidogrel-blood. ADP was used as a positive control. ADP exposure resulted in a mean platelet activation of 20.08% (95%CI 9.21-30.95) in control blood, 45.46% (95%CI 16.80-74.12) in aspirin-exposed blood and 36.68% (95%CI 14.03-63.33) in clopidogrel-exposed blood. 'Unactivated' exposure where blood was not exposed to any hemostatic agent but just labelled with antibodies gave a mean platelet activation of 0.78% (95%CI 0.40-1.16) in control blood, 15.94% (95% CI 4.80-27.08) in aspirin-exposed blood and 10.01% (95% CI 2.25-17.77) in clopidogrel exposed blood (Figure 3).

Discussion:

In our study crushed muscle effectively activated platelets within whole blood at a ratio of between 5.18-9.40 times greater than uncrushed muscle. Crushed muscle activated platelets to a higher degree than uncrushed muscle in both control and in patients taking platelet inhibiting medications. This indicates a consistent effect even in the face of platelet activation pathway inhibition at two different points. There are multiple methods and phases of platelet activation but the most prominent is the exposure of platelets to collagen, which is found almost everywhere in the body apart from the inner wall of the normal vascular endothelium. Platelets adhere to exposed collagen and extracellular membrane proteins on damaged endothelium despite extensive 'shear stress' caused by rapidly flowing blood(190, 191). They do this via a number of mechanisms, the most prominent being Glycoprotein Ib-IX (GPIb-IX) binding to Von Willebrands factor (vWF) and Glycoprotein IIbIIIa (GPIIbIIIa) binding to fibrin and fibrinogen(189). Crushing muscle likely results in the release of large amounts of pro-thrombotic factors such as collagen to platelets in the vicinity of the arterial injury, allowing activation and subsequent activation. This effect appears to be reflected in surgical practice where crushed muscle (generally vastus or rectus muscle) has proven to be life-saving in a number of instances where autologous muscle has been harvested emergently, crushed and applied to the bleeding point(120, 121, 138, 139). Our group has developed a pre-clinical ovine model of endoscopic carotid artery hemorrhage control for surgeons. In this model, the carotid artery of an anaesthetised merino sheep is dissected free of surrounding adventitia and a modified Sinus Model Otorhino Neuro Trainer (SIMONT, Pro Delphus, Pernambuco, Brazil) is placed over this. An incision is made in the artery and surgeons are trained to control the bleeding vessel, harvest muscle from the neck or thigh, crush it, and apply it to the bleeding artery.

We have found that crushing the muscle provides a significantly reduced time to bleeding control, lower total blood volume loss and less haemodynamic deterioration in the animal when compared to non-crushed muscle(117, 121, 122, 128, 140). Whilst the ratios of activation between crushed and uncrushed remained somewhat consistent between groups, the absolute value of the percentage of platelets activated was increased in the aspirin and clopidogrel cohorts. We included these commonly prescribed anti-platelet agents as there are instances where operations may need to be performed without the usual medical cessation period (such as in the case of pituitary apoplexy with visual impairment)(39, 123, 137, 533-536). It should also be remembered that platelet transfusions do not entirely reverse or negate the effect of these antiplatelet agents(116, 537). Blood was collected from patients already taking aspirin or clopidogrel who had not undergone a diagnosed ischaemic event in the previous 3 months). They were not placed on these medications solely for the purpose of our study. it is possible that these patients may have had more reactive platelets, predisposing them to a previous thromboembolic event and therefore already had a higher circulating percentage of activated platelets. This may potentially account for the higher absolute levels of platelet activation seen in this study. We have therefore chosen to report both absolute values and ratios. Our results may be reflective of real world practice as patients may have multiple medical comorbidities when they come to surgery.

Conclusion:

Exposing whole blood to crushed muscle supernatant causes platelet activation at a ratio of between 5.18-9.40 times greater than uncrushed muscle supernatant indicating the release of platelet activating factors by this crushing. We hypothesise that there are both

intracellular and extracellular factors at play. The extracellular proteins that are exposed to whole blood may be insufficient to activate platelets and that the exposure to intracellular components that occurs when cells are crushed increases platelet activation. These components are usually shielded from circulating blood so their exposure renders platelets more active and thrombogenic. These components likely include collagen fibres and cytoplasmic proteins. The higher absolute percentage of platelets activated in the clopidogrel and aspirin groups may be reflective of an innate propensity of these patients to arterial thrombosis but it indicates that the crushed muscle patch may still provide some in vivo platelet activating effect in the face of what is, ostensibly, significant platelet inhibition. It would be of benefit to surgeons and patients alike if a component of crushed muscle could be isolated that was efficacious in activating platelets. Further research is required to determine the precise components released by crushed muscle that cause platelet activation and whether these may be isolated or synthesised for clinical use.

Group	*Agent 1*	*Agent 2*	*Mean ratio (95% CI)*	*p*
Aspirin	Crushed	Uncrushed	6.53(3.00-14.22)	<0.0001
Clopidogrel	Crushed	Uncrushed	9.40(4.30-20.53)	<0.0001
Normal	Crushed	Uncrushed	5.18(1.90-14.16)	0.002

Table 1. Ratios of mean percentage activation in each blood group between each hemostatic agent.

Group	*Presentation*	*Mean(95%CI)*	*Median(95% CI)*
Aspirin	ADP	45.46(16.80-74.12)	54.50(7.4-68.0)
Aspirin	Crushed	31.38(14.22-48.54)	27.40(0.2-62.0)
Aspirin	Unactivated	15.94(4.80-27.08)	11.90(0.3-36.8)
Aspirin	Uncrushed	5.84(0.31-11.37)	1.90(0.4-23.4)
Clopidogrel	ADP	38.68(14.03-63.33)	46.60(5.2-54.6)
Clopidogrel	Crushed	26.08(16.02-36.14)	25.85(5.5-43.5)
Clopidogrel	Unactivated	10.01(2.25-17.77)	7.55(0.0-28.4)
Clopidogrel	Uncrushed	4.67(0.40-8.94)	2.45(0.1-19.5)
Normal	ADP	20.08(9.21-30.95)	22.00(1.1-52.3)
Normal	Crushed	6.63(0.55-12.71)	3.65(0.2-28.4)
Normal	Unactivated	0.78(0.40-1.16)	0.75(0.0-1.9)
Normal	Uncrushed	0.99(-0.44-2.42)	0.20(0.1-6.5)

Table 2. Mean percentage platelet activation divided by hemostatic agent.

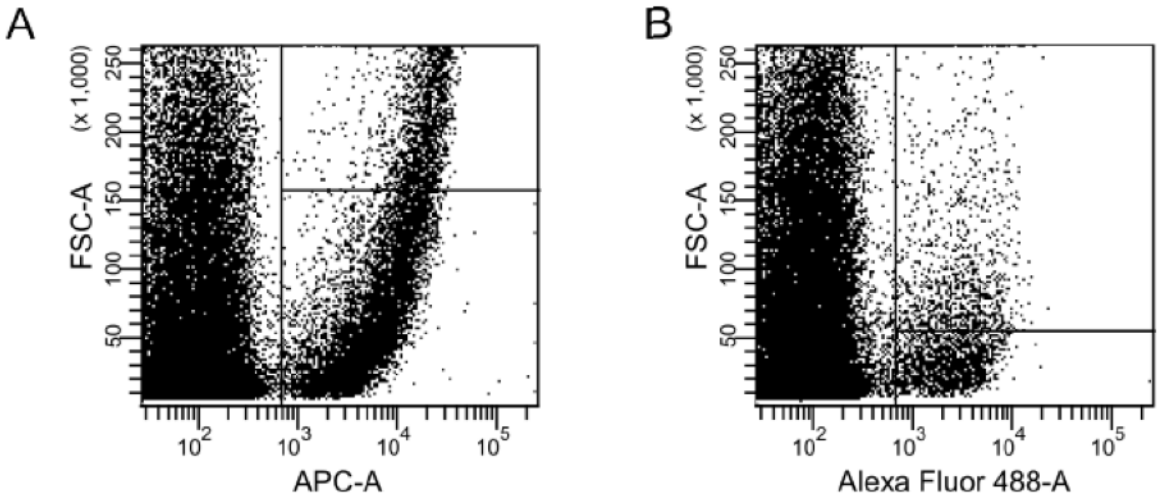


Figure 1. Platelets were identified by CD42 APC (A) and activated platelets by PAC-1 AF488 (B)

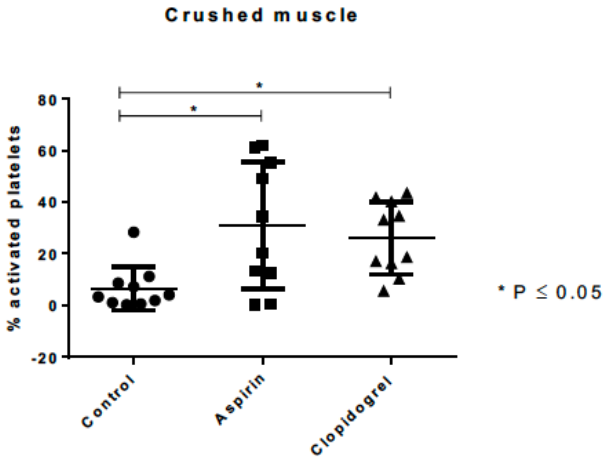


Figure 2. Percentage of activated platelets divided by blood cohort.

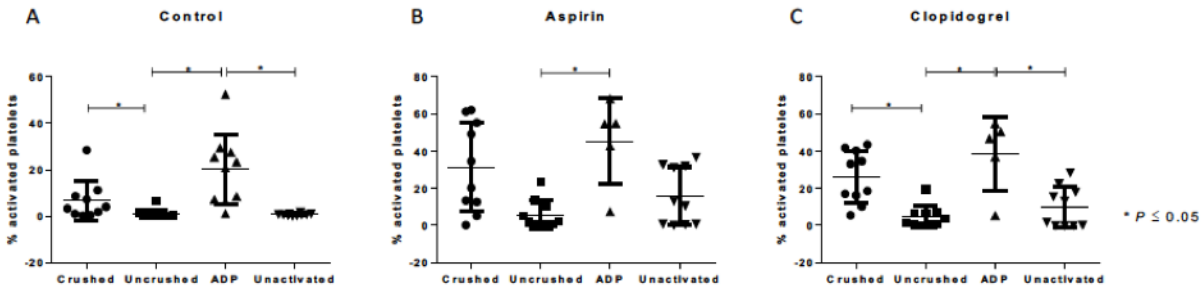


Figure 3. Percentage of activated platelets (PAC1 +) out of total platelets (CD42+), in (A) Control patients, (B) patients on Aspirin and (C) patients on Clopidogrel in crushed, uncrushed, ADP activated and un-activated patient samples. Medians with interquartile range. p≤0.05 Kruskal-Wallis

References:

1. Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. Neurosurgery. 2013;73:261

2. AlQahtani A, Castelnuovo P, Nicolai P, Prevedello DM, Locatelli D, Carrau RL. Injury of the Internal Carotid Artery During Endoscopic Skull Base Surgery: Prevention and Management Protocol. Otolaryngol Clin North Am. 2016;49:237-52.

3. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. Int Forum Allergy Rhinol. 2011;1:118-22.

4. Valentine R, Wormald PJ. A Vascular Catastrophe during Endonasal Surgery: An Endoscopic Sheep Model. Skull Base. 2011;21:109-14.

5. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. Laryngoscope. 2011;121:562-6.

6. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. Otolaryngol Clin North Am. 2011;44:1059-79.

7. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. Int Forum Allergy Rhinol. 2015;5:253-7.
8. Weidenbecher M, Huk WJ, Iro H. Internal carotid artery injury during functional endoscopic sinus surgery and its management. Eur Arch Otorhinolaryngol. 2005;262:640-5.
9. Koitschev A, Simon C, Lowenheim H, Naegele T, Ernemann U. Management and outcome after internal carotid artery laceration during surgery of the paranasal sinuses. Acta Otolaryngol. 2006;126:730-8.
10. Gardner PT, Mj ; Pant, H ; Fernandez-Miranda, Jc ; Snyderman, Ch ; Horowitz, MB. Carotid Artery Injury During Endoscopic Endonasal Skull Base Surgery: Incidence and Outcomes. Neurosurgery. 2013;73:261-9.
11. Fu M, Patel T, Baehring JM, Bulsara KR. Cavernous carotid pseudoaneurysm following transsphenoidal surgery. J Neuroimaging. 2013;23:319-25.
12. Rajiv S, Rodgers S, Bassiouni A, Vreugde S, Wormald PJ. Role of crushed skeletal muscle extract in hemostasis. Int Forum Allergy Rhinol. 2015;5:431-4.
13. Perzborn E, Heitmeier S, Laux V. Effects of Rivaroxaban on Platelet Activation and Platelet-Coagulation Pathway Interaction: In Vitro and In Vivo Studies. J Cardiovasc Pharmacol Ther. 2015;20:554-62.
14. Evangelista V, Smyth SS. Interactions Between Platelets, Leukocytes and the Endothelium. 2013:295-312.
15. Kamath S, Blann AD, Lip GY. Platelet activation: assessment and quantification. Eur Heart J. 2001;22:1561-71.
16. Padhye V, Valentine R, Paramasivan S, Jardeleza C, Bassiouni A, Vreugde S, Wormald, PJ. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. Int Forum Allergy Rhinol. 2014;4:651-7.
17. Chin OY, Ghosh R, Fang CH, Baredes S, Liu JK, Eloy JA. Internal carotid artery injury in endoscopic endonasal surgery: A systematic review. Laryngoscope. 2016;126:582-90.
18. Padhye V, Naidoo Y, Alexander H, Floreani S, Robinson S, Santoreneos S, Wickremesekera A, Brophy B, Harding M, Vrodos N, Wormald P. J. Endoscopic endonasal resection of anterior skull base meningiomas. Otolaryngol Head Neck Surg. 2012;147:575-82.
19. Padhye VV, R; Wormald, PJ. Management of Carotid Artery Injury in Endonasal Surgery. Int Arch Otorhinolaryngol 2014;18:S173-S8.
20. Berker M, Aghayev K, Saatci I, Palaoglu S, Onerci M. Overview of vascular complications of pituitary surgery with special emphasis on unexpected abnormality. Pituitary. 2010;13:160-7.
21. Chuang CC, Chen YL, Pai PC. A giant intracavernous carotid artery aneurysm embedded in pituitary macroadenoma presenting with pituitary apoplexy. Cerebrovasc Dis. 2006;21:142-4.
22. Fyrmpas G, Constantinidis J, Foroglou N, Selviaridis P. Pituitary apoplexy following endoscopic sinus surgery. J Laryngol Otol. 2010;124:677-9.
23. Harrold C, Randeva H. Conservative Versus Surgical Decompression for Pituitary Apoplexy. 2014:13-7.
24. Razak AA, Horridge M, Connolly DJ, Warren DJ, Mirza S, Muraleedharan V, Sinha, S. Comparison of endoscopic and microscopic trans-sphenoidal pituitary surgery: early results in a single centre. Br J Neurosurg. 2013;27:40-3.

25. Takeda N, Fujita K, Katayama S, Akutu N, Hayashi S, Kohmura E. Effect of transsphenoidal surgery on decreased visual acuity caused by pituitary apoplexy. Pituitary. 2010;13:154-9.
26. Zhang X, Zhang W, Fu LA, Cheng JX, Liu BL, Cao WD, Fei Z, Zhang JN, Liu WP, Zhen, HN. Hemorrhagic pituitary macroadenoma: characteristics, endoscopic endonasal transsphenoidal surgery, and outcomes. Ann Surg Oncol. 2011;18:246-52.
27. Kimpton G, Dabbous B, Leach P. New oral anticoagulant and antiplatelet agents for neurosurgeons. Br J Neurosurg. 2015;29:614-21.
28. Herbstreit FP, J. Spinal anaesthesia despite combined clopidogrel and aspirin therapy in a patient awaiting lung transplantation: effects of platelet transfusion on clotting tests. Anaesthesia. 2005;60:85-7.

Chapter 5

Stress response and communication in surgeons undergoing training in endoscopic management of major vessel haemorrhage: A mixed methods study

Alistair Jukes[1,2], Annika Mascarenhas[1], Jae Murphy[1], Lia Stepan[1], Tamara N. Munoz[3], Claudio A. Callejas[3], Rowan Valentine[1,4], PJ Wormald[1,5], Alkis J. Psaltis[1,5]

1. Department of Otolaryngology, Head and Neck Surgery. Queen Elizabeth Hospital, Woodville South, Adelaide, Australia
2. Department of Neurosurgery, Royal Adelaide Hospital, Adelaide, Australia
3. Department of Otolaryngology, Pontficia Universidad Catolica de Chile, Santiago, Chile
4. Department of Otolaryngology, Royal Adelaide Hospital, Adelaide, Australia
5. Department of Medicine - The University of Adelaide, Queen Elizabeth Hospital, Woodville South, Adelaide, Australia

Short title: Surgeon stress in vascular injury training

Keywords:
Simulation training
Residency training in rhinology
Endoscopic skull base surgery
Endoscopic minimally invasive surgery of the skull base
Endoscopic pituitary surgery

Corresponding author:
Dr Alistair K Jukes
Department of Otolaryngology, Head and Neck Surgery
Level 3
Queen Elizabeth Hospital, 28 Woodville Rd, Woodville South, 5011, Australia
Phone +61 08 82227158
Fax +61 08 82227419
alistair.jukes@adelaide.edu.au

Disclosures of potential conflicts of interest: PJ Wormald receives royalties from Medtronic ENT for instruments designed and is a consultant for Neilmed.

There are no external funding sources to declare.

Statement of Authorship

Title of Paper	Stress response and communication in surgeons undergoing training in endoscopic management of major vessel hemorrhage: a mixed methods study
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted work written in manuscript style
Publication Details	Jukes, Alistair K. ; Mascarenhas, Annika ; Murphy, Jae ; Stepan, Lia ; Muñoz, Tamara N. ; Callejas, Claudio A. ; Valentine, Rowan ; Wormald, P. J. ; Psaltis, Alkis J. International Forum of Allergy & Rhinology, June 2017, Vol.7(6), pp.576-583 [Peer Reviewed Journal]

Principal Author

Name of Principal Author (Candidate)	Jukes, Alistair
Contribution to the Paper	Study design, Ethics application, study conduct and data collection, data analysis, manuscript writing and preparation
Overall percentage (%)	85%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 2/12/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Mascarenhas, Annika
Contribution to the Paper	Study conduct and data collection
Signature	Date 2/1/18

Name of Co-Author	Murphy, Jae
Contribution to the Paper	Study conduct and data collection
Signature	Date 2/12/17

Name of Co-Author	Stepan, Lia		
Contribution to the Paper	Study conduct and data collection		
Signature		Date	2/12/17

Name of Co-Author	Munoz, Tamara		
Contribution to the Paper	Ethics application, Study conduct and data collection		
Signature		Date	1/12/17

Name of Co-Author	Callejas, Claudio		
Contribution to the Paper	Ethics application, Study conduct and data collection		
Signature		Date	13/12/17

Name of Co-Author	Valentine, Rowan		
Contribution to the Paper	Study design, Study conduct and data collection		
Signature		Date	1/12/17

Name of Co-Author	Wormald, Peter-John		
Contribution to the Paper	Study design and manuscript review		
Signature		Date	2/12/17

Name of Co-Author	Psaltis, Alkis		
Contribution to the Paper	Study design and manuscript review		
Signature		Date	2/12/17

Stress response and communication in surgeons undergoing training in endoscopic management of major vessel hemorrhage: a mixed methods study

Alistair K. Jukes, MBBS(Hons) B.LibStud[1,2], Annika Mascarenhas, MBBS[1], Jae Murphy, MBBS[1], Lia Stepan, MBBS[1], Tamara N. Muñoz, MD[3], Claudio A. Callejas, MD[3], Rowan Valentine, MBBS PhD FRACS[1,4], P.J. Wormald, MD FRACS FRCS[1,5] and Alkis J. Psaltis, MBBS PhD FRACS[1,5]

Background: **Major vessel hemorrhage in endoscopic, endonasal skull-base surgery is a rare but potentially fatal event. Surgical simulation models have been developed to train surgeons in the techniques required to manage this complication. This mixed-methods study aims to quantify the stress responses the model induces, determine how realistic the experience is, and how it changes the confidence levels of surgeons in their ability to deal with major vascular injury in an endoscopic setting.**

Methods: **Forty consultant surgeons and surgeons in training underwent training on an endoscopic sheep model of jugular vein and carotid artery injury. Pre-course and post-course questionnaires providing demographics, experience level, confidence, and realism scores were taken, based on a 5-point Likert scale. Objective markers of stress response including blood pressure, heart rate, and salivary alpha-amylase levels were measured.**

Results: **Mean "realism" score assessed posttraining showed the model to be perceived as highly realistic by the participants (score 4.02). Difference in participant self-rated pre-course and post-course confidence levels was significant ($p < 0.0001$): mean pre-course confidence level 1.66 (95% confidence interval [CI], 1.43 to 1.90); mean post-course confidence level 3.42 (95% CI, 3.19 to 3.65). Differences in subjects' heart rates (HRs) and mean arterial blood pressures (MAPs) were significant between injury models ($p = 0.0008$, $p = 0.0387$, respectively). No statistically significant difference in salivary alpha-amylase levels pretraining and posttraining was observed.**

Conclusion: **Results from this study indicate that this highly realistic simulation model provides surgeons with an increased level of confidence in their ability to deal with the rare but potentially catastrophic event of major vessel injury in endoscopic skull-base surgery.** © 2017 ARS-AAOA, LLC.

Key Words:
simulation training; residency training in rhinology; endoscopic skull-base surgery; endoscopic minimally invasive surgery of the skull base; endoscopic pituitary surgery

***How to Cite this Article*:**
Jukes AK, Mascarenhas A, Murphy J, et al. Stress response and communication in surgeons undergoing training in endoscopic management of major vessel hemorrhage: a mixed methods study. *Int Forum Allergy Rhinol.* 2017;XX: 1–8.

[1]*Department of Otolaryngology, Head and Neck Surgery, Queen Elizabeth Hospital, Adelaide, Australia;* [2]*Department of Neurosurgery, Royal Adelaide Hospital, Adelaide, Australia;* [3]*Department of Otolaryngology, Pontificia Universidad Católica de Chile, Santiago, Chile;* [4]*Department of Otolaryngology, Royal Adelaide Hospital, Adelaide, Australia;* [5]*Department of Medicine, University of Adelaide, Queen Elizabeth Hospital, Adelaide, Australia*

Correspondence to: Alistair K. Jukes, MBBS(Hons) B.LibStud, Department of Otolaryngology, Head and Neck Surgery, Level 3, Queen Elizabeth Hospital, 28 Woodville Rd, Woodville South, 5011, Australia; e-mail: alistair.jukes@adelaide.edu.au

Potential conflict of interest: P.J.W. receives royalties from Medtronic ENT for instruments designed and is a consultant for NeilMed.

Received: 11 January 2017; Revised: 4 March 2017; Accepted: 14 March 2017
DOI: 10.1002/alr.21941
View this article online at wileyonlinelibrary.com.

Surgery can be an inherently stressful activity for all concerned, especially when complications occur. The response of surgeons to complications can both help and hinder the situation. Up to a certain level, stress may improve concentration on the task at hand, focus communication, and assist in solving the problem. Beyond this, stress hinders effective responses and impairs communication and problem solving. This has been described by Stokes and Kite[1] as "the result of a mismatch between individuals' perceptions of the demands of the task or situation and their perceptions of the resources for coping with them." Simulation training is one of the ways in which surgeons can prepare themselves for encountering such situations in a safe and educational environment. Internal carotid artery (ICA) hemorrhage is a recognized complication of

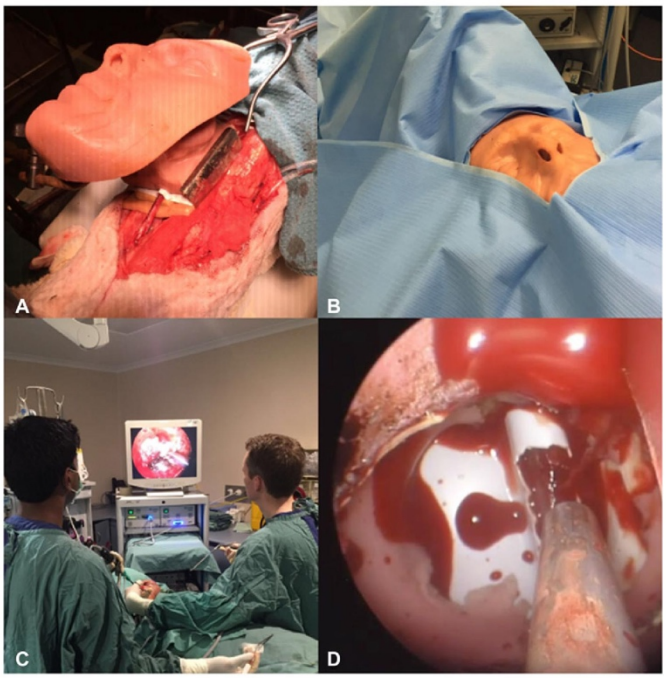


FIGURE 1. The carotid artery simulation model showing: (A) model attached to the right carotid artery; (B) model draped as the participants see it; (C) participants operating on the model; and (D) endoscopic view of the arterial injury.

endoscopic, endonasal approaches (EEAs) to the skull base with an incidence of 0.5% to 9% depending on the extent of the approach.[2–4] Valentine and Wormald have previously described and validated a sheep model of endoscopic major vessel hemorrhage.[5–8] In this model, a specifically designed, endoscopic skull-base model is placed over the internal jugular vein or common carotid artery of an anaesthetized sheep (Fig. 1). The vessel is then sharply injured and the surgeons must work as a team using the "2 surgeons, 4 hands" technique to gain control of the bleeding. This model affords a high-fidelity experience while mimicking the hemodynamic changes one would expect in a situation of major hemorrhage and incorporates the animal's innate haemostatic mechanisms to increase the reality of the situation. Previous studies have demonstrated that skills learned in simulation training successfully transfer across into the operative setting.[9–16] There are also a number of studies which have established not only a preference among students for high-fidelity training, but that this higher fidelity may result in more efficient skills acquisition provided the stress levels are not overtly high.[9,17–19] This study aimed to quantify the stress response of surgeons to this established simulation model using self-reported questionnaires, continuous heart rate recording, intermittent blood pressure recording, and salivary alpha-amylase levels (a biomarker for acute stress).[20] It further aims to assess the model's "realism" and utility in increasing confidence in dealing with this situation, and to determine if level of experience with skull-base surgery or prior experience of endoscopic carotid hemorrhage has a modulating effect on this stress response.

Subjects and methods

Ethics approval for this study was granted by the Central Adelaide Local Health Network Human Research Ethics Committee and the Scientific Ethics Committee of the Faculty of Medicine of Pontificia Universidad Católica de Chile.

Participants

This multicenter, multinational study recruited consultant otolaryngology surgeons and otolaryngology surgical trainees enrolled in the Vascular Injuries Management Workshops run in Adelaide, Australia (22 participants) and Santiago, Chile (18 participants). Participants were

excluded if they were taking medications causing alpha-adrenergic or beta-adrenergic blockage. Participants were randomly split into 2 equal groups. One-half underwent training in the morning and one-half underwent training in the afternoon. Surgeons that underwent training in the afternoon observed the ones that underwent training in the morning. The surgeons were assisted by experienced otolaryngology scrub and scout nurses and all participants encouraged to behave and communicate as they would in a routine operating theatre.

Vascular injury model

As described by Padhye et al.,[2] Valentine and Wormald,[5] and Valentine et al.,[7] merino sheep undergo bilateral neck dissection and application of a specifically designed skull-base model based on the SIMONT endoscopic trainer (Promedicus, Sao Paulo, Brazil) to first the internal jugular and then the carotid artery (Fig. 1). Participants remove a synthetic bone that overlies the vessel with both drill and bone rongeurs. Incisions are then made in the vessels and are controlled with various hemostatic agents including Floseal (Baxter, Deerfield, IL) and autologous muscle patches.

Methods

After consent, participants completed a baseline questionnaire (Fig. 2) providing basic demographics, gauging level of general endoscopic experience, prior exposure to vascular injuries, and quantifying their level of confidence in dealing with ICA injuries on a 5-point Likert scale. A baseline salivary swab for alpha-amylase was then taken and a chest strap heart rate monitor (Suunto M2, Suunto, Vantaa, Finland) was fitted to participants. Baseline blood pressure levels were taken (Health care wrist portable digital automatic blood pressure monitor; Sodial, Shanghai, China) and participants then underwent exposure to both venous and arterial injuries in the animal model. Peak heart rate (HR; bpm) was measured during each injury type and, immediately after exposure to each injury model, a second blood pressure recording was taken, along with a postexposure salivary alpha-amylase swab after the final arterial injury. Participants then filled out another questionnaire at the end of the session, self-rating their communications skills with their team, rating the "realism" of the model, rating their anxiety during the course, and quantifying their posttraining confidence level in dealing with an ICA injury. Mean arterial pressure (MAP; mmHg) was calculated as (2 × diastolic + 1 × systolic)/3.

Salivary alpha-amylase

Samples were stored frozen at −20°C until assay. On the day of assay, appropriate number of samples were thawed and analyzed using commercially available kits (Salimetrics, USA) according to the manufacturer's instructions See Appendix. Thawed samples were centrifuged at 1500*g* for 15 minutes to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before adding to the assay wells and all samples were analyzed in duplicate. Thawed and centrifuged saliva samples were diluted 1:200 by first diluting samples 1:10 with the α-amylase diluent provided followed by a 1:20 dilution of the 1:10 diluted sample resulting in a final dilution of 1:200. Eight microliters (8 μL) of diluted saliva samples were added in duplicate to individual wells of a 96-well plate using a positive displacement pipette. The plate was placed on a 37°C heating block and 320 μL of preheated (37°C) α-amylase substrate solution (provided as part of the kit) was added to each well simultaneously using a multichannel pipette. After the addition of substrate, the plate on the heating block was mixed continuously using a vortexer at 500 rpm. Optical density measurements were taken at 1-minute and 3-minute intervals using a plate reader with a filter set at 405 nm. High and low salivary alpha-amylase level controls (supplied as part of the kits) were included in each plate run to ensure a high standard of assay performance with each run.

Statistical analysis

Statistical calculations were performed on SAS version 9.3 (SAS Institute Inc., Cary, NC) with a p value of ≤ 0.05 considered significant. Questionnaires were analyzed using Mann-Whitney U tests. Wilcoxon matched pairs signed rank test was used to determine the significance of the difference between baseline HR, peak HR during venous training, and peak HR during arterial training; the significance of the difference between MAP at baseline, during venous training, and during arterial training; and the significance of the difference in pretraining and posttraining salivary alpha-amylase levels. Nonparametric Spearman tests of correlation were used to determine if there is any relationship between the physiological markers of stress and experience levels.

Results

There were 27 males and 13 females. Mean age was 31 years. Mean overall endoscopic skull-base experience level was 1.85 on a ranking scale where 1 = <10 cases; 2 = 10 to 50 cases; 3 = 50 to 100 cases; 4 = 100 to 200 cases; and 5 = >200 cases. A skull-base case was defined as a case where endoscopic drilling of the sella, clivus, olfactory groove region, or similar location was required. Functional endoscopic sinus surgery was not counted. A total of 17 surgeons had performed <10 cases; 14 surgeons had performed 10 to 50 cases; 5 surgeons had performed 50 to 100 cases; and 4 surgeons had performed 100 to 200 cases. Mean experience level in the surgeons from Chile was 1.21. Mean experience level in the Australian surgeons was 2.32.

Post-course questionnaire

The mean communication score was 4 out of a maximum score 5 (high). Mean anxiety levels during the course were 4 (high) with a mean anxiety awareness score of 3. The

Pre-Course

1 What is your overall case load of experience in skull base surgery?
(1) <10 (2) 10-50 (3) 50-100 (4) 100-200 (5) >200

2 What is your annual case load of experience in skull base surgery?
(1) <10 (2) 10-50 (3) 50-100 (4) 100-200 (5) >200

3 Have you experience or witnessed a major vascular injury during endoscopic transnasal surgery?
Yes No

4 How do you rate your anxiety levels during that event?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high N/A

5 How do you rate your confidence levels at controlling a major vascular injury?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

Post Course

1 How well do you feel you communicated with your partner during the procedure?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

2 How do you rate your anxiety levels during the procedure?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

3 How aware of feeling anxious were you during the procedure?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

4 How do you think this simulated situation compares to 'real' surgery events?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

5 How would you rate your post course anxiety levels during a major vascular injury?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

6 How do you rate your post course confidence levels at controlling a major vascular injury?
(1) Very Low (2) Low (3) Regular (4) High (5) Very high

FIGURE 2. Pretraining and posttraining questionnaire filled out by participants.

mean "realism" score assessed posttraining was 4 out of a maximum of 5 (high). Mean realism scores did not differ between participants with case experience of less than 50 cases or more than 50 cases.

Confidence levels

Questionnaire results were analyzed using the Mann-Whitney *U* test. The difference between self-rated pre-course and post-course confidence levels was significant, indicating that the course achieved one of its major aims in improving participants' confidence in being able to control major vascular bleeding ($p < 0.0001$). Mean pre-course confidence level was 1.66 (95% confidence interval [CI], 1.43 to 1.90). Mean post-course confidence level was 3.42 (95% CI, 3.19 to 3.65) (Fig. 3A). Subgroup analysis, with participants divided into experience with "less than" or "greater than" 50 skull-base cases, demonstrated that level of experience did not appear to have a significant modulating effect on participants' increased confidence posttraining ($p = 0.5323$). The increased confidence derived from the training was equivalent between groups. In addition, subgroup analysis of the morning and afternoon groups did not demonstrate a significant difference in confidence levels between watching the model and then participating or participating immediately ($p = 0.253$).

HR

Both peak HR during time of venous injury and time of arterial injury were significantly elevated when compared to the baseline HR recorded ($p < 0.0001$ for both groups). The mean HR for venous training was 91.65 bpm (95% CI, 86.39 to 96.91 bpm) and the mean HR for arterial training was 100.6 bpm (95% CI, 96.05 to 105.1 bpm). The difference between peak HR recorded during venous injury and that recorded during arterial injury was also statistically significant ($p = 0.0023$) (Fig. 3B). The mean HR during training in the morning and afternoon groups were compared to each other using an unpaired Mann-Whitney test to determine if watching the morning group had a stress-modifying effect on the afternoon group. The difference between the 2 groups was not significant for either the venous ($p = 0.118$) or arterial ($p = 0.537$) training, suggesting that observing a major vessel injury earlier in the day did not modify the stress response when participating in the injury model firsthand.

MAP

There was a significant difference in MAP between baseline and venous training ($p = 0.0234$), and baseline and arterial training ($p = 0.0176$). There was a significant difference in MAP between the venous and arterial training

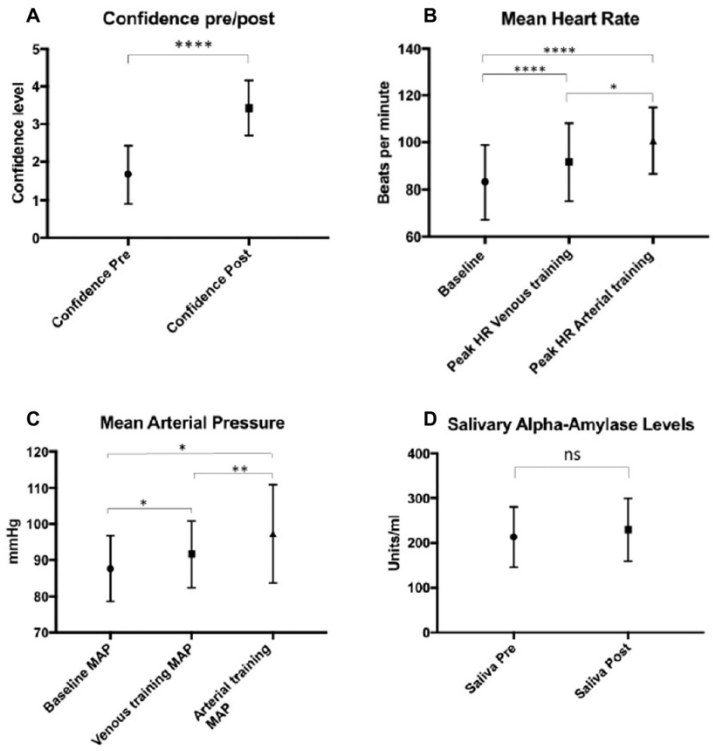


FIGURE 3. (A) Mean confidence levels pre-course and post-course. (B) Mean peak heart rate during venous and arterial training compared to mean baseline. (C) Mean arterial pressure (mmHg) during venous and arterial training compared to baseline. (D) Mean salivary alpha-amylase levels pretraining and posttraining (U/mL) (bars represent standard deviation; $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$, ns signifies $p > 0.05$).

($p = 0.0062$) with mean MAP 91.68 mmHg (95% CI, 87.61 to 95.76 mmHg) during the venous injury and 97.36 mmHg (95% CI, 91.34 to 103.40 mmHg) during the arterial injury (Fig. 3C). The MAP during training in the morning and afternoon groups were compared to each other using an unpaired Mann-Whitney test and the difference between the 2 groups was not significant for either the venous ($p = 0.3633$) or arterial ($p = 0.2952$) training.

Salivary alpha-amylase

Wilcoxon matched pairs signed rank test of the difference in salivary alpha-amylase between pretraining and posttraining was not significant ($p = 0.5389$). The mean pretraining was 213 U/mL (95% CI, 183.3 to 242.7 U/mL) and the mean posttraining was 229.2 U/mL (95% CI, 198 to 260.4 U/mL) (Fig. 3D). This was not significant when the morning and afternoon groups were analyzed separately, indicating that observing the injury model prior to actually training on it did not appear to have a moderating effect on salivary alpha-amylase levels. When participants were divided into "less than" and "greater than" 50 skull-base cases, there was not a significant difference in the rise in salivary alpha-amylase in each group ($p = 0.7451$) for the "less than" and ($p = 0.2783$) for the "greater than" group. The mean pretraining level in the "less than" group was 239 U/mL and the mean posttraining level was 243.4 U/mL. The mean pretraining level in the "greater than" group was 186 U/mL and the mean posttraining level was 215 U/mL.

Correlation between experience level and change in physiological markers

Nonparametric Spearman correlation analysis demonstrated a significant correlation between experience level and participant HR in the arterial injury training ($p = 0.0194$), with a higher HR in those with more experience. This would suggest that even in surgeons with a large experience in endoscopic surgery, this model provides a realistic simulation of major vessel hemorrhage. Analysis did not demonstrate any significant correlation between experience level and participant HR during venous training

($p = 0.1592$), experience and participant venous injury MAP ($p = 0.2770$), experience and participant arterial injury MAP ($p = 0.3171$), experience and baseline salivary alpha-amylase ($p = 0.8290$), and experience and posttraining salivary alpha-amylase ($p = 0.5087$).

Discussion

This study indicates that the Adelaide animal model is a highly realistic simulation model for the surgical training of the management of major vascular injury in endoscopic skull-base surgery. Self-reported confidence levels significantly improved after training on this model. "Realism" was rated as high on the posttraining questionnaires and the significant difference between the participant's HR and MAP between baseline and the venous and arterial injuries training suggests that the model provides an expected difference in the level of stress encountered for each of these 2 injury types, as measured objectively.

Stress, especially high levels, can negatively impact effective communications and, given that safe and successful surgery relies upon the coordinated actions of a team (anesthetist, surgeon, nurses, and orderlies), this may result in negative outcomes for patients if not dealt with appropriately.[21–23] It is difficult to quantify the stress response that occurs in surgeons when faced with a novel or challenging situation. Contemporaneous questionnaires are obviously tempered somewhat by the surgeon filling it out after having dealt with the situation and being aware of the outcome. They are also somewhat subjective. This is not entirely inappropriate as a tool for research because the stress response has a psychological component to it. It is, however, subject to biases and a relative and subjective grading system.[23] The ideal method would be to capture the subjective psychological and objective physiological responses and then correlate them with both the time course of the stressful situation and with each other, as we have attempted to do in this study.[18,24]

We opted to utilize relatively noninvasive measurement methods for HR and MAP that are widely accepted as surrogate markers of the innate human stress response.[25–27] Campbell and Ehlert's review of 49 studies of the correlation between psychological stress and anxiety and physiological markers found a significant correlation between HR and stress, especially in the "anticipation" phase, although this association diminishes with repeated exposure. To further attempt to measure the objective magnitude of this stress response, baseline and posttraining salivary alpha-amylase levels obtained. Psychoneuroendocrinology studies have demonstrated the utility of salivary alpha-amylase as a marker of immediate sympathetic nervous system activation with a reliable correlation with plasma catecholamines.[26,28–36] Human studies have confirmed that mean concentrations of salivary alpha-amylase are higher when sympathetic nervous system stimulation is increased.[32,37,38] There is a less reliable correlation with emotional state, with a number of studies that demonstrate a trend toward, rather than absolute statistical significance, depending on the precise definition of "anxiety" and "stress" used. Salivary alpha-amylase appears to hold promise as a biological marker because it reflects trends in sympathetic nervous system activation over the short term. This study did not, however, show significant change in the salivary alpha-amylase levels, neither when the group was analyzed as a whole, nor when subgroup analysis was performed according to experience level, although the mean levels did increase to a higher degree in the "greater than" group when participants were divided into groups based on experience. We hypothesize, given that the "less than" group had higher mean pretraining levels, that they may have already undergone a stress response at the prospect of the training, rather than during the training itself.

Patient outcomes are dependent on a multitude of factors in surgery. The surgeon's technical skill is but one small part of this. The nontechnical skills such as communication, planning, teamwork, and decision making are increasingly recognized as playing a crucial role.[39] When all goes well with surgery, it is relatively easy to maintain open lines of communication with all members of the surgical team—scrub and scout nurses, assistant, anesthetist, orderlies, and staff outside of theatre. It is when a potentially catastrophic event occurs that coordinated teamwork is vital and clear and concise communication is imperative.[39] Unfortunately, this is often the time when stress and anxiety may hinder optimum performance.[24,40] Human performance assessment and management in the world of aviation has much to teach surgeons about ways to train for adverse events.[23,24,28,41–46] The aviation industry has long been a proponent of simulation of not just technical skills but teamwork and communication.[47] The industry shares many things with surgery, particularly the high stakes of a bad outcome. Simulation is focused on improving people's perceived ability to cope through repeated exposure to scenarios and environments, where the consequences of task failure are minimized compared with surgery on humans. Simulation in surgery is a valuable tool. It has been incorporated into many training programs and varies widely from laparoscopic and animal training models to videotaped sessions with actors and other members of the surgical team to develop communication and teamwork skills.[7,46,48–50] It also provides surgeons with the ability to experience rare situations that they may never encounter in their entire careers, allowing them to develop strategies to safely master the event should it occur.[51] In this simulation, surgeons are taught to manipulate the endoscopic camera and keep their visual and working fields free, enabling them to control the hemorrhage expeditiously and appropriately.

Conclusion

From the point of view of surgical training, this preclinical simulation of endoscopic skull-base hemorrhage remains a valuable model that allows surgeons to be exposed to a relatively rare and potentially fatal event and equip them with

the confidence and skills required to deal with this safely. Results would suggest that participants find the course realistic, that it is able to induce a stressful response, and that it provides participants with an increased level of confidence in their ability to deal with major vessel bleeding in an endoscopic environment.

Appendix

Salivary alpha-amylase

Sample collection and storage

Samples were stored frozen at −20°C until assay. On the day of assay appropriate number of samples were thawed and analyzed using commercially available kits (Salimetrics, USA) according to the manufacturer's instructions. Thawed samples were centrifuged at 1500*g* for 15 minutes to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before being added to the assay wells and all samples were analyzed in duplicate. Thawed and centrifuged saliva samples were diluted 1:200 by first diluting samples 1:10 with the α-amylase diluent provided, followed by a 1:20 dilution of the 1:10 diluted sample, resulting in a final dilution of 1:200. Eight microliters (8 μL) of diluted saliva samples were added in duplicate to individual wells of a 96-well plate using a positive displacement pipette. The plate was placed on a 37°C heating block and 320 μL of preheated (37°C) α-amylase substrate solution (provided as part of the kit) was added to each well simultaneously using a multichannel pipette. After the addition of substrate the plate on the heating block was mixed continuously using a vortexer at 500 rpm. Optical density measurements were taken at 1-minute and 3-minute intervals using a plate reader with a filter set at 405 nm. Subtract the 1-minute reading from the 3-minute reading and multiply by the conversion factor 328 (see Calculations below). The conversion factor takes into account the 1:200 sample dilution, sample volume, and assay volume, light path for the 96-well plates used, and the millimolar absorptivity of the substrate. High and low salivary alpha-amylase level controls (supplied as part of the kits) were included in each plate run to ensure a high standard of assay performance with each run. The Salimetrics assay uses the principle that salivary alpha-amylase catalyses the substrate maltotriose linked with a chromogenic substrate, 2-chloro-p-nitrophenol. The enzymatic action of α-amylase on maltotriose releases the 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm. The amount of α-amylase activity present in the sample is directly proportional to the increase in absorbance at 405 nm over a specified period of time.

Calculations

$$\frac{\Delta \text{ Abs./min} \times \text{TV} \times \text{DF}}{\text{MMA} \times \text{SV} \times \text{LP}} = \text{U/mL of } \alpha\text{-amylase activity in sample}$$

where: ΔAbs./min = absorbance difference per minute
TV = total assay volume (0.328 mL)
DF = dilution factor
MMA = millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)
SV = sample volume (0.008 mL)
LP = light path = 0.97 (specific to plate received with kit)

$$\frac{\Delta \text{ Abs./2} \times 0.328 \times 200}{12.9 \times 0.008 \times 0.97} = \Delta\text{Abs.} \times 328^{*} = \text{U/mL } \alpha\text{-amylase activity}$$

Example:

If change in absorbance (OD change over 2 minutes) was 0.3, then 0.3 × 328 = 98.4 U/mL

Intraassay variability (within assay) 5.5%

Interassay variability (between assays) 6.2%

References

1. Stokes AF, Kite K. On grasping a nettle and becoming emotional. In: Hancock PA, Desmond PA, eds. *Stress, Workload, and Fatigue*. Mahwah, NJ: Lawrence Erlbaum Associates, Publishers; 2001:107–132.
2. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. *Int Forum Allergy Rhinol*. 2015;5:253–257.
3. Padhye V, Valentine R, Paramasivan S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. *Int Forum Allergy Rhinol*. 2014;4:651–657.
4. Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. *Neurosurgery*. 2013;73(2 Suppl Operative):ons261–ons269; discussion ons269-ons270.
5. Valentine R, Wormald PJ. A vascular catastrophe during endonasal surgery: an endoscopic sheep model. *Skull Base*. 2011;21:109–114.
6. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. *Laryngoscope*. 2011;121:562–566.
7. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. *Int Forum Allergy Rhinol*. 2011;1:118–122.
8. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. *Otolaryngol Clin North Am*. 2011;44:1059–1079.
9. Sturm LP, Windsor JA, Cosman PH, Cregan P, Hewett PJ, Maddern GJ. A systematic review of skills transfer after surgical simulation training. *Ann Surg*. 2008;248:166–179.
10. Arora S, Aggarwal R, Sirimanna P, et al. Mental practice enhances surgical technical skills: a randomized controlled study. *Ann Surg*. 2011;253:265–270.
11. Marcus HJ, Hughes-Hallett A, Cundy TP, et al. Comparative effectiveness of 3-dimensional vs 2-dimensional and high-definition vs standard-definition neuroendoscopy: a preclinical randomized crossover study. *Neurosurgery*. 2014;74:375–380; discussion 380-381.
12. Cundy TP, Gattas NE, Yang GZ, Darzi A, Najmaldin AS. Experience related factors compensate for haptic loss in robot-assisted laparoscopic surgery. *J Endourol*. 2014;28:532–538.
13. Retrosi G, Cundy T, Haddad M, Clarke S. Motion analysis-based skills training and assessment in pediatric laparoscopy: construct, concurrent, and content validity for the eoSim simulator. *J Laparoendosc Adv Surg Tech A*. 2015;25:944–950.
14. Beyer-Berjot L, Berdah S, Hashimoto DA, Darzi A, Aggarwal R. A virtual reality training curriculum for laparoscopic colorectal surgery. *J Surg Educ*. 2016;73:932–941.
15. Choudhury N, Gelinas-Phaneuf N, Delorme S, Del Maestro R. Fundamentals of neurosurgery: virtual reality tasks for training and evaluation of technical skills. *World Neurosurg*. 2013;80:e9–e19.
16. Kockro RA. Neurosurgery simulators—beyond the experiment. *World Neurosurg*. 2013;80:e101–e102.
17. Wetzel CM, Kneebone RL, Woloshynowych M, et al. The effects of stress on surgical performance. *Am J Surg*. 2006;191:5–10.
18. Hull L, Arora S, Kassab E, Kneebone R, Sevdalis N. Observational teamwork assessment for surgery: content validation and tool refinement. *J Am Coll Surg*. 2011;212:234–243.e5.
19. Hindmarsh J, Hyland L, Banerjee A. Work to make simulation work: 'realism', instructional

correction and the body in training. *Discourse Stud*. 2014;16:247–269.

20. Koh D. 0334 Use of salivary biomarkers to evaluate response to a stress management intervention. *Occup Environ Med*. 2014;71(Suppl 1):A109.
21. Larkin AC, Cahan MA, Whalen G, et al. Human Emotion and Response in Surgery (HEARS): a simulation-based curriculum for communication skills, systems-based practice, and professionalism in surgical residency training. *J Am Coll Surg*. 2010;211:285–292.
22. Solheim O. Is aneurysm surgery too exciting for our own good? *Acta Neurochir (Wien)*. 2016;158:217–219.
23. Arora S, Aggarwal R, Moran A, et al. Mental practice: effective stress management training for novice surgeons. *J Am Coll Surg*. 2011;212:225–233.
24. Hull L, Arora S, Kassab E, Kneebone R, Sevdalis N. Assessment of stress and teamwork in the operating room: an exploratory study. *Am J Surg*. 2011;201:24–30.
25. Reinhardt T, Schmahl C, Wust S, Bohus M. Salivary cortisol, heart rate, electrodermal activity and subjective stress responses to the Mannheim Multicomponent Stress Test (MMST). *Psychiatry Res*. 2012;198:106–111.
26. Allen AP, Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. *Neurosci Biobehav Rev*. 2014;38:94–124.
27. Lane RD, McRae K, Reiman EM, Chen K, Ahern GL, Thayer JF. Neural correlates of heart rate variability during emotion. *Neuroimage*. 2009;44:213–222.
28. Campbell J, Ehlert U. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology*. 2012;37:1111–1134.
29. Dantzer R, Kalin N. Salivary biomarkers of stress: cortisol and alpha-amylase. *Psychoneuroendocrinology*. 2009;34:1.
30. Diaz Gomez MM, Bocanegra Jaramillo OL, Teixeira RR, Espindola FS. Salivary surrogates of plasma nitrite and catecholamines during a 21-week training season in swimmers. *PLoS One*. 2013;8:e64043.
31. Kidd SA, Corbett BA, Granger DA, Boyce WT, Anders TF, Tager IB. Daytime secretion of salivary cortisol and alpha-amylase in preschool-aged children with autism and typically developing children. *J Autism Dev Disord*. 2012;42:2648–2658.
32. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*. 2009;34:486–496.
33. Nater UM, Rohleder N, Gaab J, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *Int J Psychophysiol*. 2005;55:333–342.
34. Payne JD, Jackson ED, Hoscheidt S, Ryan L, Jacobs WJ, Nadel L. Stress administered prior to encoding impairs neutral but enhances emotional long-term episodic memories. *Learn Mem*. 2007;14:861–868.
35. Rai B, Kaur J. Mental and physical workload, salivary stress biomarkers and taste perception: Mars desert research station expedition. *N Am J Med Sci*. 2012;4:577–581.
36. Valentin B, Grottke O, Skorning M, et al. Cortisol and alpha-amylase as stress response indicators during prehospital emergency medicine training with repetitive high-fidelity simulation and scenarios with standardized patients. *Scand J Trauma Resusc Emerg Med*. 2015;23:31.
37. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*. 2006;31:137–141.
38. van Stegeren AH, Wolf OT, Kindt M. Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. *Int J Psychophysiol*. 2008;69:33–40.
39. Arora S, Sevdalis N, Nestel D, Woloshynowych M, Darzi A, Kneebone R. The impact of stress on surgical performance: a systematic review of the literature. *Surgery*. 2010;147:318–330.e6.
40. Pinto A, Faiz O, Bicknell C, Vincent C. Acute traumatic stress among surgeons after major surgical complications. *Am J Surg*. 2014;208:642–647.
41. Gillespie BM, Withers TK, Lavin J, Gardiner T, Marshall AP. Factors that drive team participation in surgical safety checks: a prospective study. *Patient Saf Surg*. 2016;10:3.
42. Goransson K, Lundberg J, Ljungqvist O, Ohlsson E, Sandblom G. Safety hazards in abdominal surgery related to communication between surgical and anesthesia unit personnel found in a Swedish nationwide survey. *Patient Saf Surg*. 2016;10:2.
43. Underwood P, Waterson P. Systems thinking, the Swiss Cheese Model and accident analysis: a comparative systemic analysis of the Grayrigg train derailment using the ATSB, AcciMap and STAMP models. *Accid Anal Prev*. 2014;68:75–94.
44. Salmon PM, Cornelissen M, Trotter MJ. Systems-based accident analysis methods: a comparison of Accimap, HFACS, and STAMP. *Saf Sci*. 2012;50:1158–1170.
45. Tomizawa Y. Clinical benefits and risk analysis of topical hemostats: a review. *J Artif Organs*. 2005;8:137–142.
46. Thorpe P. Training and patient safety. *Br J Surg*. 2004;91:391–392.
47. Muller MP, Hansel M, Fichtner A, et al. Excellence in performance and stress reduction during two different full scale simulator training courses: a pilot study. *Resuscitation*. 2009;80:919–924.
48. Goon SS, Stamatakis EA, Adapa RM, et al. Clinical decision-making augmented by simulation training: neural correlates demonstrated by functional imaging: a pilot study. *Br J Anaesth*. 2014;112:124–132.
49. Mason V, Balloo S, Upton D, Heer K, Higton P, Shiralkar U. Surgeons' experience of learning psychological skills: a preliminary evaluation of a psychological skills training course. *Ann R Coll Surg Engl*. 2009;91:321–325.
50. Lifchez SD, Redett RJ 3rd. A standardized patient model to teach and assess professionalism and communication skills: the effect of personality type on performance. *J Surg Educ*. 2014;71:297–301.
51. Lu H, Wang L, Zhong W, et al. Establishment of swine-penetrating craniocerebral gunshot wound model. *J Surg Res*. 2015;199:698–706.

Abstract

Background: Major vessel haemorrhage in endoscopic, endonasal skull base surgery is a rare but potentially fatal event. Surgical simulation models have been developed to train surgeons in the techniques required to manage this complication. This mixed-methods study aims to quantify the stress responses the model induces, determine how realistic the experience is, and how it changes the confidence levels of surgeons in their ability to deal with major vascular injury in an endoscopic setting.

Methods: Forty consultant surgeons and surgeons in training underwent training on an endoscopic sheep model of jugular vein and carotid artery injury. Pre-and post-course questionnaires providing demographics, experience level, confidence and realism scores were taken, based on a 5-point Likert scale. Objective markers of stress response including blood pressure, heart rate and salivary alpha-amylase levels were measured.

Results: Mean 'realism' score assessed post training showed the model to be perceived as highly realistic by the participants (score 4.02). Difference in participant self-rated pre- and post-course confidence levels was significant ($P<0.0001$) (Mean pre-course confidence level 1.66 (95% CI 1.43-1.90), mean post-course confidence level 3.42 (95% CI 3.19-3.65). Differences in subject's heart rates (HR) and mean arterial blood pressures (MAP) were significant between injury models (($P=0.0008$) and ($P=0.0387$) respectively). No statistically significant difference in salivary alpha-amylase levels pre- and post-training was observed.

Conclusions: Results from this study indicate that this highly realistic simulation model provides surgeons with an increased level of confidence in their ability to deal with the rare but potentially catastrophic event of major vessel injury in endoscopic skull base surgery

Introduction

Surgery can be an inherently stressful activity for all concerned, especially when complications occur. The response of surgeons to complications can both help and hinder the situation. Up to a certain level, stress may improve concentration on the task at hand, focus communication and assist in solving the problem. Beyond this, stress hinders effective responses and impairs communication and problem solving. This has been described by Stokes and Kite as *"the result of a mismatch between individuals' perceptions of the demands of the task or situation and their perceptions of the resources for coping with them."*(490). Simulation training is one of the ways in which surgeons can prepare themselves for encountering such situations in a safe and educational environment. Internal carotid artery (ICA) haemorrhage is a recognized complication of endoscopic, endonasal approaches (EEA) to the skull base with an incidence of 0.5-9% depending on the extent of the approach(121, 122, 142). Valentine and Wormald have previously described and validated a sheep model of endoscopic major vessel haemorrhage (117-120). In this model, a specifically designed, endoscopic skull base model is placed over the internal jugular vein or common carotid artery of an anaesthetized sheep (Figure 1). The vessel is then sharply injured and the surgeons must work as a team using the '2 surgeons, 4 hands' technique to gain control of the bleeding. This model affords a high-fidelity experience whilst mimicking the haemodynamic changes one would expect in a situation of major haemorrhage and incorporates the animal's innate haemostatic mechanisms to increase the reality of the

situation. Previous studies have demonstrated that skills learned in simulation training successfully transfer across into the operative setting(510-517). There are also a number of studies which have established not only a preference amongst students for high fidelity training, but that this higher fidelity may result in more efficient skills acquisition provided the stress levels are not overtly high(510, 518, 538, 539). This study aims to quantify the stress response of surgeons to this established simulation model using self-reported questionnaires, continuous heart rate recording, intermittent blood pressure recording and salivary alpha-amylase levels (a biomarker for acute stress)(504) It further aims to assess the model's 'realism' and utility in increasing confidence in dealing with this situation, and to determine if level of experience with skull base surgery or prior experience of endoscopic carotid haemorrhage has a modulating effect on this stress response.

Materials and Methods

Ethics approval for this study was granted by the Central Adelaide Local Health Network Human Research Ethics Committee and the Scientific Ethics Committee of the Faculty of Medicine of Pontificia Universidad Catolica de Chile

Participants

This multicenter, multinational study recruited consultant otolaryngology surgeons and otolaryngology surgical trainees enrolled in the Vascular Injuries Management Workshops run in Adelaide, Australia (22 participants) and Santiago, Chile (18 participants). Participants were excluded if they were taking medications causing alpha-adrenergic or beta-adrenergic blockage. Participants were randomly split into two equal groups. Half underwent training in the morning and half underwent training in the afternoon. Surgeons that underwent

training in the afternoon observed the ones that underwent training in the morning. The surgeons were assisted by experienced otolaryngology scrub and scout nurses and all participants encouraged to behave and communicate as they would in a routine operating theatre.

Vascular Injury Model

As described by Valentine et al and Padhye et al, merino sheep undergo bilateral neck dissection and application of a specifically designed skull base model based on the SIMONT endoscopic trainer (Pro-medicus, Sao Paulo, Brazil) to first the internal jugular and then the carotid artery (Figure 1)(117, 119, 122). Participants remove a synthetic bone that overlies the vessel with both drill and bone rongeurs. Incisions are then made in the vessels and are controlled with various haemostatic agents including floseal (Baxter, Deerfield, IL) and autologous muscle patches.

Methods

Post consent, participants completed a baseline questionnaire (figure 2) providing basic demographics, gauging level of general endoscopic experience, prior exposure to vascular injuries and quantifying their level of confidence in dealing with ICA injuries on a 5- point scale. A baseline salivary swab for alpha-amylase was then taken and a chest strap heart rate monitor (Suunto M2, Suunto, Finland) fitted to participants. Baseline blood pressure levels were taken (Health care wrist portable digital automatic blood pressure monitor, Sodial, China) and participants then underwent exposure to both venous and arterial injuries in the animal model. Peak HR was measured during each injury type and, immediately after exposure to each injury model, a second blood pressure recording was

taken, along with a post-exposure salivary alpha-amylase swab after the final arterial injury. Participants then filled out another questionnaire at the end of the session, self-rating their communications skills with their team, rating the 'realism' of the model, rating their anxiety during the course and quantifying their post-training confidence level in dealing with an ICA injury. Mean arterial pressure (MAP) was calculated as (2 x diastolic + 1 x systolic)/3.

Salivary alpha amylase

Samples were stored frozen at -20°C until assay. On the day of assay, appropriate number of samples were thawed and analysed using commercially available kits (Salimetrics, USA) according to the manufacturer's instructions. Thawed samples were centrifuged at 1500 x *g* for 15 min to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before adding to the assay wells and all samples were analysed in duplicate. Thawed and centrifuged saliva samples were diluted 1:200 by first diluting samples 1:10 with the α-amylase diluent provided followed by a 1:20 dilution of the 1:10 diluted sample resulting in a final dilution of 1:200. 8 µL of diluted saliva samples were added in duplicate to individual wells of a 96 well plate using a positive displacement pipette. The plate was placed on a 37^{o}C heating block and 320 µL of preheated (37^{o}C) α-amylase substrate solution (provided as part of the kit) was added to each well simultaneously using a multichannel pipette. After the addition of substrate, the plate on the heating block was mixed continuously using a vortexer at 500 RPM. Optical density measurements were taken at 1 min and 3 min intervals using a plate reader with a filter set at 405 nm. High and low salivary alpha amylase level controls (supplied as part of the kits) was included in each plate run to ensure a high standard of assay performance with each run.

Statistical Analysis

Statistical calculations were performed on SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA) with a P-value of ≤ 0.05 to be considered significant. Questionnaires were analysed using Mann-Whitney U tests. Wilcoxon matched pairs signed ranking test was used to determine the significance of the difference between baseline HR, peak HR during venous training and peak HR during arterial training; the significance of the difference between MAP at baseline, during venous training and during arterial training; and the significance of the difference in pre-training and post-training salivary alpha-amylase levels. Non-parametric Spearman tests of correlation were used to determine if there was any relationship between the physiological markers of stress and experience levels.

Results

There were 27 males and 13 females. Mean age was 31 years. Mean overall endoscopic skull base experience level was 1.85 on a ranking scale where 1 = <10 cases; 2 = 10-50 cases; 3 = 50-100 cases; 4 = 100-200 cases and 5 = >200 cases. A skull-base case was defined as a case where endoscopic drilling of the sella, clivus, olfactory groove region or similar was required. Functional endoscopic sinus surgery was not counted. 17 surgeons had performed <10 cases, 14 surgeons had performed 10-50 cases, 5 surgeons had performed 50-100 cases and 4 had performed 100-200 cases. Mean experience level in the surgeons from Chile was 1.21. Mean experience level in the Australian surgeons was 2.32.

Post-course Questionnaire: The mean communication score was 4 out of a maximum score 5(high). Mean anxiety levels during the course were 4 (high) with a mean anxiety awareness score of 3. The mean 'realism' score assessed post training was 4 out of a maximum of 5

(High). Mean realism scores did not differ between participants with case experience of less than 50 cases or more than 50 cases.

Confidence levels: Questionnaire results were analysed using Mann-Whitney U test. The difference between self-rated pre- and post-course confidence levels was significant, indicating that the course achieved one of its major aims in improving participant's confidence in being able to control major vascular bleeding(P<0.0001). Mean pre-course confidence level was 1.66 (95% CI 1.43-1.90). Mean post-course confidence level was 3.42 (95% CI 3.19-3.65)(figure 3A). Sub-group analysis, with participants divided into 'less than' or 'greater than' 50 skull base cases experience, demonstrated that level of experience did not appear to have a significant modulating effect on participants increased confidence post-training (P=0.5323). The increased confidence derived from the training was equivalent between groups. In addition, sub group analysis of morning and afternoon groups did not demonstrate a significant difference in confidence levels between watching the model and then participating or participating immediately (P=0.253).

Heart rate: Both peak HR during time of venous injury and time of arterial injury were significantly elevated when compared to the baseline HR recorded (P<0.0001 for both groups). The mean HR for venous training was 91.65 (95% CI 86.39-96.91) and the mean HR for arterial was 100.6 (95%CI 96.05-105.1). The difference between peak HR recorded during venous injury and that recorded during arterial injury was also statistically significant (P=0.0023)(figure 3B). The mean HR during training in the morning and afternoon groups were compared to each other using an unpaired Mann-Whitney test to determine if watching the morning group had a stress modifying effect on the afternoon group. The

difference between the two groups was not significant for either the venous (P=0.118) or arterial (P=0.537) training, suggesting observing a major vessel injury earlier in the day did not modify the stress response when participating in the injury model first hand.

Mean Arterial Pressure (MAP): There was a significant difference between baseline and venous training (P=0.0234), and baseline and arterial training (P=0.0176). There was a significant difference in MAP between the venous and arterial training(P=0.0062) with mean MAP 91.68mmHg (95%CI 87.61-95.76) during the venous injury and 97.36mmHg (95%CI 91.34-103.40) during the arterial injury(figure 3C). The MAP during training in the morning and afternoon groups were compared to each other using an unpaired Mann-Whitney test and the difference between the two groups was not significant for either the venous (P=0.3633) or arterial (P=0.2952) training.

Salivary Alpha Amylase: Wilcoxon matched pairs signed ranking test of the difference in salivary alpha-amylase between pre- and post-training was not significant (P=0.5389). The mean pre-training was 213 (95% CI 183.3-242.7) and the mean post training was 229.2 (95% CI 198-260.4) (figure 3D). This was not significant when the morning and afternoon groups were analysed separately, indicating that observing the injury model prior to actually training on it did not appear to have a moderating effect on salivary alpha-amylase levels. When participants were divided into 'less than' and 'greater than' 50 skull base cases, there was not a significant difference in the rise in salivary alpha amylase in each group (P=0.7451) for the 'less than' and (P=0.2783) for the 'greater than' group. The mean pre-training level in the 'less than' group was 239 and the mean post-training level was 243.4.

The mean pre-training level in the 'greater than' group was 186 and the mean post-training level was 215.

Correlation between experience level and change in physiological markers

Non-parametric Spearman correlation analysis demonstrated a significant correlation between experience level and participant HR in the arterial injury (P=0.0194) with a higher HR in those with more experience. This would suggest that even in surgeons with a large experience in endoscopic surgery, this model provides a realistic simulation of major vessel haemorrhage. Analysis did not demonstrate any significant correlation between experience level and participant HR during venous training (P=0.1592), experience and participant venous injury MAP (P=0.2770), experience and participant arterial injury MAP (P=0.3171), experience and baseline salivary alpha-amylase (P=0.8290) and experience and post-training salivary alpha-amylase (P=0.5087).

Discussion

This study indicates that the Adelaide animal model is a highly realistic, simulation model for the surgical training of the management of major vascular injury in endoscopic skull base surgery. Self-reported confidence levels significantly improved after training on this model. 'Realism' was rated as high on the post-training questionnaires and the significant difference between the participant's HR and MAP between baseline, and the venous and arterial injuries training, suggests that the model provides an expected difference in the level of stress encountered for each of these two injury types, as measured objectively.

Stress, especially high levels, can negatively impact upon effective communications and, given that safe and successful surgery relies upon the coordinated actions of a team (anaesthetist, surgeon, nurses, and orderlies), this may result in negative outcomes for patients if not dealt with appropriately (484, 520, 521). It is difficult to quantify the stress response that occurs in surgeons when faced with a novel or challenging situation. Contemporaneous questionnaires are obviously tempered somewhat by the surgeon filling it out after having dealt with the situation and being aware of the outcome. They are also somewhat subjective. This is not entirely inappropriate as a tool for research as the stress response has a psychological component to it. It is, however, subject to biases and a relative and subjective grading system (484). The ideal method would be to capture the subjective psychological and objective physiological responses and then correlate them with both the time course of the stressful situation and with each other, as we have attempted to do in this study (480, 518).

We opted to utilize relatively non-invasive measurement of HR and MAP that are widely accepted as surrogate markers of the innate human stress response (491, 540, 541). Campbell and Ehlert's review of 49 studies of the correlation between psychological stress and anxiety and physiological markers found a significant correlation between HR and stress, especially in the 'anticipation' phase, albeit that this association diminishes with repeated exposure. To further attempt to measure the objective magnitude of this stress response, baseline and post-training salivary alpha amylase levels were performed. Psychoneuroendocrinology studies have demonstrated the utility of salivary alpha-amylase as a marker of immediate sympathetic nervous system activation with a reliable correlation with plasma catecholamines(485, 491, 492, 495-501). Human studies have confirmed that

mean concentrations of salivary alpha-amylase are higher when sympathetic nervous system stimulation is increased (497, 502, 503). There is a less reliable correlation with emotional state, with a number of studies that demonstrate a trend towards, rather than absolute statistical significance, depending on the precise definition of 'anxiety' and 'stress' used. Salivary alpha-amylase appears to hold promise as a biological marker as it reflects trends in sympathetic nervous system activation over the short term. This study did not, however, show significant change in the salivary alpha amylase levels when the group was analysed as a whole, nor when sub-group analysis was performed according to experience level although the mean levels did increase to a higher degree in the 'greater than' group when participants were divided into groups based on experience. We hypothesise, given that the 'less than' group had higher mean pre-training levels, that they may have already undergone a stress response at the prospect of the training, rather than the training itself.

Patient outcomes are dependent on a multitude of factors in surgery. The surgeon's technical skill is but one small part of this. The non-technical skills such as communication, planning, teamwork and decision making are increasingly recognised as playing a crucial role (479). When all goes well with surgery, it is relatively easy to maintain open lines of communication with all members of the surgical team – scrub and scout nurses, assistant, anaesthetist, orderlies and staff outside of theatre. It is when a potentially catastrophic event occurs that coordinated teamwork is vital and clear and concise communication imperative (479). Unfortunately, this is often the time when stress and anxiety may hinder optimum performance (480, 481). Human performance assessment and management in the world of aviation has much to teach surgeons about ways to train for adverse events (104, 105, 284, 480, 484-488). The aviation industry has long been a proponent of simulation of

not just technical skills but teamwork and communication (482). The industry shares many parallels with surgery, particularly the high stakes of a bad outcome. Simulation is focused on improving people's perceived ability to cope through repeated exposure to scenarios and environments, where the consequences of task failure are minimized compared with surgery on humans. Simulation in surgery is a valuable tool. It has been incorporated into many training programs and varies widely from laparoscopic and animal training models to videotaped sessions with actors and other members of the surgical team to develop communication and team work skills (119, 488, 507-509). It also provides surgeons with the ability to experience rare situations that they may never encounter in their entire careers allowing them to develop strategies to safely master the event should it occur (273). In this simulation, surgeons are taught to manipulate the endoscopic camera and keep their visual and working fields free, enabling them to control the haemorrhage expeditiously and appropriately.

Conclusion

From the point of view of surgical training, this pre-clinical simulation of endoscopic skull base haemorrhage remains a valuable model that allows surgeons to be exposed to a relatively rare and potentially fatal event and equip them with the confidence and skills required to deal with this safely. Results would suggest that participants find the course realistic, that it is able to induce a stressful response, and that it provides participants with an increased level of confidence in their ability to deal with major vessel bleeding in an endoscopic environment.

References:

1. Stokes AF, Kite K. On grasping a nettle and becoming emotional. In: Desmond PAHPA, editor. Stress, workload, and fatigue. New Jersey: Mahwah, NJ: L. Erlbaum; 2001.
2. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. Int Forum Allergy Rhinol. 2015;5(3):253-7.
3. Padhye V, Valentine R, Paramasivan S, Jardeleza C, Bassiouni A, Vreugde S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. Int Forum Allergy Rhinol. 2014;4(8):651-7.
4. Gardner PT, Mj ; Pant, H ; Fernandez-Miranda, Jc ; Snyderman, Ch ; Horowitz, MB. Carotid Artery Injury During Endoscopic Endonasal Skull Base Surgery: Incidence and Outcomes
. Neurosurgery. 2013;73(Dec):261-9.
5. Valentine R, Wormald PJ. A Vascular Catastrophe during Endonasal Surgery: An Endoscopic Sheep Model. Skull Base. 2011;21(2):109-14.
6. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. Laryngoscope. 2011;121(3):562-6.
7. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. Int Forum Allergy Rhinol. 2011;1(2):118-22.
8. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. Otolaryngol Clin North Am. 2011;44(5):1059-79.
9. Sturm LP, Windsor JA, Cosman PH, Cregan P, Hewett PJ, Maddern GJ. A systematic review of skills transfer after surgical simulation training. Ann Surg. 2008;248(2):166-79.
10. Arora S, Aggarwal R, Sirimanna P, Moran A, Grantcharov T, Kneebone R, et al. Mental practice enhances surgical technical skills: a randomized controlled study. Ann Surg. 2011;253(2):265-70.
11. Marcus HJ, Hughes-Hallett A, Cundy TP, Di Marco A, Pratt P, Nandi D, et al. Comparative effectiveness of 3-dimensional vs 2-dimensional and high-definition vs standard-definition neuroendoscopy: a preclinical randomized crossover study. Neurosurgery. 2014;74(4):375-80; discussion 80-1.
12. Cundy TP, Gattas NE, Yang GZ, Darzi A, Najmaldin AS. Experience related factors compensate for haptic loss in robot-assisted laparoscopic surgery. J Endourol. 2014;28(5):532-8.
13. Retrosi G, Cundy T, Haddad M, Clarke S. Motion Analysis-Based Skills Training and Assessment in Pediatric Laparoscopy: Construct, Concurrent, and Content Validity for the eoSim Simulator. J Laparoendosc Adv Surg Tech A. 2015;25(11):944-50.
14. Beyer-Berjot L, Berdah S, Hashimoto DA, Darzi A, Aggarwal R. A Virtual Reality Training Curriculum for Laparoscopic Colorectal Surgery. J Surg Educ. 2016.
15. Choudhury N, Gelinas-Phaneuf N, Delorme S, Del Maestro R. Fundamentals of neurosurgery: virtual reality tasks for training and evaluation of technical skills. World Neurosurg. 2013;80(5):e9-19.
16. Kockro RA. Neurosurgery simulators--beyond the experiment. World Neurosurg. 2013;80(5):e101-2.
17. Wetzel CM, Kneebone RL, Woloshynowych M, Nestel D, Moorthy K, Kidd J, et al. The effects of stress on surgical performance. Am J Surg. 2006;191(1):5-10.

18. Hull L, Arora S, Kassab E, Kneebone R, Sevdalis N. Observational teamwork assessment for surgery: content validation and tool refinement. J Am Coll Surg. 2011;212(2):234-43 e1-5.
19. Hindmarsh J, Hyland L, Banerjee A. Work to make simulation work: 'Realism', instructional correction and the body in training. Discourse Studies 2014;16(2):247-69.
20. Koh D. 0334 Use of salivary biomarkers to evaluate response to a stress management intervention. Occupational and Environmental Medicine. 2014;71(Suppl_1):A109-A.
21. Larkin AC, Cahan MA, Whalen G, Hatem D, Starr S, Haley HL, et al. Human Emotion and Response in Surgery (HEARS): a simulation-based curriculum for communication skills, systems-based practice, and professionalism in surgical residency training. J Am Coll Surg. 2010;211(2):285-92.
22. Solheim O. Is aneurysm surgery too exciting for our own good? Acta Neurochir (Wien). 2016;158(2):217-9.
23. Arora S, Aggarwal R, Moran A, Sirimanna P, Crochet P, Darzi A, et al. Mental practice: effective stress management training for novice surgeons. J Am Coll Surg. 2011;212(2):225-33.
24. Hull L, Arora S, Kassab E, Kneebone R, Sevdalis N. Assessment of stress and teamwork in the operating room: an exploratory study. Am J Surg. 2011;201(1):24-30.
25. Reinhardt T, Schmahl C, Wust S, Bohus M. Salivary cortisol, heart rate, electrodermal activity and subjective stress responses to the Mannheim Multicomponent Stress Test (MMST). Psychiatry Res. 2012;198(1):106-11.
26. Allen AP, Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci Biobehav Rev. 2014;38:94-124.
27. Lane RD, McRae K, Reiman EM, Chen K, Ahern GL, Thayer JF. Neural correlates of heart rate variability during emotion. Neuroimage. 2009;44(1):213-22.
28. Campbell J, Ehlert U. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? Psychoneuroendocrinology. 2012;37(8):1111-34.
29. Dantzer R, Kalin N. Salivary biomarkers of stress: cortisol and alpha-amylase. Psychoneuroendocrinology. 2009;34(1):1.
30. Diaz Gomez MM, Bocanegra Jaramillo OL, Teixeira RR, Espindola FS. Salivary surrogates of plasma nitrite and catecholamines during a 21-week training season in swimmers. PLoS One. 2013;8(5):e64043.
31. Kidd SA, Corbett BA, Granger DA, Boyce WT, Anders TF, Tager IB. Daytime secretion of salivary cortisol and alpha-amylase in preschool-aged children with autism and typically developing children. J Autism Dev Disord. 2012;42(12):2648-58.
32. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology. 2009;34(4):486-96.
33. Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. Int J Psychophysiol. 2005;55(3):333-42.
34. Payne JD, Jackson ED, Hoscheidt S, Ryan L, Jacobs WJ, Nadel L. Stress administered prior to encoding impairs neutral but enhances emotional long-term episodic memories. Learn Mem. 2007;14(12):861-8.

35. Rai B, Kaur J. Mental and physical workload, salivary stress biomarkers and taste perception: Mars desert research station expedition. N Am J Med Sci. 2012;4(11):577-81.
36. Valentin B, Grottke O, Skorning M, Bergrath S, Fischermann H, Rortgen D, et al. Cortisol and alpha-amylase as stress response indicators during pre-hospital emergency medicine training with repetitive high-fidelity simulation and scenarios with standardized patients. Scand J Trauma Resusc Emerg Med. 2015;23:31.
37. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. Psychoneuroendocrinology. 2006;31(1):137-41.
38. van Stegeren AH, Wolf OT, Kindt M. Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. Int J Psychophysiol. 2008;69(1):33-40.
39. Arora S, Sevdalis N, Nestel D, Woloshynowych M, Darzi A, Kneebone R. The impact of stress on surgical performance: a systematic review of the literature. Surgery. 2010;147(3):318-30, 30 e1-6.
40. Pinto A, Faiz O, Bicknell C, Vincent C. Acute traumatic stress among surgeons after major surgical complications. Am J Surg. 2014;208(4):642-7.
41. Gillespie BM, Withers TK, Lavin J, Gardiner T, Marshall AP. Factors that drive team participation in surgical safety checks: a prospective study. Patient Saf Surg. 2016;10:3.
42. Goransson K, Lundberg J, Ljungqvist O, Ohlsson E, Sandblom G. Safety hazards in abdominal surgery related to communication between surgical and anesthesia unit personnel found in a Swedish nationwide survey. Patient Saf Surg. 2016;10:2.
43. Underwood P, Waterson P. Systems thinking, the Swiss Cheese Model and accident analysis: a comparative systemic analysis of the Grayrigg train derailment using the ATSB, AcciMap and STAMP models. Accid Anal Prev. 2014;68:75-94.
44. Salmon PM, Cornelissen M, Trotter MJ. Systems-based accident analysis methods: A comparison of Accimap, HFACS, and STAMP. Safety Science. 2012;50(4):1158-70.
45. Tomizawa Y. Clinical benefits and risk analysis of topical hemostats: a review. J Artif Organs. 2005;8(3):137-42.
46. Thorpe P. Training and patient safety. Br J Surg. 2004;91(4):391-2.
47. Muller MP, Hansel M, Fichtner A, Hardt F, Weber S, Kirschbaum C, et al. Excellence in performance and stress reduction during two different full scale simulator training courses: a pilot study. Resuscitation. 2009;80(8):919-24.
48. Goon SS, Stamatakis EA, Adapa RM, Kasahara M, Bishop S, Wood DF, et al. Clinical decision-making augmented by simulation training: neural correlates demonstrated by functional imaging: a pilot study. Br J Anaesth. 2014;112(1):124-32.
49. Mason V, Balloo S, Upton D, Heer K, Higton P, Shiralkar U. Surgeons' experience of learning psychological skills: a preliminary evaluation of a psychological skills training course. Ann R Coll Surg Engl. 2009;91(4):321-5.
50. Lifchez SD, Redett RJ, 3rd. A standardized patient model to teach and assess professionalism and communication skills: the effect of personality type on performance. J Surg Educ. 2014;71(3):297-301.
51. Lu H, Wang L, Zhong W, Qi R, Li N, You W, et al. Establishment of swine-penetrating craniocerebral gunshot wound model. J Surg Res. 2015;199(2):698-706.

Appendix

Salivary alpha amylase:

Sample collection and storage.

Samples were stored frozen at -20°C until assay. On the day of assay appropriate number of samples were thawed and analysed using commercially available kits (Salimetrics, USA) according to the manufacturer's instructions. Thawed samples were centrifuged at 1500 x *g* for 15 min to collect clear saliva and this saliva was used without further processing for all assays. All samples were brought to room temperature before adding to the assay wells and all samples were analysed in duplicate. Thawed and centrifuged saliva samples were diluted 1:200 by first diluting samples 1:10 with the α-amylase diluent provided followed by a 1:20 dilution of the 1:10 diluted sample resulting in a final dilution of 1:200. 8 µL of diluted saliva samples were added in duplicate to individual wells of a 96 well plate using a positive displacement pipette. The plate was placed on a 37^{o}C heating block and 320 µL of preheated (37^{o}C) α-amylase substrate solution (provided as part of the kit) was added to each well simultaneously using a multichannel pipette. After the addition of substrate the plate on the heating block was mixed continuously using a vortexer at 500 RPM. Optical density measurements were taken at 1 min and 3 min intervals using a plate reader with a filter set at 405 nm. Subtract the 1 min readings from the 3 min reading and multiply by the conversion factor 328 (see below). The conversion factor takes into account the 1:200 sample dilution, sample volume and assay volume, light path for the plates 96 well plates used, and the millimolar absorptivity of the substrate. High and low salivary alpha amylase level controls (supplied as part of the kits) was included in each plate run to ensure a high standard of assay performance with each run. The Salimatrics assay uses the principle that sAA catalyses the substrate maltotriose linked with a chromagenic substrate, 2-chloro-p-

nitrophenol. The enzymatic action of α-amylase on maltotriose releases the 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm. The amount of α-amylase activity present in the sample is directly proportional to the increase in absorbance at 405 nm over a specified period of time.

Calculations

$$\frac{\Delta \text{ Abs./min} \times TV \times DF}{MMA \times SV \times LP} = \text{U/mL of } \alpha\text{-amylase activity in sample}$$

Where: ΔAbs./min = Absorbance difference per minute

TV = Total assay volume (0.328 mL)

DF = Dilution factor

MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)

SV = Sample volume (0.008 mL)

LP = Light path = 0.97(specific to plate received with kit)

$$\frac{\Delta Abs./2 \times 0.328 \times 200}{12.9 \times 0.008 \times 0.97} = \Delta Abs. \times 328^* = \text{U/mL } \alpha\text{-amylase activity}$$

Example

If change in absorbance (OD change over 2 minutes) was 0.3, then 0.3 x 328 = 98.4 U/mL

Intra assay variability (within assay) 5.5%

Inter assay variability (between assays) 6.2%

Chapter 6

Thesis Summary

As endoscopic approaches to the skull base become more commonplace, it seems reasonable to assume that the risk of potential major vessel injury will also increase. Surgeons are attempting ever more complex operations through endoscopic approaches that were once the exclusive domain of the open cranial skull base surgeon. Not only are these venturing beyond the lateral boundaries of the carotid arteries; they also extend ever more anterior and posterior with consequent exposure to anterior communicating and basilar vascular complexes. The good news is that as surgeons gain more experience, confidence and skill with these operations, the ability to identify 'at risk' areas and the confidence to deal with vascular injury increases(129, 542-545).

This thesis has accomplished a number of aims. The first was to determine the feasibility of the use of self-assembling nano-peptides as haemostats in arterial injury. This is described in detail in chapter 2. Whilst the literature shows that there is promise in both laboratory settings and in small, low pressure animal models, our pilot study demonstrated a high flow, high pressure bleed is not able to be stopped by this particular self-assembling nanopeptide(546). We utilized a validated animal model with which we have much familiarity. An identical mix of 10ml of RADA nano-haemostat was immediately applied to the injury. There was no discernible decrease in the bleeding rate. RADA-16 appeared to be washed away by the pressure of the bleeding (Systolic range 70-90mmHg). We were unable to control the bleeding and after a period of 90 seconds were forced to use a crushed

muscle patch to stop the bleeding which worked to good effect as previously reported(117-120). This was repeated in a second sheep with identical results.

There is much more work to be done in this particular field in terms of further refining of peptides to allow more durable and adherent haemostatic agents to be formed. There are many questions to answer before nano-haemostat technology can be utilized in human surgery routinely, namely; Which formula is best and why? What rate of flow can it stop? Does it need to be impregnated in a carrier? Does it preserve vessel lumen patency and thus allow distal brain perfusion? Does the scaffold last long enough for more permanent healing to occur? Do pseudo-aneurysms form after its use? Are there long-term effects on native tissues and what happens to the breakdown products? Is there pseudo-aneurysm formation? Do surrounding neurons/glial cells contain any evidence of abnormal protein inclusions? And finally, is there evidence of distal embolic phenomena? This study was published in 2016(546).

Chapter 3 describes the results of a second study with several aims. The first was to determine the relative haemostatic capabilities of 3 commercially available products to control major vessel arterial injury (2 in combination). Again, this utilized the sheep model of carotid artery haemorrhage. Fibrin/thrombin patch (Tachosil [Baxter, Deerfield, IL]) and fibrin/thrombin glue (Evicel [Ethicon, Sommerville, NJ]), combined with surgicel snow [Ethicon, Sommerville, NJ]) as a carrier agent, were applied to a standardised carotid artery injury.

The results from this study demonstrated both decreased time to haemostasis and decreased blood volume loss for both products when compared with muscle patch, which is considered the current gold standard in the literature and from previous studies within our department. Muscle patch is easily obtainable, autologous and thus costs little apart from possible increases in operative time to harvest and close the wound. It does, however require a second or third surgeon to harvest and there is a not insignificant risk of pseudoaneurysm formation. Tachosil is primarily used in extra-cranial surgery and whilst there are case reports of off-label use in intracranial regions (such as superior sagittal sinus tears), there is little data as to its safety when exposed long term to neural tissue. Evicel/Snow suffers from the technical problem of adherence to instruments when attempting to withdraw these from the nasal cavity although we have found that the addition of a second layer of snow obviates this to some degree.

A secondary aim was the development of a novel beta-chitin patch [Department of Chemistry, University of Otago, Dunedin, New Zealand] as a haemostatic agent. This was developed after literature review and previous experience within our department and proved to be a very effective haemostatic agent with no signs of allergy or ill effect in a recovery model. It compared very favourably to gold standard muscle patch with significantly decreased times to haemostasis and blood volume loss. Animals were recovered to a 3-month pre-determined endpoint and underwent magnetic resonance angiography to determine the incidence of carotid pseudoaneurysm formation. One animal died of a ruptured pseudoaneurym in the fibrin/thrombin patch group at day 11 post operatively however no other animal suffered this complication indicating that the incidence of pseudoaneurysm formation is not greater than muscle patch. This study has

been submitted for publication. Further studies of the intra-cranial uses of these patches on vascular injury could focus on smaller arterial injury models as the avulsion of perforators and exposure to anterior communicating and basilar complexes becomes more common with extended endonasal approaches.

Crushed autologous muscle is used in skull base surgery in the acute phase of major arterial hemorrhage to stop bleeding. The mechanism of this is not yet clear but is thought to involve the formation of a platelet plug, that seals the vessel wall defect but still allows ongoing blood flow to the brain. Chapter 4 describes a flow cytometry study, published in 2017, designed to replicate the in vivo actions of crushed muscle on platelets in whole blood. It compared the ratio of activation of platelets exposed to crushed and uncrushed muscle supernatant in control patients and those on antiplatelet agents. This study demonstrated that crushed muscle activated platelets to a higher degree than uncrushed muscle. This ratio was 5.18 times greater in control blood ($P=0.002$), 6.53 times greater in aspirin-exposed blood ($P<0.0001$) and 9.4 times greater in clopidogrel exposed blood ($P<0.0001$). Crushed muscle caused a consistently increased ratio of platelet activation when compared with uncrushed muscle across all groups, adding to the evidence that at least part of its clinical effect is the result of platelet activation(547). This is likely due to the exposure of collagen and other extracellular matrix components to the circulating platelets and their subsequent activation with formation of a platelet fibrin plug.

Surgery can be an inherently stressful activity for all concerned, especially when complications occur. Major vessel haemorrhage in endoscopic, endonasal skull base surgery is a rare but potentially fatal event. Live animal surgical simulation models have been

developed to train surgeons in the techniques required to manage this complication. Chapter 5 describes a mixed-methods study, published in 2017, which aimed to quantify the stress responses the model induces, determine how realistic the experience is, and how it changes the confidence levels of surgeons in their ability to deal with major vascular injury in an endoscopic setting. Forty consultant surgeons and surgeons in training underwent training on an endoscopic sheep model of jugular vein and carotid artery injury. Pre-and post-course questionnaires providing demographics, experience level, confidence and realism scores were taken, based on a 5-point Likert scale. Objective markers of stress response including blood pressure, heart rate and salivary alpha-amylase levels were measured. Mean 'realism' score assessed post training showed the model to be perceived as highly realistic by the participants (score 4.02). Difference in participant self-rated pre- and post-course confidence levels was significant ($P<0.0001$) (Mean pre-course confidence level 1.66 (95% CI 1.43-1.90), mean post-course confidence level 3.42 (95% CI 3.19-3.65). Differences in subject's heart rates (HR) and mean arterial blood pressures (MAP) were significant between injury models (($P=0.0008$) and ($P=0.0387$) respectively). No statistically significant difference in salivary alpha-amylase levels pre- and post-training was observed, although we hypothesis that this occurred as a result of an already heightened baseline level of stress as part of the anticipated course participation. If this study was to be repeated then perhaps performing baseline salivary alpha-amylase sampling a day or two prior to the course would have provided a different result. Results from this study indicate that this is a highly realistic simulation model that not only causes heightened stress levels in participants, but that it also provides surgeons with an increased level of confidence in their ability to deal with the rare but potentially catastrophic event of major vessel injury in endoscopic skull base surgery(129).

Appendix 1 is a book chapter published during the period of candidacy in a textbook describing management of internal carotid artery injury. This synthesizes an extensive review of the literature including case reports, case series and previous published management strategies, the results of several studies into the management of this complication within our department and the development of a management algorithm able to be utilised by surgeons, anaesthetists and their respective teams.

This thesis describes novel techniques for large vessel, high pressure, high volume haemorrhage control. There are, however, other vascular injuries that can occur in endoscopic skull base surgery such as small vessel perforator injuries. These injuries can be just as devastating as carotid injury as often there is little or no collateral supply to eloquent brain. Further work is required to develop techniques and products to manage these injuries in a safe and durable manner.

Appendix 1

Book Chapter – Management of ICA injury

Statement of Authorship

Title of Paper	Management of Internal Carotid Artery Injury
Publication Status	[X] Published [ ] Accepted for Publication [ ] Submitted for Publication [ ] Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Jukes A; Wormald P. J.Management of Internal Carotid Artery Injury In: Juvenile Nasopharyngeal Angiofibroma. Janakiram T, editor. 1st Edition: Thieme; 2017.

Principal Author

Name of Principal Author (Candidate)	Jukes, Alistair		
Contribution to the Paper	Literature review and manuscript writing		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	2/12/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Wormald, Peter-John		
Contribution to the Paper	Manuscript editing		
Signature		Date	2/12/17

12

Management of Internal Carotid Artery Injury

Alistair K. Jukes and
Peter-John Wormald

12

Management of Internal Carotid Artery Injury

Introduction

Internal carotid artery (ICA) bleeding in endoscopic endonasal surgery is one of the most feared complications. There are a wide range of pathologies that involve the major vessels of the skull base, which include sinus, nasopharyngeal, neural and dural-based tumors, bony skull lesions, cavernous sinus pathologies, and, importantly, vascular aneurysms and malformations.[1-3] The skull base is an anatomically constrained area with brain parenchyma, neural and vascular structures, bony prominences and canals, and dural reflections that make it one of the most difficult areas in which a surgeon can operate. In addition to this, with a major vascular injury and the level of difficulty increases enormously as does the potential for a poor outcome for the patient.

Incidence

The incidence of major vessel injury in uncomplicated midline endoscopic skull base surgery varies in the literature but ranges somewhere between 0.58 and 1.1%.[4-9] This is in contrast to the much lower rate in endoscopic sinus surgery (<0.25%) in which the sphenoid sinus is either not entered or not drilled extensively.[7] These rates are much higher in the case of extended endoscopic approaches (EEAs) in which the instruments and endoscope may come into more direct contact with the carotid artery or require more extensive bone removal. These EEAs generally extend the surgical corridor more rostral or caudal and may, through the use of angled scopes also extend the boundaries of the approach laterally. Couldwell et al, Cavallo et al, and Gardner et al report rates of 5 to 9% in series of craniopharyngiomas, chordomas, and chondrosarcomas, which, by virtue of their location and tumor consistency, invariably require more bone removal or dissection.[4-7, 10-13] Surgeons who have been involved with more than 500 transsphenoidal approaches are thought to have a greater than 50% chance of having seen a carotid artery injury.[14] Given the increasing use of endoscopic transsphenoidal surgery to access more complex pathologies, it is reasonable to assume that rates will only increase despite the author's knowledge of predisposing factors.

Initial Planning and Management

The most important initial management step occurs before the operation begins and requires that the surgeon be proactive in assessing the likelihood of the occurrence of injury. Tumors that encase the carotid, or are at least adherent in a plane of more than 120 degrees, and those tumors in which the surgical intent is curative, total resection are at a much higher risk. These cases should be discussed in a multidisciplinary meeting with skull base otolaryngologists, skull base neurosurgeons, radiologists, endovascular radiologists/neurosurgeons, and ophthalmologists. The authors cannot stress enough

the value of developing a team-based approach to skull-base surgery, and that extended endonasal cases should not be attempted before developing a collaborative team able within the institution. Additionally, team-based training in vascular injuries courses on animal models (**Figs. 12.1, 12.2**), such as that developed by Valentine and Wormald, is invaluable and allows surgical teams to practice managing this stressful scenario.[4-6]

Fig. 12.1 Endoscopic trainer utilized sheep carotid artery injury model.

Fig. 12.2 Training on the animal model of endoscopic hemorrhage control.

Risk Factors

It is important to review the preoperative imaging closely to determine whether there are any predisposing factors that may increase the risk of ICA injury and allow modifications to surgical technique at any predetermined "danger points." These factors may be divided into anatomical factors, tumor factors, and patient factors.

Anatomical Factors

The wall of the sphenoid sinus is deficient in 4 to 22% of cases, leaving only the mucosa or dura to surround the carotid. Even if present, the wall should not be considered thick enough to protect the artery from the drill or other instruments.[15] Bone windows on preoperative computed tomography (CT) may assist with this but should not be relied on.

The carotids may also deviate toward the midline. The ICAs are usually at least 12 mm apart but have been described as close together as 4 mm in their cavernous segment and, rarely, even touching each other. These so-called "kissing carotids" have been considered as having contributed to ICA injury in several case reports.[16] Magnetic resonance angiography (MRA) may be performed, but even on plain magnetic resonance imaging (MRI) flow voids may be seen on axial slices and the distance between these measured.

There may be bony septations and spicules within the sphenoid and cavernous sinuses that, while not being dangerous in themselves, may either require the use of instrumentation to remove with consequent risk of ICA injury by either bone fragments or the instruments themselves.

Cavernous segment ICA aneurysms make up to 12% of total intracranial aneurysms in some case series and require the surgeon to carefully plan any endoscopic approach.[17-20] It is advisable to consider treating these prior to attempting any endoscopic endonasal skull base approaches.[21-23] These may be treated with flow diverting stents with or without coil insertion into the fundus; however, it is important to recognize that this will require the patient to take antiplatelet therapy for at least 3 to 6 months post stent insertion that may factor into further surgical planning.

Tumor Factors

Tumors may themselves adhere to the ICA, and any extension into the cavernous sinus should be approached with caution, especially if this extends greater than 120 degrees around the ICA. There is a risk of tumor adherence to the ICA. This not only predisposes the ICA to injury when the tumor is dissected off the carotid, but this dissection may result in vasospasm as the carotid is handled.[24] This may manifest as neurologic injury or as elevated blood pressure as the patient attempts to maintain cerebral perfusion pressure with impaired autoregulation.

The tumor size is an independent risk factor. Tumors that require extensive bone removal for exposure will tend to require exposure of more of the ICA.

There also appears to be an increased risk of carotid injury with pituitary tumors secreting growth hormone or adrenocorticotrophic-releasing hormone (ACTH).[25,26] This may not be only due to tumor size or invasion but also due to as yet undefined influences of insulin-like growth factor 1 (IGF-1) or cortisol on the arterial wall.[7]

Patient Factors

Previous transsphenoidal surgery may result in aberrant anatomy and adhesions. It may also be difficult to ascertain the precise amount of anatomical disruption and bone removal that has previously occurred. Previous radiation to the region may result in scarring, adhesions, or more friable vessels. Bromocriptine therapy appears to increase the risk of ICA injury, possibly because of adhesions and fibrosis, and acromegaly patients have a tendency toward more ectatic and tortuous carotid arteries.[7,24] **Table 12.1** summarizes the risk factors in ICA injury.

Table12.1 Factors contributing to increased risk of internal carotid artery injury

Anatomical	Sphenoid wall defect Carotid proximity Bony septations and spicules Cavernous segment ICA aneurysm
Tumor	Encasement of ICA Surrounding > 120 degrees ACTH-secreting tumors
Patient	Previous surgery Previous radiotherapy Bromocriptine therapy Acromegaly

Abbreviations: ACTH, adrenocorticotrophic-releasing hormone; ICA, internal carotid artery.

Operation Theater Setup

Ideally factors such as anatomical variation or the possibility of tumor erosion into the artery or cavernous sinus will have alerted the surgeon to the danger of injury to the carotid artery; however, this is not always possible or indeed reliable.[27,28] The entire surgical team must be alert to the possibility of injury. A preoperative briefing involving all staff that identifies the risks involved and the plan, should such an injury occur, is a highly valuable step. The patient should always be cross-matched and blood available, especially in more extended endonasal approaches. Anesthetic staff should be aware of the possibility of ICA injury and have a plan in place in the event that it occurs. Large-bore IV access and invasive arterial monitoring should be considered prior to it being required. Neuronavigation should be utilized as, while it does not replace a thorough knowledge of anatomy, it gives the surgeon greater confidence in identifying anatomical structures intraoperatively. A Doppler ultrasound device with an angled probe may also be useful to identify the ICA intraoperatively.

Theater staff should have patties, gauze, and oxidized cellulose (authors prefer Surgicel snow [Ethicon, NJ, United States] for its absorptive capacity) on the setup for all cases and the endoscope should have a lens-cleaning system attached. There should be two 10F suction devices on the setup. These suction devices should be checked to ensure that they work and do not require changing immediately prior to any bone removal and at regular intervals. Authors advocate the use of blunt instruments, such as suction freer dissectors and pituitary ring curettes, when working adjacent to the ICA and that bone removal should take place with diamond rather than cutting burrs. It may also be advisable that when using Kerrison's punch, a twisting or pulling motion is not used, but that the sharp edges of the instrument are allowed to completely transect the bone before removal.[24] These should all be ready on the instrument tray at the commencement of the case. The patient should be draped to allow access to the lateral thigh or abdomen for muscle harvesting as required. It may also be advisable for the angiography service to be made aware that the operation is planned in case they are required post any injury.

Injury Types

It is important to be aware of the different injury types that are seen in injury to the carotid vessel. These range from linear incisions to stellate or large wall defects caused by Kerrison-type punches or high-speed drill injury. While initial management and resuscitation is the same, the likelihood that different techniques may work varies accordingly and decision making should reflect this.[8]

Initial Management

The immediate management of injury to the carotid artery in endoscopic surgery is a team-centered approach involving coordination between nursing, anesthetic, and surgical staff.[27] Communication with the anesthetic staff early is vital and the staff should immediately begin volume resuscitation of the patient before their hemodynamic parameters are affected. It is important to maintain normotension or even mild hypertension. Cerebral perfusion is vital to maintain despite the temptation to lower the blood pressure to assist hemostasis. Theater staff should check the suction to ensure that the bag is not becoming full, which may lead to a stoppage just when it is required the most. Theater staff should also activate the on-call angiography team.

ICA injury is only possible to manage with a "two-surgeon, four-hand" approach. A second surgeon's assistance must be immediately sought if they are not already in the room. A third surgeon may also be utilized to harvest muscle from the abdomen or lateral thigh. Visualization is key to management and the endoscope lens must remain free of blood. To achieve this, the second surgeon introduces the suction down the side with most bleeding, clearing the majority of the blood and allowing the first surgeon to advance the second suction just ahead of the endoscope down the other nostril and into the surgical field. The second surgeon's suction is used to direct the blood flow away from the endoscope lens. Once the site of bleeding is visualized, the second surgeon moves the suction directly over the bleeding point of the vessel. This suction is hovered over the bleed point collecting all the blood from the injury. The need to have space in the surgical field for both surgeons to operate is important so that when the surgical approach to the lesion is planned creation of such space should be part of the approach. Routine removal of the posterior septum and floor of sphenoid to create a single posterior cavity is helpful as the surgeons may use both the nostrils to pass instruments and the endoscope.

Once visualization has been achieved, pressure can be applied to the bleeding point with patties, gauze, or Surgicel snow as a temporizing measure. It is tempting to pack the entire nasopharynx in an attempt to stop the bleeding, but this should be avoided as much as possible as it impedes access to the bleeding point. Depending on the point reached in the operation, this practice may stop the bleeding from exiting the nose, but blood may be directed intracranially with detrimental results. Overpacking may also completely occlude the ICA and any other vessels exposed with potentially devastating results. In theory, acute unilateral ICA blood flow disruption should result in contralateral ICA and vertebral artery flow reaching the hemispheres through the circle of Willis. In practice, however, this is highly variable. Techniques to control hemorrhage in endoscopic skull base surgery must take this factor into account and maintain patency of flow through the ICA as much as possible. The evidence from neurosurgical literature regarding the temporary proximal clipping of feeding vessels indicates that interruption to the blood supply of less than 3 to 5 minutes is generally well tolerated with the brain likely deriving oxygen and glucose from collateral perfusion; however, longer times risk permanent neuron and astrocyte death.[29-31] Care should also be taken when extrapolating these data to entire anterior circulation or whole hemisphere models that would occur with sudden, complete ICA occlusion.

Options for Hemostasis

Traditionally, 1 to 2 cm^3 of the muscle has been harvested from the abdomen or thigh. This is crushed between two hard surfaces, placed over the bleeding point, and held in place for at least 5 to 7 minutes (although this may take up to 12 minutes to adhere).[4-7] It is important to hold this in place with sufficient pressure to stem the bleeding but not to completely occlude the artery (**Fig. 12.3**). The crushed muscle is thought to activate a platelet/fibrin plug that seals the artery while the high flow within the lumen maintains patency of the parent vessel.[32] No attempt should be made to remove the muscle once hemostasis has been achieved. This muscle patch should be reinforced with oxidized cellulose, and if the carotid injury is within the sphenoid sinus, a pedicled septal flap is placed over the injury. If the lesion is intracranial, the combination of oxidized cellulose and fibrin glue is used.[24]

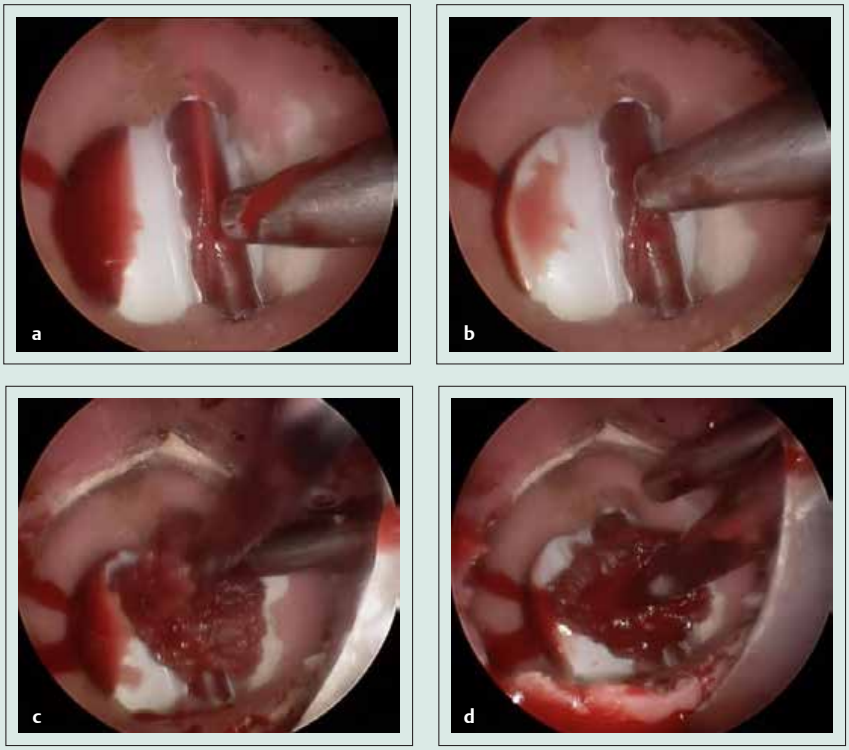


Fig. 12.3 Hemorrhage control in a sheep model. **(a)** Blood jet from internal carotid artery. **(b)** Sucker in contralateral nostril used to direct flow away from endoscope lens. **(c)** Crushed muscle patch being applied. **(d)** Crushed muscle held in place for 5 to 7 minutes.

If the lesion is in the sphenoid, nasal packing may then be placed; however, it is important that this is not packed too tightly as this may occlude distal ICA flow. This method is suitable for both linear and stellate or jagged-type injuries.

If the injury is clearly able to be visualized, accessed with instruments, direct vessel closure may be attempted. This is usually more applicable for linear injuries. Padhye et al have shown in a sheep model of hemorrhage that a curved T2 aneurysm clip may be applied to the wall of the vessel without occluding the lumen.[9] Direct closure may also be achieved with a "pincer"-type metal clip (AnastoClip, LeMaitre Vascular, MA, United States). The vessel walls are held together by a Wormald endovascular clamp or aneurysm clip and the AnastoClips applied sequentially along the injury (**Fig. 12.4**).

Current and Future Studies in Animal Models

There are several other commercially available products that have been trialed with varying degrees of success. In general, the authors have found that the high-flow/high-pressure bleed will tend to wash away thrombin-based powders such as Floseal (Baxter, IL, United States) or Surgiflow (Ethicon, NJ, United States) and fibrin/thrombin glues. Animals trials within the author's department have demonstrated that it is possible to stop carotid bleeding with fibrin/thrombin glue, however, only if the initial blood jet is able to be controlled with Surgicel snow and the glue is then able to be applied

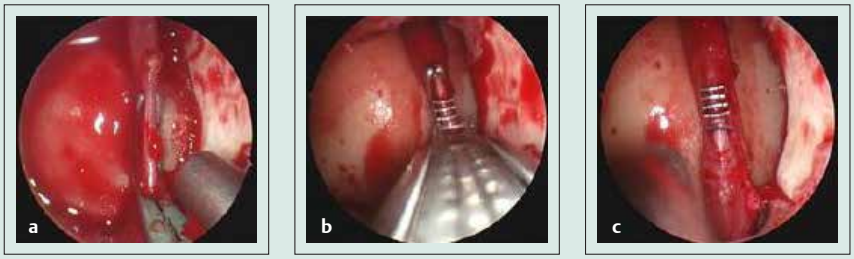


Fig. 12.4 **(a)** Wormald vascular clamp across carotid laceration in a sheep model of hemorrhage. **(b)** AnastoClips being applied with clamp in situ. **(c)** AnastoClips post clamp removal.[11]

without being washed away. Pressure is applied with instruments for 5 to 7 minutes. The authors do not yet have data of the long-term efficacy of this or the rate of pseudoaneurysm formation. Another option is human-derived fibrin/thrombin embedded in a collagen-based patch such as Tachosil (Baxter). This has an active and nonactive side, and the authors have found it useful to fold this over upon itself so that all sides are effectively hemostatic. Again, the authors have found this to work in an animal model but, as yet, have no long-term data of its efficacy or pseudoaneurysm formation.

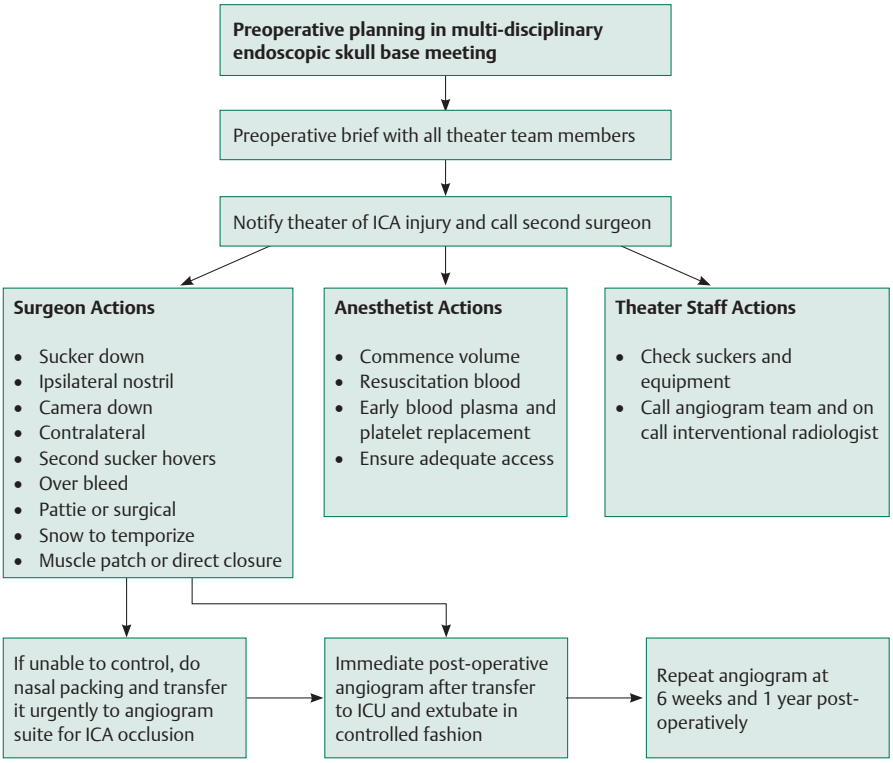
Bipolar cautery devices have been designed specifically for endoscopic endonasal surgery and attempts have been made to use this technique on carotid injury.[33] While this may work for smaller bleeds, there is a risk of the cautery device becoming adherent to the bleeding vessel with subsequent tearing when attempts are made to remove the instrument. There is some experimental evidence to suggest a higher rate of secondary bleeds and complete vessel occlusion than other methods and the authors do not recommend its use in ICA bleeding.[24]

Endovascular Control

If hemostasis is not able to be achieved with these methods, endovascular intervention may be urgently required to occlude the ICA. It is important that endoscopic techniques are attempted first as endovascular intervention tends to result in artery sacrifice. Balloon or coil occlusion should be performed at the site of injury to prevent ongoing hemorrhage from antegrade or retrograde filling. It is also important to avoid balloon or coil migration and subsequent occlusion of the ophthalmic artery (a complication discussed later). It is important to emphasize that endovascular intervention at this point is a lifesaving procedure and that a presacrifice balloon test occlusion is not likely to change management at this stage, in contrast to the elective setting. **Flowchart 12.1** shows hemorrhage control in ICA injury.

Postoperative Management

Once hemostasis has been achieved, the patient should undergo a formal angiogram to determine perfusion through the site of injury, ongoing bleeding, and whether a dissection, pseudoaneurysm, or carotid-cavernous fistula has formed.[27] It is advisable to perform this immediately under the same anesthetic to avoid blood pressure fluctuation or Valsalva being performed by the patient on emergence and extubation, which may potentially cause the patch to "blow off" the vessel. The surgeon should attend the initial formal angiogram to determine whether the ICA is patent, especially if large amounts of nasal packing have been left in situ. If this is "overpacked," the patient may need some of this removed to allow distal flow in the ICA. It is important to note that while the authors have discussed the obvious and immediate ICA bleed, a smaller, unrecognized injury may occur and manifest in the weeks or months following surgery.



Flowchart 12.1 Hemorrhage control in internal carotid artery injury. ICA, internal carotid artery.

The decision to extubate the patient depends on whether any vascular anomaly is discovered at the initial postoperative angiogram. If none is discovered, a reasonable option is to wake the patient slowly in intensive care unit (ICU), avoiding blood pressure fluctuations, and ensuring that cerebral perfusion is maintained.

If an arterial wall injury such as a dissection flap is seen on formal angiogram, it is reasonable to assume that the intima has been disrupted with potential for thrombosis and subsequent embolism to occur. Some authors advocate intravenous heparin administration at the time of injury.[27] Much of the rationale behind this has been extrapolated from stroke literature, which tends to limit external validity when applying these data to the ICA injury patient population. The Cervical Artery Dissection in Stroke Study (CADISS) trial, designed to determine the optimal treatment for carotid dissection, was unable to recommend whether antiplatelet agents or anticoagulant agents should be used.[34] Author's practice has been to wait until the immediate postoperative angiogram to make a judgment regarding the risks and benefits of anticoagulation at this point.

Complications

After an ICA injury there is the potential for the development of a pseudoaneurysm. The putative mechanism of development is damage to the vessel in the initial phase with subsequent arterial pulsations forcing blood between layers (sometimes known as *false aneurysm*) or, more commonly in this context, between the damaged vessel and the patch or packing that has been applied.

This grows larger over time as the systolic pressure forces further tracking of blood along this dissection layer.[35] The most common etiology worldwide is in penetrating cranial trauma such as stabbing or shrapnel/bullet wounds and the majority of the literature supporting treatment is extrapolated from this.[36,37] Post-ICA injury, it is generally considered that the incidence of subsequent pseudoaneurysm development is in the order of 10 to 35%; however, some series report up to 66%.[8,11,38,39] This is partly a function of the low case numbers in each case series reported and partly because no reliable animal model of this exists. A pooled analysis of case series would seem to indicate that a reasonable estimate would make the rate somewhere between 10 and 35% across all patients with a patent ICA.[38] The formal angiogram should be repeated; authors advocate for this at 1 week postoperatively and then again at 6 weeks and 1 year postoperatively.[8,9,40-43]

The treatment of pseudoaneurysm is dependant on size, location, and whether it has ruptured prior to treatment. The pseudoaneurysm is usually coiled and/or the artery stented as first-line management (**Fig. 12.5**). Given that even a balloon test occlusion cannot reliably predict a patient's reliance on blood flow from a particular carotid artery, consideration should be given to preserving the carotid, if possible.[44] It is possible to perform an endovascular sacrifice of the carotid artery by completely occluding it with coils; however, this leaves the patient at risk of developing an infarction distal to this.[27] The most common intervention is stenting of the parent vessel. Direct open repair and remodeling can be considered but is generally not possible in the cavernous segment of the carotid.[9,42,43,45-50] It may also be possible to use a flow-diverting stent that directs flow beyond the pseudoaneurysm and causes stasis and subsequent thrombosis within the aneurysm.[45] It is important to be aware of the location of the ophthalmic artery in relation to the pseudoaneurysm. The ophthalmic artery typically leaves the ICA a few millimeters beyond any injury to the cavernous segment and stent migration may occur, occluding this artery and resulting in potential blindness or even death. Additionally, rates of stroke following carotid stenting can be as high as 4.5% in the first 30 days and patients are generally treated with dual-antiplatelet agents (aspirin and clopidogrel) or similar agents that may delay or preclude further surgery.[22,23,51-54]

Another vascular complication is the development of a carotid—cavernous fistula. In this situation, arterial blood escapes from a defect in the ICA and enters directly into the venous channels of the cavernous sinus. Symptoms and signs of this include proptosis, chemosis, conjunctival injection, ocular bruit, blindness due to venous hypertension, ophthalmoplegia due to cranial nerve defects, pulsatile tinnitus, venous infarct of the cortex, and pain.[55] The initial imaging is often CT or MRI that demonstrate characteristic features of proptosis and "corkscrewing" of engorged vessels; however, diagnosis requires formal angiogram and demonstration of early venous filling as blood exits the ICA.[55-58] Treatment is performed via endovascular approach with obliteration of the fistula via coils, glue, stenting, or combination of these.[40,55,56,58-60]

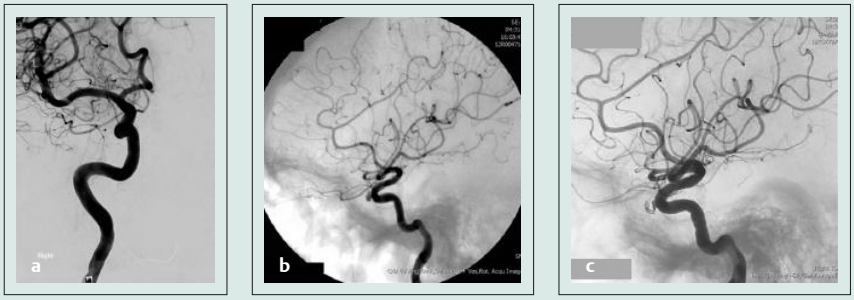


Fig. 12.5 Pseudoaneurysm post internal carotid artery injury. **(a)** Initial angiogram with no aneurysm. **(b)** Angiogram at 1 week showing cavernous segment pseudoaneurysm. **(c)** Coiling of pseudoaneurysm.

Conclusion

ICA injury is a potentially devastating complication of endoscopic endonasal surgery. It is crucial to have a plan in place before this potentially disastrous situation occurs. Good communication and teamwork is vital to ensure that the situation is rapidly brought under control. It is helpful to have a trained team for this eventuality and ensure that team members' roles are clearly defined before this situation is encountered. Training has been shown to improve the outcome for injuries managed by surgeons subsequent to training.[9,24] The muscle patch is certainly the simplest and most studied hemostatic material utilized in this setting; however, if the injury is able to be both visualized and accessed by instruments, direct laceration closure with AnastoClip (LeMaitre vascular) or a curved T2 aneurysm clip may be attempted. It is important that whatever technique is used, that vessel lumen patency is maintained and ongoing flow to allow distal perfusion of the brain occurs. Endovascular sacrifice of the vessel is the last resort and one that may potentially lead to neurologic deficit, even if one had performed a balloon test occlusion previously. It is important to realize that stopping the hemorrhage is merely the first step in the management of these patients and they will require ongoing angiographic follow-up to ensure that they do not develop pseudoaneurysm, carotid-cavernous fistulae, or have a delayed ICA rupture. It is crucial that endoscopic, endonasal skull base operations, even those that are considered comparatively simple, are performed by a dedicated skull base team and that all cases are discussed and individualized risk analysis is performed preoperatively.

References

1. Sindou M. Practical Handbook of Neurosurgery Sindou M, editor. Heidelberg, Germany: Springer-Verlag; 2009
2. Koutourousiou M, Snyderman CH, Fernandez-Miranda J, Gardner PA. Skull base chordomas. Otolaryngol Clin North Am 2011;44(5):1155–1171
3. Rhoton AL Jr. The anterior and middle cranial base. Neurosurgery 2002; 51(4, Suppl)S273–S302
4. Valentine R, Wormald PJ. A Vascular Catastrophe during Endonasal Surgery: An Endoscopic Sheep Model. Skull Base 2011;21(2):109–114
5. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. Laryngoscope 2011;121(3):562–566
6. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. Int Forum Allergy Rhinol 2011;1(2):118–122
7. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. Otolaryngol Clin North Am 2011;44(5):1059–1079
8. Padhye V, Valentine R, Paramasivan S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. Int Forum Allergy Rhinol 2014;4(8):651–657
9. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. Int Forum Allergy Rhinol 2015;5(3):253–257
10. Couldwell WTWM, Weiss MH, Rabb C, Liu JK, Apfelbaum RI, Fukushima T. Variations on the standard transsphenoidal approach to the sellar region, with emphasis on the extended approaches and parasellar approaches: surgical experience in 105 cases. Neurosurgery 2004;55(3):539–547, discussion 547–550
11. Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. Neurosurgery 2013; 73(2, Suppl Operative)ons261–ons269, discussion ons269–ons270
12. Dehdashti AR, Karabatsou K, Ganna A, Witterick I, Gentili F. Expanded endoscopic endonasal approach for treatment of clival chordomas: early results in 12 patients. Neurosurgery 2008;63(2):299–307, discussion 307–309
13. Cavallo LM, Solari D, Esposito F, Cappabianca P. The endoscopic endonasal approach for the management of craniopharyngiomas involving the third ventricle. Neurosurg Rev 2013;36(1):27–37, discussion 38
14. Ciric I, Ragin A, Baumgartner C, Pierce D. Complications of transsphenoidal surgery: results of a national survey, review of the literature, and personal experience. Neurosurgery 1997;40(2):225–236, discussion 236–237
15. Weidenbecher M, Huk WJ, Iro H. Internal carotid artery injury during functional endoscopic sinus surgery and its management. Eur Arch Otorhinolaryngol 2005;262(8):640–645
16. Rhoton AL Jr. The cavernous sinus, the cavernous venous plexus, and the carotid collar. Neurosurgery 2002; 51(4, Suppl)S375–S410
17. Joo W, Funaki T, Yoshioka F, Rhoton AL Jr. Microsurgical anatomy of the carotid cave. Neurosurgery 2012; 70(2, Suppl Operative)300–311, discussion 311–312

18. Peris-Celda M, Da Roz L, Monroy-Sosa A, Morishita T, Rhoton AL Jr. Surgical anatomy of endoscope-assisted approaches to common aneurysm sites. Neurosurgery 2014;10(Suppl 1):121–144, discussion 144
19. Larson JJ, Tew JM Jr, Tomsick TA, van Loveren HR. Treatment of aneurysms of the internal carotid artery by intravascular balloon occlusion: long-term follow-up of 58 patients. Neurosurgery 1995;36(1):26–30, discussion 30 (discussion)
20. Brown B, Hanel RA. Endovascular management of cavernous and paraclinoid aneurysms. Neurosurg Clin N Am 2014;25(3):415–424
21. Yamada S, Yamada SM, Hirohata T, et al. Endoscopic extracapsular removal of pituitary adenoma: the importance of pretreatment of an adjacent unruptured internal carotid artery aneurysm. Case Rep Neurol Med 2012;2012:891847
22. Hamasaki O, Ikawa F, Hidaka T, Kurokawa Y, Yonezawa U. Extracranial internal carotid artery pseudoaneurysm associated with neurofibromatosis type 1 treated with endovascular stenting and coil embolization. Vasc Endovascular Surg 2014;48(2):176–179
23. Berne JD, Reuland KR, Villarreal DH, McGovern TM, Rowe SA, Norwood SH. Internal carotid artery stenting for blunt carotid artery injuries with an associated pseudoaneurysm. J Trauma 2008;64(2):398–405
24. Padhye V, Valentine R, Wormald PJ. Management of carotid artery injury in endonasal surgery. Int Arch Otorhinolaryngol 2014;18(Suppl 2):S173–S178
25. Chaurasia PK, Singh D, Meher S, Saran RK, Singh H. Epistaxis as first clinical presentation in a child with giant prolactinoma: Case report and review of literature. J Pediatr Neurosci 2011;6(2):134–137
26. Chuang CC, Chen YL, Pai PC. A giant intracavernous carotid artery aneurysm embedded in pituitary macroadenoma presenting with pituitary apoplexy. Cerebrovasc Dis 2006;21(1-2):142–144
27. AlQahtani A, Castelnuovo P, Nicolai P, Prevedello DM, Locatelli D, Carrau RL. Injury of the Internal Carotid Artery During Endoscopic Skull Base Surgery: Prevention and Management Protocol. Otolaryngol Clin North Am 2016;49(1):237–252
28. Chin OY, Ghosh R, Fang CH, Baredes S, Liu JK, Eloy JA. Internal carotid artery injury in endoscopic endonasal surgery: A systematic review. Laryngoscope 2016;126(3):582–590
29. Andrade-Barazarte H, Luostarinen T, Goehre F, et al. Transient Cardiac Arrest Induced by Adenosine: A Tool for Contralateral Clipping of Internal Carotid Artery-Ophthalmic Segment Aneurysms. World Neurosurg 2015; 84(6):1933–1940
30. Lee SH, Kwun BD, Kim JU, et al. Adenosine-induced transient asystole during intracranial aneurysm surgery: indications, dosing, efficacy, and risks. Acta Neurochir (Wien) 2015;157(11):1879–1886, discussion 1886
31. Rangel-Castilla L, Russin JJ, Britz GW, Spetzler RF. Update on transient cardiac standstill in cerebrovascular surgery. Neurosurg Rev 2015;38(4):595–602
32. Rajiv S, Rodgers S, Bassiouni A, Vreugde S, Wormald PJ. Role of crushed skeletal muscle extract in hemostasis. Int Forum Allergy Rhinol 2015;5(5):431–434
33. Hernesniemi J, Romani R, Niemelä M. Skull base and aneurysm surgery. Surg Neurol 2009;71(1):30–31
34. Markus HS, Hayter E, Levi C, Feldman A, Venables G, Norris J; CADISS trial investigators. Antiplatelet treatment compared with anticoagulation treatment for cervical artery dissection (CADISS): a randomised trial. Lancet Neurol 2015;14(4):361–367
35. Nazari P, Kasliwal MK, Wewel JT, Dua SG, Chen M. Delayed Intracerebral Hemorrhage from a Pseudoaneurysm Following a Depressed Skull Fracture. Neurointervention 2016;11(1):42–45
36. Dubey A, Sung WS, Chen YY, et al. Traumatic intracranial aneurysm: a brief review. J Clin Neurosci 2008; 15(6):609–612
37. Kim KA, Wang MY, McNatt SA, et al. Vector analysis correlating bullet trajectory to outcome after civilian through-and-through gunshot wound to the head: using imaging cues to predict fatal outcome. Neurosurgery 2005;57(4):737–747, discussion 737–747
38. Gardner PA, Vaz-Guimaraes F, Jankowitz B, et al. Endoscopic Endonasal Clipping of Intracranial Aneurysms: Surgical Technique and Results. World Neurosurg 2015;84(5):1380–1393
39. Kassam AB, Prevedello DM, Carrau RL, et al. The front door to meckel's cave: an anteromedial corridor via expanded endoscopic endonasal approach- technical considerations and clinical series. Neurosurgery 2009; 64(3, Suppl)ons71–ons82, discussion ons82–ons83
40. Iancu D, Lum C, Ahmed ME, et al. Flow diversion in the treatment of carotid injury and carotid-cavernous fistula after transsphenoidal surgery. Interv Neuroradiol 2015;21(3):346–350
41. Griauzde J, Gemmete JJ, Pandey AS, McKean EL, Sullivan SE, Chaudhary N. Emergency reconstructive endovascular management of intraoperative complications involving the internal carotid artery from trans-sphenoidal surgery. J Neurointerv Surg 2015;7(1):67–71
42. Nerva JD, Morton RP, Levitt MR, et al. Pipeline Embolization Device as primary treatment for blister aneurysms and iatrogenic pseudoaneurysms of the internal carotid artery. J Neurointerv Surg 2015;7(3):210–216
43. Lee CH, Chen SM, Lui TN. Posterior Cerebral Artery Pseudoaneurysm, a Rare Complication of Pituitary Tumor Transsphenoidal Surgery: Case Report and Literature Review. World Neurosurg 2015;84(5):1493.e1–1493.e3
44. Griessenauer CJ, Foreman P, Deveikis JP, Harrigan MR. Endovascular tools available for the treatment of cerebrovascular disease. Neurosurg Clin N Am 2014;25(3):387–394
45. Giorgianni A, Pellegrino C, Minotto R, et al. Flow-diverter stenting of post-traumatic bilateral anterior cerebral artery pseudoaneurysm: A case report. Interv Neuroradiol 2015;21(1):23–28

46. Munich SA, Cress MC, Rangel-Castilla L, Krishna C, Siddiqui AH, Snyder KV. Importance of repeat angiography in the diagnosis of iatrogenic anterior cerebral artery territory pseudoaneurysm following endoscopic sinus surgery. BMJ Case Rep 2015;2015:201
47. Morken MH, Cappelen J, Kvistad KA, Søvik E, Stensæth KH. Acute endovascular repair of iatrogenic right internal carotid arterial laceration. Acta Radiol Short Rep 2013;2(5):2047981613496088
48. Chen Z, Zhang J, Miao H, Niu Y, Feng H, Zhu G. Delayed rupture of iatrogenic cerebral pseudoaneurysms after neurosurgical procedures: report of two cases. Clin Neurol Neurosurg 2013;115(8):1552–1554
49. Mortazavi MM, Verma K, Tubbs RS, Harrigan M. Pediatric traumatic carotid, vertebral and cerebral artery dissections: a review. Childs Nerv Syst 2011;27(12):2045–2056
50. Brzozowski K, Frankowska E, Piasecki P, Zięcina P, Zukowski P, Bogusławska-Walecka R. The use of routine imaging data in diagnosis of cerebral pseudoaneurysm prior to angiography. Eur J Radiol 2011;80(3):e401–e409
51. Wholey MH, Wholey MH, Eles G. Endovascular procedures for carotid artery occlusive disease. Tech Vasc Interv Radiol 2004;7(4):168–186
52. Zhang J, Zhang X, Guo Q, et al. Surgical treatment of giant fusiform aneurysm of extracranial internal carotid artery in a child: 1 case report and literature review. Surg Neurol 2007;68(3):329–333, discussion 334
53. Faggioli GL, Ferri M, Serra C, et al. The residual risk of cerebral embolism after carotid stenting: the complex interplay between stent coverage and aortic arch atherosclerosis. Eur J Vasc Endovasc Surg 2009;37(5):519–524
54. Cohen DJ, Stolker JM, Wang K, et al; CREST Investigators. Health-related quality of life after carotid stenting versus carotid endarterectomy: results from CREST (Carotid Revascularization Endarterectomy Versus Stenting Trial). J Am Coll Cardiol 2011;58(15):1557–1565
55. Barry RC, Wilkinson M, Ahmed RM, et al. Interventional treatment of carotid cavernous fistula. J Clin Neurosci 2011;18(8):1072–1079
56. Wang W, Li YD, Li MH, et al. Endovascular treatment of post-traumatic direct carotid-cavernous fistulas: A single-center experience. J Clin Neurosci 2011;18(1):24–28
57. Berker M, Aghayev K, Saatci I, Palaoğlu S, Onerci M. Overview of vascular complications of pituitary surgery with special emphasis on unexpected abnormality. Pituitary 2010;13(2):160–167
58. Tytle TLP, Kumar P. Carotid cavernous fistula. Seminars in Cerebrovascular Diseases and Stroke. 2001;1(1):83–111
59. Venkataramaiah S, Sriganesh K. Images in clinical medicine. Carotid cavernous fistula. N Engl J Med 2014;371(19):1832
60. Romani R, Elsharkawy A, Laakso A, Kangasniemi M, Hernesniemi J. Tailored anterior clinoidectomy through the lateral supraorbital approach: experience with 82 consecutive patients. World Neurosurg 2012;77(3-4):512–517

References

1. Campero A, Campero AA, Socolovsky M, Martins C, Yasuda A, Basso A, et al. The transzygomatic approach. J Clin Neurosci. 2010;17(11):1428-33.
2. Patel CR, Fernandez-Miranda JC, Wang WH, Wang EW. Skull Base Anatomy. Otolaryngol Clin North Am. 2016;49(1):9-20.
3. de Notaris M, Palma K, Serra L, Ensenat J, Alobid I, Poblete J, et al. A three-dimensional computer-based perspective of the skull base. World Neurosurg. 2014;82(6 Suppl):S41-8.
4. Davies A, Dale O, Renowden S. Spontaneous rupture of an intra-cavernous internal carotid artery aneurysm presenting with massive epistaxis. J Laryngol Otol. 2011;125(10):1070-2.
5. Prestigiacomo CJ, Dagi TF. The history of skull base surgery. Neurosurg Focus. 2012;33(2):Introduction.
6. Ceylan S, Anik I, Koc K, Cabuk B. Extended endoscopic transsphenoidal approach infrachiasmatic corridor. Neurosurg Rev. 2015;38(1):137-47; discussion 47.
7. Gardner PA, Vaz-Guimaraes F, Jankowitz B, Koutourousiou M, Fernandez-Miranda JC, Wang EW, et al. Endoscopic Endonasal Clipping of Intracranial Aneurysms: Surgical Technique and Results. World Neurosurg. 2015;84(5):1380-93.
8. Krischek B, Carvalho FG, Godoy BL, Kiehl R, Zadeh G, Gentili F. From craniofacial resection to endonasal endoscopic removal of malignant tumors of the anterior skull base. World Neurosurg. 2014;82(6 Suppl):S59-65.
9. Koutourousiou M, Snyderman CH, Fernandez-Miranda J, Gardner PA. Skull base chordomas. Otolaryngol Clin North Am. 2011;44(5):1155-71.
10. Panzer S, Borumandi F, Wanek J, Papageorgopoulou C, Shved N, Colacicco G, et al. "Modeling ancient Egyptian embalming": radiological assessment of experimentally mummified human tissue by CT and MRI. Skeletal Radiol. 2013;42(11):1527-35.
11. Saleem SN, Hawass Z. Variability in brain treatment during mummification of royal Egyptians dated to the 18th-20th dynasties: MDCT findings correlated with the archaeologic literature. AJR Am J Roentgenol. 2013;200(4):W336-44.
12. Wade AD, Nelson AJ. Radiological evaluation of the evisceration tradition in ancient Egyptian mummies. Homo. 2013;64(1):1-28.
13. Wade AD, Nelson AJ, Garvin GJ. A synthetic radiological study of brain treatment in ancient Egyptian mummies. Homo. 2011;62(4):248-69.
14. Schmidt RF, Choudhry OJ, Takkellapati R, Eloy JA, Couldwell WT, Liu JK. Hermann Schloffer and the origin of transsphenoidal pituitary surgery. Neurosurg Focus. 2012;33(2):E5.
15. Scott Stevenson RG, Douglas A History of Oto-Laryngology. Edinburgh: ES Livingstone; 1949.
16. Bedrosian JC, Anand VK, Schwartz TH. The endoscopic endonasal approach to repair of iatrogenic and noniatrogenic cerebrospinal fluid leaks and encephaloceles of the anterior cranial fossa. World Neurosurg. 2014;82(6 Suppl):S86-94.
17. Cavallo LM, Solari D, Esposito F, Cappabianca P. The endoscopic endonasal approach for the management of craniopharyngiomas involving the third ventricle. Neurosurg Rev. 2013;36(1):27-37; discussion 8.

18. Kasemsiri P, Carrau RL, Ditzel Filho LF, Prevedello DM, Otto BA, Old M, et al. Advantages and limitations of endoscopic endonasal approaches to the skull base. World Neurosurg. 2014;82(6 Suppl):S12-21.
19. Stokken JK, Halderman A, Recinos PF, Woodard TD, Sindwani R. Strategies for Improving Visualization During Endoscopic Skull Base Surgery. Otolaryngol Clin North Am. 2016;49(1):131-40.
20. Zacharia BE, Romero FR, Rapoport SK, Raza SM, Anand VK, Schwartz TH. Endoscopic Endonasal Management of Metastatic Lesions of the Anterior Skull Base: Case Series and Literature Review. World Neurosurg. 2015;84(5):1267-77.
21. Zhang Q, Wang Z, Guo H, Kong F, Chen G, Bao Y, et al. Resection of anterior cranial base meningiomas with intra- and extracranial involvement via a purely endoscopic endonasal approach. ORL J Otorhinolaryngol Relat Spec. 2012;74(4):199-207.
22. de Almeida JR, Vescan AD. Outcomes and Quality of Life Following Skull Base Surgery. Current Otorhinolaryngology Reports. 2013;1(4):214-20.
23. Snyderman CP, H; Carrau, R; Prevedello, D; Gardner, P; Kassam, A. What Are the Limits of Endoscopic Sinus Surgery?: The Expanded Endonasal Approach to the Skull Base. Keio J Med. 2009;58(3):152-60.
24. Romani R, Elsharkawy A, Laakso A, Kangasniemi M, Hernesniemi J. Tailored anterior clinoidectomy through the lateral supraorbital approach: experience with 82 consecutive patients. World Neurosurg. 2012;77(3-4):512-7.
25. Bien AG, Bowdino B, Moore G, Leibrock L. Utilization of preoperative cerebrospinal fluid drain in skull base surgery. Skull Base. 2007;17(2):133-9.
26. Cho JM, Ahn JY, Chang JH, Kim SH. Prevention of cerebrospinal fluid rhinorrhea after transsphenoidal surgery by collagen fleece coated with fibrin sealant without autologous tissue graft or postoperative lumbar drainage. Neurosurgery. 2011;68(1 Suppl Operative):130-6; discussion 6-7.
27. Hirono S, Kawauchi D, Higuchi Y, Setoguchi T, Kihara K, Horiguchi K, et al. Life-Threatening Intracranial Hypotension after Skull Base Surgery with Lumbar Drainage. J Neurol Surg Rep. 2015;76(1):e83-6.
28. Sugita K. Microneurosurgical Atlas. Germany: Springer-Verlag; 1985.
29. Sindou M. Practical Handbook of Neurosurgery Sindou M, editor. Heidelberg, Germany: Springer-Verlag; 2009.
30. Di Ieva A, Tam M, Tschabitscher M, Cusimano MD. A journey into the technical evolution of neuroendoscopy. World Neurosurg. 2014;82(6):e777-89.
31. Zada G, Liu C, Apuzzo ML. "Through the looking glass": optical physics, issues, and the evolution of neuroendoscopy. World Neurosurg. 2013;79(2 Suppl):S3-13.
32. Karppinen A, Kivipelto L, Vehkavaara S, Ritvonen E, Tikkanen E, Kivisaari R, et al. Transition From Microscopic to Endoscopic Transsphenoidal Surgery for Nonfunctional Pituitary Adenomas. World Neurosurg. 2015;84(1):48-57.
33. Schroeder HW. General principles and intraventricular neuroendoscopy: endoscopic techniques. World Neurosurg. 2013;79(2 Suppl):S14 e23-8.
34. Ammirati M, Wei L, Ciric I. Short-term outcome of endoscopic versus microscopic pituitary adenoma surgery: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2013;84(8):843-9.
35. Duz B, Harman F, Secer HI, Bolu E, Gonul E. Transsphenoidal approaches to the pituitary: a progression in experience in a single centre. Acta Neurochir (Wien). 2008;150(11):1133-8; discussion 8-9.

36. Horowitz PM, DiNapoli V, Su SY, Raza SM. Complication Avoidance in Endoscopic Skull Base Surgery. Otolaryngol Clin North Am. 2016;49(1):227-35.
37. Gaab MR. Instrumentation: endoscopes and equipment. World Neurosurg. 2013;79(2 Suppl):S14 e1-21.
38. Kahilogullari G, Beton S, Al-Beyati ES, Kantarcioglu O, Bozkurt M, Kantarcioglu E, et al. Olfactory functions after transsphenoidal pituitary surgery: endoscopic versus microscopic approach. Laryngoscope. 2013;123(9):2112-9.
39. Razak AA, Horridge M, Connolly DJ, Warren DJ, Mirza S, Muraleedharan V, et al. Comparison of endoscopic and microscopic trans-sphenoidal pituitary surgery: early results in a single centre. Br J Neurosurg. 2013;27(1):40-3.
40. Takemura Y, Inoue T, Morishita T, Rhoton AL, Jr. Comparison of microscopic and endoscopic approaches to the cerebellopontine angle. World Neurosurg. 2014;82(3-4):427-41.
41. Quinones-Hinojosa A, Ware ML, Sanai N, McDermott MW. Assessment of image guided accuracy in a skull model: comparison of frameless stereotaxy techniques vs. frame-based localization. J Neurooncol. 2006;76(1):65-70.
42. Dixon BJ, Chan H, Daly MJ, Vescan AD, Witterick IJ, Irish JC. The effect of augmented real-time image guidance on task workload during endoscopic sinus surgery. Int Forum Allergy Rhinol. 2012;2(5):405-10.
43. Mert A, Micko A, Donat M, Maringer M, Buehler K, Sutherland GR, et al. An advanced navigation protocol for endoscopic transsphenoidal surgery. World Neurosurg. 2014;82(6 Suppl):S95-105.
44. Garcia AS, Rhoton AL, Jr. Speculum opening in transsphenoidal surgery. Neurosurgery. 2006;59(1 Suppl 1):ONS35-40; discussion ONS35-40.
45. Alfieri AH-D, J. Endoscopic Endonasal Cavernous Sinus Surgery: An Anatomic Study. Neurosurgery. 2001;48(4):827-37.
46. Yasuda A, Campero A, Martins C, Rhoton AL, Ribas GC. The Medial Wall of the Cavernous Sinus: Microsurgical Anatomy. Neurosurgery. 2004;55(1):179-90.
47. Chen LH, X; Mao, Y; Zhou, L. Reconsideration of Cavernous Sinus Surgeries. Neurosurgery. 2014;61(1):130-4.
48. Essayed WI, Singh H, Lapadula G, Almodovar-Mercado GJ, Anand VK, Schwartz TH. Endoscopic endonasal approach to the ventral brainstem: anatomical feasibility and surgical limitations. J Neurosurg. 2017;127(5):1139-46.
49. Bhatki AM, Pant H, Snyderman CH, Carrau RL, Kassam AB, Prevedello DM, et al. Reconstruction of the cranial base after endonasal skull base surgery: Local tissue flaps. Operative Techniques in Otolaryngology-Head and Neck Surgery. 2010;21(1):74-82.
50. Rhoton A. The Anterior and Middle Cranial Base. Neurosurgery. 2002;51(Supp1):273-302.
51. Castelnuovo P, Dallan I, Tschabitscher M. Skull Base Segment. 2013:59-80.
52. de Lara D, Ditzel Filho LF, Prevedello DM, Carrau RL, Kasemsiri P, Otto BA, et al. Endonasal endoscopic approaches to the paramedian skull base. World Neurosurg. 2014;82(6 Suppl):S121-9.
53. Rhoton A. The Cavernous Sinus, The Cavernous Venous Plexus, and the Carotid Collar. Neurosurgery. 2002;51(Supp1)(1):375-410.
54. Saito T, Yoneoka Y, Watanabe J, Kikuchi B, Hasegawa H, Fujii Y. Unruptured medial paraclinoid internal carotid artery aneurysm embedded within symptomatic nonfunctioning

pituitary giant adenoma: A high-risk comorbid pathology in transsphenoidal surgery and its countermeasures. Interdisciplinary Neurosurgery. 2017;10:138-41.
55. George B, Ferrario CA, Blanquet A, Kolb F. Cavernous Sinus Exenteration for Invasive Cranial Base Tumors. Neurosurgery. 2003;52(4):772-82.
56. Matsuo S, Komune N, Iihara K, Rhoton AL. Translateral Orbital Wall Approach to the Orbit and Cavernous Sinus. Operative Neurosurgery. 2015:1.
57. Barges-Coll J, Fernandez-Miranda JC, Prevedello DM, Gardner P, Morera V, Madhok R, et al. Avoiding injury to the abducens nerve during expanded endonasal endoscopic surgery: anatomic and clinical case studies. Neurosurgery. 2010;67(1):144-54; discussion 54.
58. Iancu D, Lum C, Ahmed ME, Glikstein R, Dos Santos MP, Lesiuk H, et al. Flow diversion in the treatment of carotid injury and carotid-cavernous fistula after transsphenoidal surgery. Interv Neuroradiol. 2015;21(3):346-50.
59. Griessenauer CJ, Mortazavi MM, Loukas M, Shoja MM, Watanabe K, Tubbs RS. Heinrich Bircher (1850-1923) and the first description of a surgical approach to the cavernous sinus. Childs Nerv Syst. 2013;29(10):1923-5.
60. Anqi X, Zhang S, Jiahe X, Chao Y. Cavernous sinus cavernous hemangioma: imaging features and therapeutic effect of Gamma Knife radiosurgery. Clin Neurol Neurosurg. 2014;127:59-64.
61. Hayashi M, Chernov M, Tamura N, Nagai M, Yomo S, Ochiai T, et al. Gamma Knife robotic microradiosurgery of pituitary adenomas invading the cavernous sinus: treatment concept and results in 89 cases. J Neurooncol. 2010;98(2):185-94.
62. Metellus P, Regis J, Muracciole X, Fuentes S, Dufour H, Nanni I, et al. Evaluation of Fractionated Radiotherapy and Gamma Knife Radiosurgery in Cavernous Sinus Meningiomas: Treatment Strategy. Neurosurgery. 2005;57(5):873-86.
63. Tang X, Wu H, Wang B, Zhang N, Dong Y, Ding J, et al. A new classification and clinical results of Gamma Knife radiosurgery for cavernous sinus hemangiomas: a report of 53 cases. Acta Neurochir (Wien). 2015;157(6):961-9; discussion 9.
64. Iaconetta G, de Notaris M, Cavallo LM, Benet A, Ensenat J, Samii M, et al. The oculomotor nerve: microanatomical and endoscopic study. Neurosurgery. 2010;66(3):593-601; discussion
65. Solares CA, Ong YK, Carrau RL, Fernandez-Miranda J, Prevedello DM, Snyderman CH, et al. Prevention and management of vascular injuries in endoscopic surgery of the sinonasal tract and skull base. Otolaryngol Clin North Am. 2010;43(4):817-25.
66. Joo W, Funaki T, Yoshioka F, Rhoton AL, Jr. Microsurgical anatomy of the carotid cave. Neurosurgery. 2012;70(2 Suppl Operative):300-11; discussion 11-2.
67. Castelnuovo P, Dallan I, Tschabitscher M. Intracranial Segment. 2013:81-158.
68. Rhoton A. The Supratentorial Arteries. Neurosurgery. 2002;51(Supp1):53-120.
69. Durukan A, Tatlisumak T. Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol Biochem Behav. 2007;87(1):179-97.
70. Wells AJ, Vink R, Blumbergs PC, Brophy BP, Helps SC, Knox SJ, et al. A surgical model of permanent and transient middle cerebral artery stroke in the sheep. PLoS One. 2012;7(7):e42157.
71. Wells AJ, Vink R, Helps SC, Knox SJ, Blumbergs PC, Turner RJ. Elevated Intracranial Pressure and Cerebral Edema following Permanent MCA Occlusion in an Ovine Model. PLoS One. 2015;10(6):e0130512.

72. Raybaud C. Normal and abnormal embryology and development of the intracranial vascular system. Neurosurg Clin N Am. 2010;21(3):399-426.
73. d'Avella E, De Notaris M, Ensenat J, Berenguer J, Gragnaniello C, Mavar M, et al. The extended endoscopic endonasal transplanum transtuberculum approach to the anterior communicating artery complex: anatomic study. Acta Neurochir (Wien). 2015;157(9):1495-503; discussion 503.
74. Peris-Celda M, Da Roz L, Monroy-Sosa A, Morishita T, Rhoton AL, Jr. Surgical anatomy of endoscope-assisted approaches to common aneurysm sites. Neurosurgery. 2014;10 Suppl 1:121-44; discussion 44.
75. Pare JR, Kahn JH. Basic neuroanatomy and stroke syndromes. Emerg Med Clin North Am. 2012;30(3):601-15.
76. Cavallo LM, Solari D, Esposito F, Villa A, Minniti G, Cappabianca P. The role of the endoscopic endonasal route in the management of craniopharyngiomas. World Neurosurg. 2014;82(6 Suppl):S32-40.
77. Lai LT, Morgan MK, Dalgorf D, Bokhari A, Sacks PL, Sacks R, et al. Cadaveric study of the endoscopic endonasal transtubercular approach to the anterior communicating artery complex. J Clin Neurosci. 2014;21(5):827-32.
78. Domenico Solari MAV, MD; Michelangelo De Angelis, MD; Felice Esposito, MD, PhD; Luigi Maria Cavallo, MD, PhD; Paolo Cappabianca, MD. Anatomy and Surgery of the Endoscopic Endonasal Approach to the Skull Base. Translational Medicine. 2012;2(5):36-46.
79. Rhoton A. The cerebellar arteries Neurosurgery 2000;47(3):S29-S68.
80. Rhoton A. Micro-operative techniques. In: Youmans J, editor. Neurological Surgery. 2. Philedelphia: W.B. Saunders Co; 1990. p. 941-91.
81. Tatagiba MS, Roser F, Hirt B, Ebner FH. The retrosigmoid endoscopic approach for cerebellopontine-angle tumors and microvascular decompression. World Neurosurg. 2014;82(6 Suppl):S171-6.
82. Drazin D, Zhuang L, Schievink WI, Mamelak AN. Expanded endonasal approach for the clipping of a ruptured basilar aneurysm and feeding artery to a cerebellar arteriovenous malformation. J Clin Neurosci. 2012;19(1):144-8.
83. Perttu J. Lindsberg M, Johanna Pekkola, MD, Daniel Strbian, MD, MSc (Stroke Med), Tiina Sairanen, MD, MSc, (Stroke Med), Heinrich P. Mattle, MD, Gerhard Schroth, MD. Time window for recanalization in basilar artery occlusion. Speculative synthesis. Neurology 2015;85(November 17):1806-15.
84. Somanna S, Babu RA, Srinivas D, Narasinga Rao KV, Vazhayil V. Extended endoscopic endonasal transclival clipping of posterior circulation aneurysms-an alternative to the transcranial approach. Acta Neurochir (Wien). 2015.
85. Zaidi HA, Awad AW, Chowdhry SA, Fusco D, Nakaji P, Spetzler RF. Microvascular decompression for hemifacial spasm secondary to vertebrobasilar dolichoectasia: surgical strategies, technical nuances and clinical outcomes. J Clin Neurosci. 2015;22(1):62-8.
86. Wells A, Abou-Hamden A. Neurosurgery in Ischemic Stroke. 2014:1-32.
87. Rickards CA. Cerebral Blood-Flow Regulation During Hemorrhage. . Comprehensive Physiology. 2015;5:1585-621.
88. Phillis JOR, M. Energy Utilisation in the Ischaemic/Reperfused Brain. International Review of Neurobiology 2002;51:377-415.
89. Kim KM, Kang HS, Lee WJ, Cho YD, Kim JE, Han MH. Clinical significance of the circle of Willis in intracranial atherosclerotic stenosis. J Neurointerv Surg. 2016;8(3):251-5.

90. Rhoton AL, Jr. The cerebrum. Anatomy. Neurosurgery. 2007;61(1 Suppl):37-118; discussion -9.
91. Castelnuovo P, Dallan I, Tschabitscher M. Cervical Segment. 2013:1-57.
92. Andrade-Barazarte H, Luostarinen T, Goehre F, Kivelev J, Jahromi BR, Ludtka C, et al. Transient Cardiac Arrest Induced by Adenosine: A Tool for Contralateral Clipping of Internal Carotid Artery-Ophthalmic Segment Aneurysms. World Neurosurg. 2015;84(6):1933-40.
93. Lee SH, Kwun BD, Kim JU, Choi JH, Ahn JS, Park W, et al. Adenosine-induced transient asystole during intracranial aneurysm surgery: indications, dosing, efficacy, and risks. Acta Neurochir (Wien). 2015;157(11):1879-86; discussion 86.
94. Rangel-Castilla L, Russin JJ, Britz GW, Spetzler RF. Update on transient cardiac standstill in cerebrovascular surgery. Neurosurg Rev. 2015;38(4):595-602.
95. Fox AJ VF, Pelz DM, Peerless SJ, Ferguson GG, Drake CG, et al. . Use of detachable balloons for proximal artery occlusion in the treatment of unclippable cerebral aneurysms. J Neurosurg 1987;66:40-6.
96. Larson JJ TJJ, Tomsick TA, van Loveren HR. Treatment of aneurysms of the internal carotid artery by intravascular balloon occlusion: long-term follow-up of 58 patients. Neurosurgery. 1995;36:26-30 (discussion).
97. Marshall RS LR, Young WL, Solomon RA, Joshi S, Duong DH, et al. . Clinical utility of quantitative cerebral blood flow measurements during internal carotid artery test occlusions. . Neurosurgery. 2002;50:996-1004.
98. Roski RA SR, Nulsen FE Late complications of carotid ligation in the treatment of intracranial aneurysms. J Neurosurg. 1981;54:583-7.
99. Rhoton AL. Operative Techniques and Instrumentation for Neurosurgery. Neurosurgery. 2003;53(4):907-34.
100. Woertgen C RR, Albert R, Schebesch KM, Ullrich OW. Effects of temporary clipping during aneurysm surgery.
. Neurol Res. 2008;30(June):542-6.
101. Osterhage K, Czorlich P, Burkhardt TR, Rotermund R, Grzyska U, Flitsch J. Symptomatic Vasospasms as a life-threatening complication after transsphenoidal surgery. World Neurosurg. 2017.
102. Duffield S, Whitty SJ. Developing a systemic lessons learned knowledge model for organisational learning through projects. International Journal of Project Management. 2015;33(2):311-24.
103. Li Y, Thimbleby H. Hot cheese: a processed Swiss cheese model. J R Coll Physicians Edinb. 2014;44(2):116-21.
104. Salmon PM, Cornelissen M, Trotter MJ. Systems-based accident analysis methods: A comparison of Accimap, HFACS, and STAMP. Safety Science. 2012;50(4):1158-70.
105. Underwood P, Waterson P. Systems thinking, the Swiss Cheese Model and accident analysis: a comparative systemic analysis of the Grayrigg train derailment using the ATSB, AcciMap and STAMP models. Accid Anal Prev. 2014;68:75-94.
106. de Angelis M, Cappabianca P. Gutta cavat lapidem: the reconstruction of the skull base after endoscopic endonasal surgery. World Neurosurg. 2015;83(2):136-7.
107. Solari D, Chiaramonte C, Di Somma A, Dell'Aversana Orabona G, de Notaris M, Angileri FF, et al. Endoscopic anatomy of the skull base explored through the nose. World Neurosurg. 2014;82(6 Suppl):S164-70.
108. Deopujari CE, Karmarkar VS, Shah NJ. Endoscopic approaches to the craniovertebral junction and odontoid process. World Neurosurg. 2014;82(6 Suppl):S49-53.

109. Castelnuovo P, Battaglia P, Turri-Zanoni M, Tomei G, Locatelli D, Bignami M, et al. Endoscopic endonasal surgery for malignancies of the anterior cranial base. World Neurosurg. 2014;82(6 Suppl):S22-31.
110. Barkhoudarian G, Zada G, Laws ER. Endoscopic endonasal surgery for nonadenomatous sellar/parasellar lesions. World Neurosurg. 2014;82(6 Suppl):S138-46.
111. Kassam AB, Prevedello DM, Carrau RL, Snyderman CH, Gardner P, Osawa S, et al. The front door to meckel's cave: an anteromedial corridor via expanded endoscopic endonasal approach- technical considerations and clinical series. Neurosurgery. 2009;64(3 Suppl):ons71-82; discussion ons-3.
112. Osawa S, Rhoton AL, Jr., Seker A, Shimizu S, Fujii K, Kassam AB. Microsurgical and endoscopic anatomy of the vidian canal. Neurosurgery. 2009;64(5 Suppl 2):385-411; discussion -2.
113. Welch KC, Palmer JN. Intraoperative emergencies during endoscopic sinus surgery: CSF leak and orbital hematoma. Otolaryngol Clin North Am. 2008;41(3):581-96, ix-x.
114. Kabil MS, Shahinian HK. Fully endoscopic supraorbital resection of congenital middle cranial fossa arachnoid cysts: report of 2 cases. Pediatr Neurosurg. 2007;43(4):316-22.
115. Hernesniemi J, Romani R, Niemela M. Skull base and aneurysm surgery. Surg Neurol. 2009;71(1):30-1.
116. Kimpton G, Dabbous B, Leach P. New oral anticoagulant and antiplatelet agents for neurosurgeons. Br J Neurosurg. 2015;29(5):614-21.
117. Valentine R, Wormald PJ. A Vascular Catastrophe during Endonasal Surgery: An Endoscopic Sheep Model. Skull Base. 2011;21(2):109-14.
118. Valentine R, Wormald PJ. Controlling the surgical field during a large endoscopic vascular injury. Laryngoscope. 2011;121(3):562-6.
119. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. Int Forum Allergy Rhinol. 2011;1(2):118-22.
120. Valentine R, Wormald PJ. Carotid artery injury after endonasal surgery. Otolaryngol Clin North Am. 2011;44(5):1059-79.
121. Padhye V, Valentine R, Paramasivan S, Jardeleza C, Bassiouni A, Vreugde S, et al. Early and late complications of endoscopic hemostatic techniques following different carotid artery injury characteristics. Int Forum Allergy Rhinol. 2014;4(8):651-7.
122. Padhye V, Murphy J, Bassiouni A, Valentine R, Wormald PJ. Endoscopic direct vessel closure in carotid artery injury. Int Forum Allergy Rhinol. 2015;5(3):253-7.
123. Berker M, Aghayev K, Saatci I, Palaoglu S, Onerci M. Overview of vascular complications of pituitary surgery with special emphasis on unexpected abnormality. Pituitary. 2010;13(2):160-7.
124. Couldwell WT WM, Rabb C, Liu JK, Apfelbaum RI, Fukushima T:. Variations on the standard transsphenoidal approach to the sellar region, with emphasis on the extended approaches and parasellar approaches: surgical experience in 105 cases. . Neurosurgery 2004;55:539-50.
125. Gardner PA, Tormenti MJ, Pant H, Fernandez-Miranda JC, Snyderman CH, Horowitz MB. Carotid artery injury during endoscopic endonasal skull base surgery: incidence and outcomes. Neurosurgery. 2013;73(2 Suppl Operative):ons261-9; discussion ons9-70.
126. Dehdashti AK, K ; Ganna, A ; Witterick, I ; Gentili, F ; Cappabianca, P ; Schwartz,Th ; Prevedello, DM ; Gardner, PA ; Kassam, AB ; Frank, G. Expanded endoscopic endonasal

approach for treatment of clival chordomas: Early results in 12 patients. Neurosurgery. 2008;63(2):299-309.
127. Ciric IR, Ann; Baumgartner, Craig; Pierce, Debi. Complications of Transsphenoidal Surgery: Results of a National Survey, Review of the Literature, and Personal Experience. Neurosurgery. 1997;40(2):225-37.
128. Padhye VV, R; Wormald, PJ. Management of Carotid Artery Injury in Endonasal Surgery. Int Arch Otorhinolaryngol 2014;18(S02):S173-S8.
129. Jukes AK, Mascarenhas A, Murphy J, Stepan L, Muñoz TN, Callejas CA, et al. Stress response and communication in surgeons undergoing training in endoscopic management of major vessel hemorrhage: a mixed methods study. International Forum of Allergy & Rhinology. 2017.
130. Weidenbecher M, Huk WJ, Iro H. Internal carotid artery injury during functional endoscopic sinus surgery and its management. Eur Arch Otorhinolaryngol. 2005;262(8):640-5.
131. Gowdh NS, Gill FJ, Regan LA, Wilkie SW. Kissing carotid arteries: an unusual cause of prevertebral swelling. BMJ Case Rep. 2014;2014.
132. Brown B, Hanel RA. Endovascular management of cavernous and paraclinoid aneurysms. Neurosurg Clin N Am. 2014;25(3):415-24.
133. Yamada S, Yamada SM, Hirohata T, Ishii Y, Hoya K, Murakami M, et al. Endoscopic extracapsular removal of pituitary adenoma: the importance of pretreatment of an adjacent unruptured internal carotid artery aneurysm. Case Rep Neurol Med. 2012;2012:891847.
134. Hamasaki O, Ikawa F, Hidaka T, Kurokawa Y, Yonezawa U. Extracranial internal carotid artery pseudoaneurysm associated with neurofibromatosis type 1 treated with endovascular stenting and coil embolization. Vasc Endovascular Surg. 2014;48(2):176-9.
135. Berne JD, Reuland KR, Villarreal DH, McGovern TM, Rowe SA, Norwood SH. Internal carotid artery stenting for blunt carotid artery injuries with an associated pseudoaneurysm. J Trauma. 2008;64(2):398-405.
136. Pramod Kumar Chaurasia; Daljit Singh SMRSHS. CASE REPORT: Epistaxis as first clinical presentation in a child with giant prolactinoma: Case report and review. Journal of Pediatric Neurosciences. 2011;Journal of Pediatric Neurosciences(2):134-7.
137. Chuang CC, Chen YL, Pai PC. A giant intracavernous carotid artery aneurysm embedded in pituitary macroadenoma presenting with pituitary apoplexy. Cerebrovasc Dis. 2006;21(1-2):142-4.
138. AlQahtani A, Castelnuovo P, Nicolai P, Prevedello DM, Locatelli D, Carrau RL. Injury of the Internal Carotid Artery During Endoscopic Skull Base Surgery: Prevention and Management Protocol. Otolaryngol Clin North Am. 2016;49(1):237-52.
139. Chin OY, Ghosh R, Fang CH, Baredes S, Liu JK, Eloy JA. Internal carotid artery injury in endoscopic endonasal surgery: A systematic review. Laryngoscope. 2016;126(3):582-90.
140. Padhye V, Naidoo Y, Alexander H, Floreani S, Robinson S, Santoreneos S, et al. Endoscopic endonasal resection of anterior skull base meningiomas. Otolaryngol Head Neck Surg. 2012;147(3):575-82.
141. Wormald P-J, Robinson S. Transnasal endoscopic resection of benign tumors occurring from or extending into the infratemporal fossa. Operative Techniques in Otolaryngology-Head and Neck Surgery. 2006;17(2):97-101.
142. Gardner PT, Mj ; Pant, H ; Fernandez-Miranda, Jc ; Snyderman, Ch ; Horowitz, MB. Carotid Artery Injury During Endoscopic Endonasal Skull Base Surgery: Incidence and Outcomes

. Neurosurgery. 2013;73(Dec):261-9.
143. Alobaid A, Dehdashti AR. Hemostasis in Skull Base Surgery. Otolaryngol Clin North Am. 2016;49(3):677-90.
144. Zhao YC, Psaltis AJ. Hemostasis in sinus surgery. Curr Opin Otolaryngol Head Neck Surg. 2016;24(1):26-30.
145. Rajiv S, Rodgers S, Bassiouni A, Vreugde S, Wormald PJ. Role of crushed skeletal muscle extract in hemostasis. Int Forum Allergy Rhinol. 2015;5(5):431-4.
146. Cinar CB, H; Parildar, M; Oran, I. Endovascular Management of Vascular Injury during Transphenoidal Surgery. Interventional Neuroradiology. 2013;19:102-9.
147. Sylvester PT, Moran CJ, Derdeyn CP, Cross DT, Dacey RG, Zipfel GJ, et al. Endovascular management of internal carotid artery injuries secondary to endonasal surgery: case series and review of the literature. J Neurosurg. 2016;125(5):1256-76.
148. Munich SA, Cress MC, Rangel-Castilla L, Krishna C, Siddiqui AH, Snyder KV. Importance of repeat angiography in the diagnosis of iatrogenic anterior cerebral artery territory pseudoaneurysm following endoscopic sinus surgery. BMJ Case Rep. 2015;2015.
149. investigators TCt. Antiplatelet treatment compared with anticoagulation treatment for cervical artery dissection (CADISS): a randomised trial. The Lancet Neurology. 2015;14(4):361-7.
150. Jukes AW, P. J. Management of Internal Carotid Artery Injury In: Janakiram T, editor. Juvenile Nasopharyngeal Angiofibroma. 1: Thieme; 2017.
151. Kraak RP, de Boer HH, Elias J, Ambarus CA, van der Wal AC, de Winter RJ, et al. Coronary Artery Vessel Healing Pattern, Short and Long Term, After Implantation of the Everolimus-Eluting Bioresorbable Vascular Scaffold. J Am Heart Assoc. 2015;4(11).
152. Hou Y, Carrim N, Wang Y, Gallant RC, Marshall A, Ni H. Platelets in hemostasis and thrombosis: Novel mechanisms of fibrinogen-independent platelet aggregation and fibronectin-mediated protein wave of hemostasis. J Biomed Res. 2015;29.
153. Brott TG, Howard G, Roubin GS, Meschia JF, Mackey A, Brooks W, et al. Long-Term Results of Stenting versus Endarterectomy for Carotid-Artery Stenosis. N Engl J Med. 2016;374(11):1021-31.
154. Spence JD, Naylor AR. Endarterectomy, Stenting, or Neither for Asymptomatic Carotid-Artery Stenosis. N Engl J Med. 2016;374(11):1087-8.
155. Stevic I, Chan HH, Chan AK. Carotid artery dissections: thrombosis of the false lumen. Thromb Res. 2011;128(4):317-24.
156. Mortazavi MM, Verma K, Tubbs RS, Harrigan M. Pediatric traumatic carotid, vertebral and cerebral artery dissections: a review. Childs Nerv Syst. 2011;27(12):2045-56.
157. Morken MH, Cappelen J, Kvistad KA, Sovik E, Stensaeth KH. Acute endovascular repair of iatrogenic right internal carotid arterial laceration. Acta Radiol Short Rep. 2013;2(5):2047981613496088.
158. McMonagle MS, Matthew. At a Glance : Vascular and Endovascular Surgery at a Glance Wiley-Blackwell; 2013.
159. Ploeg M, Saey V, Delesalle C, Grone A, Ducatelle R, de Bruijn M, et al. Thoracic aortic rupture and aortopulmonary fistulation in the Friesian horse: histomorphologic characterization. Vet Pathol. 2015;52(1):152-9.
160. Bauriedel G, Jabs A, Skowasch D, Hutter R, Badimon JJ, Fuster V, et al. Dendritic cells in neointima formation after rat carotid balloon injury. Journal of the American College of Cardiology. 2003;42(5):930-8.

161. Steinberg J, Cheung V, Goel G, Pannell JS, Nation J, Khalessi A. Vessel-preserving stent-assisted coil embolization of an extracranial internal carotid artery pseudoaneurysm that developed after tonsillectomy in a pediatric patient: initial case report. J Neurosurg Pediatr. 2016:1-5.
162. Enamorado JE, Witsch J, Aviles AH, Perez RH. Neurological picture. Acute appearance of a carotid pseudoaneurysm during coughing. J Neurol Neurosurg Psychiatry. 2015;86(1):120.
163. Mathur NN, Joshi RR, Nepal A, Rauniyar RK. Common carotid artery pseudoaneurysm formation following foreign body ingestion. J Laryngol Otol. 2010;124(6):684-6.
164. Nazari P, Kasliwal MK, Wewel JT, Dua SG, Chen M. Delayed Intracerebral Hemorrhage from a Pseudoaneurysm Following a Depressed Skull Fracture. Neurointervention. 2016;11(1):42-5.
165. Dubey A, Sung WS, Chen YY, Amato D, Mujic A, Waites P, et al. Traumatic intracranial aneurysm: a brief review. J Clin Neurosci. 2008;15(6):609-12.
166. Kim KA, Wang MY, McNatt SA, Pinsky G, Liu CY, Giannotta SL, et al. Vector Analysis Correlating Bullet Trajectory to Outcome after Civilian Through-and-Through Gunshot Wound to the Head: Using Imaging Cues to Predict Fatal Outcome. Neurosurgery. 2005:737-47.
167. Fu M, Patel T, Baehring JM, Bulsara KR. Cavernous carotid pseudoaneurysm following transsphenoidal surgery. J Neuroimaging. 2013;23(3):319-25.
168. Griauzde J, Gemmete JJ, Pandey AS, McKean EL, Sullivan SE, Chaudhary N. Emergency reconstructive endovascular management of intraoperative complications involving the internal carotid artery from trans-sphenoidal surgery. J Neurointerv Surg. 2015;7(1):67-71.
169. Nerva JD, Morton RP, Levitt MR, Osbun JW, Ferreira MJ, Ghodke BV, et al. Pipeline Embolization Device as primary treatment for blister aneurysms and iatrogenic pseudoaneurysms of the internal carotid artery. J Neurointerv Surg. 2015;7(3):210-6.
170. Lee CH, Chen SM, Lui TN. Posterior Cerebral Artery Pseudoaneurysm, a Rare Complication of Pituitary Tumor Transsphenoidal Surgery: Case Report and Literature Review. World Neurosurg. 2015;84(5):1493 e1-3.
171. Jensen ME. Imaging of Traumatic Arterial Injuries to the Cervical Vessels. 2016:23-35.
172. Griessenauer CJ, Foreman P, Deveikis JP, Harrigan MR. Endovascular tools available for the treatment of cerebrovascular disease. Neurosurg Clin N Am. 2014;25(3):387-94.
173. Giorgianni A, Pellegrino C, Minotto R, Mercuri A, Frattini L, Baruzzi F, et al. Flow-diverter stenting of post-traumatic bilateral anterior cerebral artery pseudoaneurysm: A case report. Interventional Neuroradiology. 2015;21(1):23-8.
174. Chen Z, Zhang J, Miao H, Niu Y, Feng H, Zhu G. Delayed rupture of iatrogenic cerebral pseudoaneurysms after neurosurgical procedures: report of two cases. Clin Neurol Neurosurg. 2013;115(8):1552-4.
175. Brzozowski K, Frankowska E, Piasecki P, Ziecina P, Zukowski P, Boguslawska-Walecka R. The use of routine imaging data in diagnosis of cerebral pseudoaneurysm prior to angiography. Eur J Radiol. 2011;80(3):e401-9.
176. Markiewicz MP, Phillip; Bryant, Curtis; Cunningham, James; Dagan, Roi; Sandhu, Sukhwinder; Siragusa, Daniel; Gopinath, Arun; Fernandes, Rui. Preoperative Protective Endovascular Covered Stent Placement Followed by Surgery for Management of the Cervical Common and Internal Carotid Arteries with Tumor Encasement. Journal of Neurological Surgery Part B Skull Base. 2017;78((01)):052-8.

177. Wholey MH, Wholey MH, Eles G. Endovascular procedures for carotid artery occlusive disease. Tech Vasc Interv Radiol. 2004;7(4):168-86.
178. Zhang J, Zhang X, Guo Q, Cao W, Zhang Q, Gao D, et al. Surgical treatment of giant fusiform aneurysm of extracranial internal carotid artery in a child: 1 case report and literature review. Surg Neurol. 2007;68(3):329-33; discussion 34.
179. Faggioli GL, Ferri M, Serra C, Biagini E, Manzoli L, Lodi R, et al. The residual risk of cerebral embolism after carotid stenting: the complex interplay between stent coverage and aortic arch atherosclerosis. Eur J Vasc Endovasc Surg. 2009;37(5):519-24.
180. Cohen DJ, Stolker JM, Wang K, Magnuson EA, Clark WM, Demaerschalk BM, et al. Health-related quality of life after carotid stenting versus carotid endarterectomy: results from CREST (Carotid Revascularization Endarterectomy Versus Stenting Trial). J Am Coll Cardiol. 2011;58(15):1557-65.
181. Yonekawa YS, Y; Keller, E; Tsukahara, T. Trends in Surgical Stroke Management. New York: SpringerWein; 2005.
182. Barry RC, Wilkinson M, Ahmed RM, Lim CS, Parker GD, McCluskey PJ, et al. Interventional treatment of carotid cavernous fistula. J Clin Neurosci. 2011;18(8):1072-9.
183. Wang W, Li YD, Li MH, Tan HQ, Gu BX, Wang J, et al. Endovascular treatment of post-traumatic direct carotid-cavernous fistulas: A single-center experience. J Clin Neurosci. 2011;18(1):24-8.
184. Tytle TLP, Pavan Kumar. Carotid cavernous fistula. Seminars in Cerebrovascular Diseases and Stroke. 2001;1(1):83-111.
185. Venkataramaiah S, Sriganesh K. Images in clinical medicine. Carotid cavernous fistula. N Engl J Med. 2014;371(19):1832.
186. Lang M, Habboub G, Mullin JP, Rasmussen PA. A brief history of carotid-cavernous fistula. J Neurosurg. 2016:1-7.
187. Mackman N. The many faces of tissue factor. J Thromb Haemost. 2009;7 Suppl 1:136-9.
188. Bouchard BA, Silveira JR, Tracy PB. Interactions Between Platelets and the Coagulation System. 2013:425-51.
189. Kamath S, Blann AD, Lip GY. Platelet activation: assessment and quantification. Eur Heart J. 2001;22(17):1561-71.
190. Perzborn E, Heitmeier S, Laux V. Effects of Rivaroxaban on Platelet Activation and Platelet-Coagulation Pathway Interaction: In Vitro and In Vivo Studies. J Cardiovasc Pharmacol Ther. 2015;20(6):554-62.
191. Evangelista V, Smyth SS. Interactions Between Platelets, Leukocytes and the Endothelium. 2013:295-312.
192. White JG. Platelets. 3rd ed. Minnesota Elsevier; 2013.
193. Lord MS, Cheng B, McCarthy SJ, Jung M, Whitelock JM. The modulation of platelet adhesion and activation by chitosan through plasma and extracellular matrix proteins. Biomaterials. 2011;32(28):6655-62.
194. Fischer TH, Thatte HS, Nichols TC, Bender-Neal DE, Bellinger AD, Vournakis JN. Synergistic platelet integrin signaling and factor XII activation in poly-N-acetyl glucosamine fiber-mediated hemostasis. Biomaterials. 2005;26(27):5433-43.
195. Cosemans JM, Iserbyt BF, Deckmyn H, Heemskerk JW. Multiple ways to switch platelet integrins on and off. J Thromb Haemost. 2008;6(8):1253-61.
196. Tynngard N, Lindahl TL, Ramstrom S. Assays of different aspects of haemostasis - what do they measure? Thromb J. 2015;13:8.

197. Dopheide SY, C; Jackson, S. Dynamic aspects platelet adhesion under flow. Clinical and Experimental Pharmacology and Physiology. 2001;28:355-63.
198. Modery-Pawlowski CL, Tian LL, Pan V, McCrae KR, Mitragotri S, Sen Gupta A. Approaches to synthetic platelet analogs. Biomaterials. 2013;34(2):526-41.
199. De Candia E. Mechanisms of platelet activation by thrombin: a short history. Thromb Res. 2012;129(3):250-6.
200. Agrawal P, Soni S, Mittal G, Bhatnagar A. Role of polymeric biomaterials as wound healing agents. Int J Low Extrem Wounds. 2014;13(3):180-90.
201. Oz M, Rondinone, Joseph, Shargill, Narinder. Floseal Matrix: New Generation Topical Hemostatic Sealant. J Card Surg. 2003;18:486-93.
202. Huber K, Hamad B, Kirkpatrick P. Fresh from the pipeline. Ticagrelor. Nat Rev Drug Discov. 2011;10(4):255-6.
203. James S, Angiolillo DJ, Cornel JH, Erlinge D, Husted S, Kontny F, et al. Ticagrelor vs. clopidogrel in patients with acute coronary syndromes and diabetes: a substudy from the PLATelet inhibition and patient Outcomes (PLATO) trial. Eur Heart J. 2010;31(24):3006-16.
204. Montalescot G, Lassen JF, Hamm CW, Lapostolle F, Silvain J, ten Berg JM, et al. Ambulance or in-catheterization laboratory administration of ticagrelor for primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: rationale and design of the randomized, double-blind Administration of Ticagrelor in the cath Lab or in the Ambulance for New ST elevation myocardial Infarction to open the Coronary artery (ATLANTIC) study. Am Heart J. 2013;165(4):515-22.
205. Basabe-Desmonts L, Meade G, Kenny D. New trends in bioanalytical microdevices to assess platelet function. Expert Rev Mol Diagn. 2010;10(7):869-74.
206. Nurden A, Nurden P. Advances in our understanding of the molecular basis of disorders of platelet function. J Thromb Haemost. 2011;9 Suppl 1:76-91.
207. Furlan JC, Hawryluk GW, Austin J, Fehlings MG. Spinal haemorrhage during anticoagulant regimen for thromboprophylaxis: a unique form of central nervous system haemorrhage. J Neurol Neurosurg Psychiatry. 2012;83(7):746-52.
208. Beynon C, Unterberg AW, Sakowitz OW. Point of care coagulation testing in neurosurgery. J Clin Neurosci. 2015;22(2):252-7.
209. Lai A, Davidson N, Galloway SW, Thachil J. Perioperative management of patients on new oral anticoagulants. Br J Surg. 2014;101(7):742-9.
210. Ault KA, Mitchell J. Chapter 17 Analysis of Platelets by Flow Cytometry. 1994;42:275-94.
211. Jastrzebska M, Chelstowski K, Wodecka A, Siennicka A, Clark J, Nowacki P. Factors influencing multiplate whole blood impedance platelet aggregometry measurements, during aspirin treatment in acute ischemic stroke: a pilot study. Blood Coagul Fibrinolysis. 2013;24(8):830-8.
212. Hoffmann JJ, van den Broek NM, Curvers J. Reference intervals of reticulated platelets and other platelet parameters and their associations. Arch Pathol Lab Med. 2013;137(11):1635-40.
213. Gremmel T, Koppensteiner R, Panzer S. Comparison of Aggregometry with Flow Cytometry for the Assessment of Agonists -Induced Platelet Reactivity in Patients on Dual Antiplatelet Therapy. PLoS One. 2015;10(6):e0129666.
214. Berny-Lang MA, Frelinger AL, Barnard MR, Michelson AD. Flow Cytometry. 2013:581-602.

215. van Velzen JF, Laros-van Gorkom BA, Pop GA, van Heerde WL. Multicolor flow cytometry for evaluation of platelet surface antigens and activation markers. Thromb Res. 2012;130(1):92-8.
216. Michelson AD. Evaluation of Platelet Function by Flow Cytometry. Pathophysiology of Haemostasis and Thrombosis. 2006;35((1-2)):67-82.
217. Brown MW, Carl. Flow Cytometry: Principles and Clinical Applications in Hematology. Clinical Chemistry. 2000;46(8(B)):1221-9.
218. Saboor MM, M; Ilyas, S. New Horizons in Platelets Flow Cytometry The Malaysian Journal of Medical Sciences. 2013;20(2):62-6.
219. Becton DaC. Monoclonal antibodies detecting human antigens - PAC1. 2015.
220. Rondina MT, Grissom CK, Men S, Harris ES, Schwertz H, Zimmerman GA, et al. Whole blood flow cytometry measurements of in vivo platelet activation in critically-Ill patients are influenced by variability in blood sampling techniques. Thromb Res. 2012;129(6):729-35.
221. Gabay M, Boucher BA. An essential primer for understanding the role of topical hemostats, surgical sealants, and adhesives for maintaining hemostasis. Pharmacotherapy. 2013;33(9):935-55.
222. Ham SL, W; Weaver, F. Thrombin use in surgery; an evidence-based review of its clinical use. Journal of Blood Medicine. 2010;1:135-42.
223. Yao HH, Hong MK, Drummond KJ. Haemostasis in neurosurgery: what is the evidence for gelatin-thrombin matrix sealant? J Clin Neurosci. 2013;20(3):349-56.
224. Spencer HT, Hsu JT, McDonald DR, Karlin LI. Intraoperative anaphylaxis to gelatin in topical hemostatic agents during anterior spinal fusion: a case report. Spine J. 2012;12(8):e1-6.
225. Tackett SM, Calcaterra D, Magee G, Lattouf OM. Real-world outcomes of hemostatic matrices in cardiac surgery. J Cardiothorac Vasc Anesth. 2014;28(6):1558-65.
226. Echave MO, I; Casado, M. Use of Floseal, a human gelatine-thrombin matrix sealant, in surgery: a systematic review. BMC Surgery. 2014;14:111.
227. Ereth MH, Schaff M, Ericson EF, Wetjen NM, Nuttall GA, Oliver WC, Jr. Comparative safety and efficacy of topical hemostatic agents in a rat neurosurgical model. Neurosurgery. 2008;63(4 Suppl 2):369-72; discussion 72.
228. de Bruin R, van Furth WR, Verbaan D, Georgalas C, Fokkens WF, Reinartz SM. Initial experiences with endoscopic rhino-neurosurgery in Amsterdam. Eur Arch Otorhinolaryngol. 2014;271(6):1525-32.
229. Gazzeri R, Galarza M, Neroni M, Alfieri A, Esposito S. Minimal craniotomy and matrix hemostatic sealant for the treatment of spontaneous supratentorial intracerebral hemorrhage. J Neurosurg. 2009;110(5):939-42.
230. Cappabianca P, Esposito F, Esposito I, Cavallo LM, Leone CA. Use of a thrombin-gelatin haemostatic matrix in endoscopic endonasal extended approaches: technical note. Acta Neurochir (Wien). 2009;151(1):69-77; discussion
231. Ethicon. Surgiflow haemostatic matrix kit with thrombin. 2016.
232. Doria C, Fischer CP, Wood CG, Li PM, Marra S, Hart J. Phase 3, randomized, double-blind study of plasma-derived human thrombin versus bovine thrombin in achieving hemostasis in patients undergoing surgery. Curr Med Res Opin. 2008;24(3):785-94.
233. Ballard JL, Weaver FA, Singla NK, Chapman WC, Alexander WA. Safety and immunogenicity observations pooled from eight clinical trials of recombinant human thrombin. J Am Coll Surg. 2010;210(2):199-204.

234. Birth M, Figueras J, Bernardini S, Troen T, Gunther K, Mirza D, et al. Collagen fleece-bound fibrin sealant is not associated with an increased risk of thromboembolic events or major bleeding after its use for haemostasis in surgery: a prospective multicentre surveillance study. Patient Saf Surg. 2009;3(1):13.
235. Bochicchio GV, Gupta N, Porte RJ, Renkens KL, Pattyn P, Topal B, et al. The FINISH-3 trial: a phase 3, international, randomized, single-blind, controlled trial of topical fibrocaps in intraoperative surgical hemostasis. J Am Coll Surg. 2015;220(1):70-81.
236. Streiff MN, P. Acquired FV Inhibitors: a needless iatrogenic complication of bovine thrombin exposure. Transfusion 2002;42:18-26.
237. Safaee M, Sun MZ, Oh T, Aghi MK, Berger MS, McDermott MW, et al. Use of thrombin-based hemostatic matrix during meningioma resection: a potential risk factor for perioperative thromboembolic events. Clin Neurol Neurosurg. 2014;119:116-20.
238. Prabhu V. Venous Thromboembolism Prophylaxis in Neurosurgery. 2016. In: Anticoagulation and Hemostasis in Neurosurgery [Internet]. SpringerLink; [327-42].
239. Santoreneos S. In: Jukes A, editor. Description of neurosurgical DVT prophylaxis protocol ed2016.
240. Chakrabarti D, N GK, Masapu D. Adverse Hemodynamic Event Due to Floseal Hemostatic Matrix Application. J Neurosurg Anesthesiol. 2016;28(1):88-9.
241. Hanna EM, Martinie JB, Swan RZ, Iannitti DA. Fibrin sealants and topical agents in hepatobiliary and pancreatic surgery: a critical appraisal. Langenbecks Arch Surg. 2014;399(7):825-35.
242. Hickerson WL, Nur I, Meidler R. A comparison of the mechanical, kinetic, and biochemical properties of fibrin clots formed with two different fibrin sealants. Blood Coagul Fibrinolysis. 2011;22(1):19-23.
243. Bivalacqua TJ, Guzzo TJ, Schaeffer EM, Gebska MA, Champion HC, Burnett AL, et al. Application of Evicel to cavernous nerves of the rat does not influence erectile function in vivo. Urology. 2008;72(5):1169-73.
244. Isaacs JE, McDaniel CO, Owen JR, Wayne JS. Comparative analysis of biomechanical performance of available "nerve glues". J Hand Surg Am. 2008;33(6):893-9.
245. Chalmers RT, Darling Iii RC, Wingard JT, Chetter I, Cutler B, Kern JA, et al. Randomized clinical trial of tranexamic acid-free fibrin sealant during vascular surgical procedures. Br J Surg. 2010;97(12):1784-9.
246. Epstein NE. Dural repair with four spinal sealants: focused review of the manufacturers' inserts and the current literature. Spine J. 2010;10(12):1065-8.
247. Yang MB, Melia M, Lambert SR, Chiang MF, Simpson JL, Buffenn AN. Fibrin glue for closure of conjunctival incision in strabismus surgery: a report by the american academy of ophthalmology. Ophthalmology. 2013;120(9):1935-41.
248. Budde S, Noll Y, Zieglschmid V, Schroeder C, Koch A, Windhagen H. Determination of the efficacy of EVICEL on blood loss in orthopaedic surgery after total knee replacement: study protocol for a randomised controlled trial. Trials. 2015;16:299.
249. Masel JL. Transurethral Use of Evicel((R)) Fibrin Sealant. Urol Case Rep. 2016;4:5-7.
250. Esposito F, Angileri FF, Kruse P, Cavallo LM, Solari D, Esposito V, et al. Fibrin Sealants in Dura Sealing: A Systematic Literature Review. PLoS One. 2016;11(4):e0151533.
251. Cappabianca P, Cavallo LM, Valente V, Romano I, D'Enza AI, Esposito F, et al. Sellar repair with fibrin sealant and collagen fleece after endoscopic endonasal transsphenoidal surgery. Surg Neurol. 2004;62(3):227-33; discussion 33.

252. Miscusi M, Polli FM, Forcato S, Coman MA, Ricciardi L, Ramieri A, et al. The use of surgical sealants in the repair of dural tears during non-instrumented spinal surgery. Eur Spine J. 2014;23(8):1761-6.
253. Sanders-Taylor C, Anaizi A, Kosty J, Zimmer LA, Theodosopoulos PV. Sellar Reconstruction and Rates of Delayed Cerebrospinal Fluid Leak after Endoscopic Pituitary Surgery. J Neurol Surg B Skull Base. 2015;76(4):281-5.
254. Agency Em. Evicel Human Fibrinogen/Human Thrombin Product Statement. Human Medicines 2006 [Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000898/WC500030828.pdf.
255. Ezra M. Evicel for Dural Sealing. In: Jukes A, editor. Personal correspondance ed. Adelaide 2016. p. 1.
256. Dhillon S. Fibrin Sealant (Evicel[Quixil,Crosseal————————————]) A Review of its Use as Supportive Treatment for Haemostasis in Surgery. Drugs. 2011;71(14):1893-915.
257. Chapman WC, Singla N, Genyk Y, McNeil JW, Renkens KL, Jr., Reynolds TC, et al. A phase 3, randomized, double-blind comparative study of the efficacy and safety of topical recombinant human thrombin and bovine thrombin in surgical hemostasis. J Am Coll Surg. 2007;205(2):256-65.
258. Taylor LM, Mueller-Velten G, Koslow A, Hunter G, Naslund T, Kline R. Prospective randomized multicenter trial of fibrin sealant versus thrombin-soaked gelatin sponge for suture- or needle-hole bleeding from polytetrafluoroethylene femoral artery grafts. Journal of Vascular Surgery. 2003;38(4):766-71.
259. Tavanaiepour D, Jernigan S, Abolfotoh M, Al-Mefty O. Fibrin Glue Injection for Cavernous Sinus Hemostasis Associated with Cranial Nerve Deficit: A Case Report. J Neurol Surg Rep. 2015;76(1):e72-4.
260. Kassam A, Horowitz M, Carrau R, Snyderman C, Welch W, Hirsch B, et al. Use of Tisseel Fibrin Sealant in Neurosurgical Procedures: Incidence of Cerebrospinal Fluid Leaks and Cost-Benefit Analysis in a Retrospective Study. Neurosurgery. 2003;52(5):1102-5.
261. Alan A. Milne WGM, Sarah J. Reading and C. Vaughan Ruckley. Fibrin Sealant Reduces Suture Line Bleeding During Carotid Endarterectomy: A Randomised Trial. Eur J Vasc Endovascu Surg. 1995;10:91-4.
262. Lowe J LJ, Levitsky S. Evaluation of the topical hemostatic efficacy and safety of TISSEEL VH S/D fibrin sealant compared with currently licensed TISSEEL VH in patients undergoing cardiac surgery: a phase 3, randomized, double-blind clinical study. J Cadiovasc Surg. 2007;48:323-31.
263. Dickneite G, Metzner H, Pfeifer T, Kroez M, Witzke G. A comparison of fibrin sealants in relation to their in vitro and in vivo properties. Thromb Res. 2003;112(1-2):73-82.
264. Dickneite G, Metzner HJ, Kroez M, Hein B, Nicolay U. The Importance of Factor XIII as a Component of Fibrin Sealants. Journal of Surgical Research. 2002;107(2):186-95.
265. Sintler MP, Mahmood A, Smith SR, Simms MH, Vohra RK. Randomized trial comparing Quixil surgical sealant with Kaltostat hemostatic dressing to control suture line bleeding after carotid endarterectomy with ePTFE patch reconstruction. World J Surg. 2005;29(10):1259-62.
266. Vaiman M. Effectiveness of second-generation fibrin glue in endonasal operations. Otolaryngology - Head and Neck Surgery. 2002;126(4):388-91.

267. Vaiman M. Effect of Modern Fibrin Glue on Bleeding after Tonsillectomy and Adenoidectomy. Ann otot Rhinol Larvngol 2003;112:410-4.
268. Vaiman M. Use of Fibrin Glue as a hemostatic in Endoscopic sinus surgery. Ann otot Rhinol Larvngol. 2005;114:237-41.
269. Horowitz B, Busch M. Estimating the pathogen safety of manufactured human plasma products: application to fibrin sealants and to thrombin. Transfusion. 2008;48(8):1739-53.
270. Ettinger A, Miklauz MM, Hendrix BK, Bihm DJ, Maldonado-Codina G, Goodrich RP. Protein stability of previously frozen plasma, riboflavin and UV light-treated, refrozen and stored for up to 2 years at -30 degrees C. Transfus Apher Sci. 2011;44(1):25-31.
271. Hellstern P, Solheim BG. The Use of Solvent/Detergent Treatment in Pathogen Reduction of Plasma. Transfus Med Hemother. 2011;38(1):65-70.
272. Cheng CM-MK, S. A Review of Three Stand-Alone Topical Thrombins for Surgical Hemostasis. Clinical Therapeutics. 2009;31(1):32-41.
273. Lu H, Wang L, Zhong W, Qi R, Li N, You W, et al. Establishment of swine-penetrating craniocerebral gunshot wound model. J Surg Res. 2015;199(2):698-706.
274. Yuval Ran MEH, MD; Saleh Daher, MD; Ori Ganor, MD; Jonathan Kohn;, Yana Yegorov CB, MD; Nachman Ash, MD; Gil Hirschhorn, MD. QuikClot Combat Gauze Use for Hemorrhage Control in Military Trauma: January 2009 Israel Defense Force Experience in the Gaza Strip—A Preliminary Report of 14 Cases. Prehosp Disaster Medicine. 2010;25(6):584-5.
275. Fox CJ, Gillespie DL, Cox ED, Kragh JF, Jr., Mehta SG, Salinas J, et al. Damage control resuscitation for vascular surgery in a combat support hospital. J Trauma. 2008;65(1):1-9.
276. Pursifull NF, Morris MS, Harris RA, Morey AF. Damage control management of experimental grade 5 renal injuries: further evaluation of FloSeal gelatin matrix. J Trauma. 2006;60(2):346-50.
277. Fischer TH, Connolly R, Thatte HS, Schwaitzberg SS. Comparison of structural and hemostatic properties of the poly-N-acetyl glucosamine Syvek Patch with products containing chitosan. Microsc Res Tech. 2004;63(3):168-74.
278. Najjar SF, Healey NA, Healey CM, McGarry T, Khan B, Thatte HS, et al. Evaluation of Poly-N-Acetyl Glucosamine as a Hemostatic Agent in Patients Undergoing Cardiac Catheterization: A Double-Blind, Randomized Study. The Journal of Trauma: Injury, Infection, and Critical Care. 2004;57(Supplement):S38-S41.
279. Pusateri AE, Modrow HE, Harris RA, Holcomb JB, Hess JR, Mosebar RH, et al. Advanced hemostatic dressing development program: animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in Swine. J Trauma. 2003;55(3):518-26.
280. Valeri CR, Srey R, Tilahun D, Ragno G. In Vitro Effects of Poly-N-Acetyl Glucosamine on the Activation of Platelets in Platelet-Rich Plasma with and without Red Blood Cells. The Journal of Trauma: Injury, Infection, and Critical Care. 2004;57(Supplement):S22-S5.
281. Vournakis JN, Demcheva M, Whitson A, Guirca R, Pariser ER. Isolation, Purification, and Characterization of Poly-N-Acetyl Glucosamine Use as a Hemostatic Agent. The Journal of Trauma: Injury, Infection, and Critical Care. 2004;57(Supplement):S2-S6.
282. Salerno TG, C; Suarez, M; Panos, A. Control of Troublesome Bleeding During Repair of Acute Type A Dissection with Use of Modified Rapid Deployment Hemostat (MRDH). J Card Surg. 2009;24:722-4.

283. Technologies MP. Syvek NT 2016 [Product Disclosure Statement]. Available from: http://syvek.com/syvek-nt.html.
284. Tomizawa Y. Clinical benefits and risk analysis of topical hemostats: a review. J Artif Organs. 2005;8(3):137-42.
285. Bruce L. Palmer M, D. Scott Gantt, DO, Mark E. Lawrence, DO, M. Hassan Rajab, PhD, MPH, and Gregory J. Dehmer, MD. Effectiveness and Safety of Manual Hemostasis Facilitated by the SyvekPatch With One Hour of Bedrest After Coronary Angiography Using Six-French Catheters. The American Journal of Cardiology 2004;93(Jan 1).
286. Chan MWMS, Steven D. MD; Demcheva, Marina PhD; Vournakis, John PhD; Finkielsztein, Sergio BA; Connolly, Raymond J. PhD. Comparison of Poly-N-acetyl Glucosamine (P-GlcNAc) with Absorbable Collagen (Actifoam), and Fibrin Sealant (Bolheal) for Achieving Hemostasis in a Swine Model of Splenic Hemorrhage. The Journal of Trauma: Injury, Infection, and Critical Care. 2000;4(3):454-8.
287. Ethicon. EVARREST Prescribing information 2016 [Available from: http://hostedvl106.quosavl.com/qb/doc/n9kjvl0hvn14pei0kgrktcibp8.
288. EVARREST[R] Fibrin Sealant Patch. Surgical Products. 2015;2(May 2015).
289. Corral M, Ferko N, Hollmann S, Hogan A, Jamous N, Batiller J, et al. Clinician reported ease of use for a novel fibrin sealant patch for hemostasis: results from four randomized controlled trials. Curr Med Res Opin. 2016;32(2):367-75.
290. "EVARREST[R] Fibrin Sealant Patch a Potential Solution for Aortic Reconstruction Surgery." Clinical Trials Week 2015;222.
291. Matonick JP, Hammond J. Hemostatic efficacy of EVARREST, Fibrin Sealant Patch vs. TachoSil(R) in a heparinized swine spleen incision model. J Invest Surg. 2014;27(6):360-5.
292. Spotnitz W. Fibrin sealant patches: powerful and easy-to-use hemostats. Open Access Surgery. 2014:71.
293. Kivelev J, Gohre F, Niemela M, Hernesniemi J. Experiences with TachoSil(R) in microneurosurgery. Acta Neurochir (Wien). 2015;157(8):1353-7; discussion 7.
294. Colombo GL, Bettoni D, Di Matteo S, Grumi C, Molon C, Spinelli D, et al. Economic and outcomes consequences of TachoSil(R): a systematic review. Vasc Health Risk Manag. 2014;10:569-75.
295. TachoSil® Fibrin Sealant Patch - Indications and detailed important risk information U.S: Baxter Pharmaceutical; [Available from: http://tachosilus.com/important-risk-information.html.
296. Alizadeh Ghavidel A, Mirmesdagh Y, Samiei N, Gholampour Dehaki M. Haemostatic Role of TachoSil Surgical Patch in Cardiac Surgery. J Cardiovasc Thorac Res. 2014;6(2):91-5.
297. Siddiqui S, Keita L, Veerasingham D. Successful management of penetrating cardiac injury using TachoSil®. Injury Extra. 2012;43(12):142-3.
298. Celiento M, Scioti G, Pratali S, Bortolotti U. Repair of coronary artery perforation following angioplasty using TachoSil patches. Interact Cardiovasc Thorac Surg. 2010;10(2):328-30.
299. Agger P, Langhoff J, Smerup MH, Hasenkam JM. Comparison between TachoComb and TachoSil for surgical hemostasis in arterial bleeding: an animal experimental study. J Trauma. 2010;68(4):838-42.
300. Hajj-Chahine J, Jayle C, Tomasi J, Corbi P. TachoSil to prevent postoperative pericardial adhesions. Ann Thorac Surg. 2014;97(1):378-9.

301. Kuschel TJ, Gruszka A, Hermanns-Sachweh B, Elyakoubi J, Sachweh JS, Vazquez-Jimenez JF, et al. Prevention of postoperative pericardial adhesions with TachoSil. Ann Thorac Surg. 2013;95(1):183-8.
302. Antonelli A, Minervini A, Mari A, Bertolo R, Bianchi G, Lapini A, et al. TriMatch comparison of the efficacy of FloSeal versus TachoSil versus no hemostatic agents for partial nephrectomy: results from a large multicenter dataset. Int J Urol. 2015;22(1):47-52.
303. Grottke O, Braunschweig T, Daheim N, Coburn M, Grieb G, Rossaint R, et al. Effect of TachoSil in a coagulopathic pig model with blunt liver injuries. J Surg Res. 2011;171(1):234-9.
304. Dregelid EB, Pedersen G. Treatment of major vein injury with the hemostatic fleece TachoSil by interposing a peritoneal patch to avoid vein thrombosis: A feasibility study in pigs. J Emerg Trauma Shock. 2011;4(1):77-81.
305. Santulli P, Marcellin L, Touboul C, Ballester M, Darai E, Rouzier R. Experience with TachoSil in obstetric and gynecologic surgery. Int J Gynaecol Obstet. 2011;113(2):112-5.
306. Raga F, Sanz-Cortes M, Bonilla F, Casan EM, Bonilla-Musoles F. Reducing blood loss at myomectomy with use of a gelatin-thrombin matrix hemostatic sealant. Fertil Steril. 2009;92(1):356-60.
307. De Rosa P, Valeriani G, Barbato G, Cerbone V, Ciccone M, Russo E, et al. Postexplant residual cavity hemostasis with a TachoSil patch. Transplant Proc. 2011;43(4):1069-71.
308. De Rosa P, Russo E, Altieri R, Cerbone V, Papale PA, Santopietro A, et al. Saccular aneurysm of graft renal artery: case report. Transplant Proc. 2011;43(4):1213-4.
309. Sagalowsky AI. Editorial Comment on: Efficacy and safety of TachoSil as haemostatic treatment versus standard suturing in kidney tumour resection: a randomised prospective study. Eur Urol. 2007;52(4):1162-3.
310. Siemer S, Lahme S, Altziebler S, Machtens S, Strohmaier W, Wechsel HW, et al. Efficacy and safety of TachoSil as haemostatic treatment versus standard suturing in kidney tumour resection: a randomised prospective study. Eur Urol. 2007;52(4):1156-63.
311. Birk DM, Tobin MK, Moss HE, Feinstein E, Charbel FT, Alaraj A. Improvement in venous outflow following superior sagittal sinus decompression after a gunshot wound to the head: case report. J Neurosurg. 2015;123(1):81-5.
312. Lisy M, Kahlil M, Stock UA, Wildhirt SM. Fibrin sealant patch for repair of acute type a aortic dissection. J Card Surg. 2013;28(6):736-41.
313. Maplethorpe C. STN125351/172 TachoSil (Fibrin Sealant Patch) (Takeda Pharma A/S) as an adjunct to hemostasis for adult and pediatric hepatic resection surgery: FDA; 2014 [Available from: http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/UCM458010.pdf.
314. Cohen IK, E; Glaser, T. Actin and myosin from blood platelets or muscle are potent aggregating agents. Thrombosis Research. 1976;8:383-92.
315. Kim KD, Wright NM. Polyethylene glycol hydrogel spinal sealant (DuraSeal Spinal Sealant) as an adjunct to sutured dural repair in the spine: results of a prospective, multicenter, randomized controlled study. Spine (Phila Pa 1976). 2011;36(23):1906-12.
316. Lih E, Lee JS, Park KM, Park KD. Rapidly curable chitosan-PEG hydrogels as tissue adhesives for hemostasis and wound healing. Acta Biomater. 2012;8(9):3261-9.
317. Liu J, Ni B, Zhu L, Yang J, Cao X, Zhou W. Mitomycin C-polyethylene glycol controlled-release film inhibits collagen secretion and induces apoptosis of fibroblasts in the early wound of a postlaminectomy rat model. Spine J. 2010;10(5):441-7.

318. Levi M. Disseminated intravascular coagulation. Critical Care Medicine. 2007;35(9):2191-5.
319. Levi M. Disseminated Intravascular Coagulation. Journal of Coagulation Disorders 2011;3(1 October):55-64.
320. Pang KP, Tan NG, Chia KH, Tan HM, Tseng GY. Migrating foreign body into the common carotid artery. Otolaryngol Head Neck Surg. 2005;132(4):667-8.
321. Tang IP, Singh S, Shoba N, Rahmat O, Shivalingam S, Gopala KG, et al. Migrating foreign body into the common carotid artery and internal jugular vein--a rare case. Auris Nasus Larynx. 2009;36(3):380-2.
322. Oser AB, Moran CJ, Cross DT, Thompson RW. Shotgun Pellet Embolization to the lntracranial Internal Carotid Artery: Report of a Case and Review of the Literature. Emergency Radiology. 1994;1(4 (August)):200-5.
323. Vascik JT, John. Foreign body embolisation of the middle cerebral artery: Review of the literature and guidelines for management. Neurosurgery 1982;11(4):532-6.
324. Song JS, Jayashree; Gordon, David; Newell, David; Baxter, Alexander. Internal carotid pseudoanurysm and cerebral infarction from shotgun pellet penetration and embolisation. American Journal Roentgenology. 1999;173(October):1116.
325. Vaquero-Puerta C, San Norberto EM, Merino B, Gonzalez-Fajardo JA, Taylor J. Shotgun wound and pellet embolism to the intracranial carotid artery. J Vasc Surg. 2012;55(2):535-7.
326. Abdullah A, Sachithanandan S, Tan OK, Chan YM, Khoo D, Mohamed Zawawi F, et al. Cerebral embolism following N-butyl-2-cyanoacrylate injection for esophageal postbanding ulcer bleed: a case report. Hepatol Int. 2009;3(3):504-8.
327. Mehta RI, Mehta RI, Solis OE, Jahan R, Salamon N, Tobis JM, et al. Hydrophilic polymer emboli: an under-recognized iatrogenic cause of ischemia and infarct. Mod Pathol. 2010;23(7):921-30.
328. Dinesh SK, Lee SY, Thomas J. A case of mistaken identity: intracranial foreign body reaction after AVM embolisation mimicking a glioma. J Clin Neurosci. 2008;15(4):463-5.
329. Krishnan LK, Mohanty M, Umashankar PR, Lal AV. Comparative evaluation of absorbable hemostats: advantages of fibrin-based sheets. Biomaterials. 2004;25(24):5557-63.
330. Gazzeri R, Galarza M, Neroni M, Alfieri A, Giordano M. Hemostatic matrix sealant in neurosurgery: a clinical and imaging study. Acta Neurochir (Wien). 2011;153(1):148-54; discussion 55.
331. Masotti L, Di Napoli M, Godoy DA, Rafanelli D, Liumbruno G, Koumpouros N, et al. The practical management of intracerebral hemorrhage associated with oral anticoagulant therapy. Int J Stroke. 2011;6(3):228-40.
332. Gazzeri R, Galarza M, Fiore C, Callovini G, Alfieri A. Use of tissue-glue-coated collagen sponge (TachoSil) to repair minor cerebral dural venous sinus lacerations: technical note. Neurosurgery. 2015;11 Suppl 2:32-6; discussion 6.
333. Lewis KM, Atlee H, Mannone A, Lin L, Goppelt A. Efficacy of hemostatic matrix and microporous polysaccharide hemospheres. J Surg Res. 2015;193(2):825-30.
334. Qin X-H, Labuda K, Chen J, Hruschka V, Khadem A, Liska R, et al. Development of Synthetic Platelet-Activating Hydrogel Matrices to Induce Local Hemostasis. Advanced Functional Materials. 2015;25(42):6606-17.

335. Gremmel T, Calatzis A, Steiner S, Kaider A, Seidinger D, Koppensteiner R, et al. Is TRAP-6 suitable as a positive control for platelet reactivity when assessing response to clopidogrel? Platelets. 2010;21(7):515-21.
336. Zhang P, Covic L, Kuliopulos A. Protease-Activated Receptors. 2013:249-59.
337. Adams MN, Ramachandran R, Yau MK, Suen JY, Fairlie DP, Hollenberg MD, et al. Structure, function and pathophysiology of protease activated receptors. Pharmacol Ther. 2011;130(3):248-82.
338. Vassallo R. Structure-Function Relationships in the Activation of Platelet Thrombin Receptors by Receptor-Derived Peptides. Journal of Biological Chemistry. 1992;267(9):6081-5.
339. Du L, Tong L, Jin Y, Jia J, Liu Y, Su C, et al. A multifunctional in situ-forming hydrogel for wound healing. Wound Repair Regen. 2012;20(6):904-10.
340. Gong SQ, Niu LN, Kemp LK, Yiu CK, Ryou H, Qi YP, et al. Quaternary ammonium silane-functionalized, methacrylate resin composition with antimicrobial activities and self-repair potential. Acta Biomater. 2012;8(9):3270-82.
341. Roughley P, Hoemann C, DesRosiers E, Mwale F, Antoniou J, Alini M. The potential of chitosan-based gels containing intervertebral disc cells for nucleus pulposus supplementation. Biomaterials. 2006;27(3):388-96.
342. Kopecek J, Yang J. Peptide-directed self-assembly of hydrogels. Acta Biomater. 2009;5(3):805-16.
343. Jeong B, Kim SW, Bae YH. Thermosensitive sol–gel reversible hydrogels. Advanced Drug Delivery Reviews. 2012;64:154-62.
344. Nie W, Yuan X, Zhao J, Zhou Y, Bao H. Rapidly in situ forming chitosan/epsilon-polylysine hydrogels for adhesive sealants and hemostatic materials. Carbohydr Polym. 2013;96(1):342-8.
345. Behrens AM, Sikorski MJ, Li T, Wu ZJ, Griffith BP, Kofinas P. Blood-aggregating hydrogel particles for use as a hemostatic agent. Acta Biomater. 2014;10(2):701-8.
346. Wu M, Ye Z, Zhu H, Zhao X. Self-Assembling Peptide Nanofibrous Hydrogel on Immediate Hemostasis and Accelerative Osteosis. Biomacromolecules. 2015;16(10):3112-8.
347. Kim Y-M, Kim C-H, Park M-R, Song S-C. Development of an Injectable Dopamine-conjugated Poly(organophophazene) Hydrogel for Hemostasis. Bulletin of the Korean Chemical Society. 2016;37(3):372-7.
348. Jewelewicz DD, Cohn SM, Crookes BA, Proctor KG. Modified rapid deployment hemostat bandage reduces blood loss and mortality in coagulopathic pigs with severe liver injury. J Trauma. 2003;55(2):275-80; discussion 80-1.
349. Okamoto Y, Yano R, Miyatake K, Tomohiro I, Shigemasa Y, Minami S. Effects of chitin and chitosan on blood coagulation. Carbohydrate Polymers. 2003;53(3):337-42.
350. Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science. 2006;31(7):603-32.
351. Wang W. A novel hydrogel crosslinked hyaluronan with glycol chitosan. J Mater Sci Mater Med. 2006;17(12):1259-65.
352. Ward KR, Tiba MH, Holbert WH, Blocher CR, Draucker GT, Proffitt EK, et al. Comparison of a new hemostatic agent to current combat hemostatic agents in a Swine model of lethal extremity arterial hemorrhage. J Trauma. 2007;63(2):276-83; discussion 83-4.

353. Hoven V, Tangpasuthadol V, Angkitpaiboon Y, Vallapa N, Kiatkamjornwong S. Surface-charged chitosan: Preparation and protein adsorption. Carbohydrate Polymers. 2007;68(1):44-53.
354. Fischer TH, Hays WE, Valeri CR. Poly-N-acetyl glucosamine fibers accelerate hemostasis in patients treated with antiplatelet drugs. J Trauma. 2011;71(2 Suppl 1):S176-82.
355. Kadokawa J-i, Takegawa A, Mine S, Prasad K. Preparation of chitin nanowhiskers using an ionic liquid and their composite materials with poly(vinyl alcohol). Carbohydrate Polymers. 2011;84(4):1408-12.
356. Fischer TH, Bode AP, Demcheva M, Vournakis JN. Hemostatic properties of glucosamine-based materials. J Biomed Mater Res A. 2007;80(1):167-74.
357. Mi F-LS, Shin-Shing; Wu, Yu-Bey; Lee, Sung-Tao; Shyong, Jen-Yeu; Huang, Rong-Nan. Fabrication and Characterisation of a sponge-like asymetric chitosan membrane as a wound dressing. Biomaterials. 2001;22:165-73.
358. Peesan M, Rujiravanit R, Supaphol P. Characterisation of beta-chitin/poly(vinyl alcohol) blend films. Polymer Testing. 2003;22(4):381-7.
359. Burkatovskaya M, Tegos GP, Swietlik E, Demidova TN, A PC, Hamblin MR. Use of chitosan bandage to prevent fatal infections developing from highly contaminated wounds in mice. Biomaterials. 2006;27(22):4157-64.
360. Ong SY, Wu J, Moochhala SM, Tan MH, Lu J. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials. 2008;29(32):4323-32.
361. Jayakumar R, Prabaharan M, Nair SV, Tamura H. Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol Adv. 2010;28(1):142-50.
362. Muzzarelli RA. Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. Mar Drugs. 2010;8(2):292-312.
363. Laurienzo P. Marine polysaccharides in pharmaceutical applications: an overview. Mar Drugs. 2010;8(9):2435-65.
364. Dai T, Tanaka M, Huang YY, Hamblin MR. Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert Rev Anti Infect Ther. 2011;9(7):857-79.
365. Loo Y, Zhang S, Hauser CA. From short peptides to nanofibers to macromolecular assemblies in biomedicine. Biotechnol Adv. 2012;30(3):593-603.
366. Thatte HS, Zagarins S, Khuri SF, Fischer TH. Mechanisms of Poly-N-Acetyl Glucosamine Polymer???Mediated Hemostasis: Platelet Interactions. The Journal of Trauma: Injury, Infection, and Critical Care. 2004;57(Supplement):S13-S21.
367. Jegatheeswaran S, Bhanot U, Siriwardena AK. In vivo evaluation of the chitosan-based haemostatic agent Omni-stat(R) in porcine liver resection and in liver injury. Eur Surg Res. 2012;49(2):73-9.
368. Horio T, Ishihara M, Fujita M, Kishimoto S, Kanatani Y, Ishizuka T, et al. Effect of photocrosslinkable chitosan hydrogel and its sponges to stop bleeding in a rat liver injury model. Artif Organs. 2010;34(4):342-7.
369. Brandenberg GL, L; Shuman, R; Malette, W; Quigley, H. Chitosan: A new topical haemostatic agent for diffuse capillary bleeding in brain tissue. Neurosurgery. 1984;15(1):10-3.

370. Reys LL, Silva SS, Oliveira JM, Caridade SG, Mano JF, Silva TH, et al. Revealing the potential of squid chitosan-based structures for biomedical applications. Biomed Mater. 2013;8(4):045002.
371. Pedrosa M, Boyano-Martinez T, Garcia-Ara C, Quirce S. Shellfish Allergy: a Comprehensive Review. Clin Rev Allergy Immunol. 2015;49(2):203-16.
372. Beaty AD, Lieberman PL, Slavin RG. Seafood allergy and radiocontrast media: are physicians propagating a myth? Am J Med. 2008;121(2):158 e1-4.
373. Turner P, Ng I, Kemp A, Campbell D. Seafood allergy in children: a descriptive study. Ann Allergy Asthma Immunol. 2011;106(6):494-501.
374. Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature. 2007;447(7140):92-6.
375. Van Dyken SJ, Mohapatra A, Nussbaum JC, Liang H-E, Locksley RM. Cooperative activation of innate lymphoid type 2 cells (ILC2) by epithelial cytokines in response to chitin. Cytokine. 2013;63(3):306.
376. Jeebhay MF, Lopata AL. Occupational allergies in seafood-processing workers. Adv Food Nutr Res. 2012;66:47-73.
377. Lam K, Schleimer R, Kern RC. The Etiology and Pathogenesis of Chronic Rhinosinusitis: a Review of Current Hypotheses. Curr Allergy Asthma Rep. 2015;15(7):41.
378. Sigsgaard T, Thorne PS, Schlunssen V, Bonlokke J, Riddervold IS, Hoppe KA, et al. The change in nasal inflammatory markers after intranasal challenges with particulate chitin and lipopolysaccharide: a randomized, double-blind, placebo-controlled, crossover study with a positive control. Int Forum Allergy Rhinol. 2015;5(8):716-23.
379. Huri E, Beyazit Y, Mammadov R, Toksoz S, Tekinay AB, Guler MO, et al. Generation of Chimeric "ABS Nanohemostat" Complex and Comparing Its Histomorphological In Vivo Effects to the Traditional Ankaferd Hemostat in Controlled Experimental Partial Nephrectomy Model. Int J Biomater. 2013;2013:949460.
380. Busilacchi A, Gigante A, Mattioli-Belmonte M, Manzotti S, Muzzarelli RA. Chitosan stabilizes platelet growth factors and modulates stem cell differentiation toward tissue regeneration. Carbohydr Polym. 2013;98(1):665-76.
381. Muzzarelli RAA. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydrate Polymers. 2009;76(2):167-82.
382. Langer RV, JP. Tissue Engineering. Science. 1983;260:920-6.
383. Ellis-Behnke R, Jonas JB. Redefining tissue engineering for nanomedicine in ophthalmology. Acta Ophthalmol. 2011;89(2):e108-14.
384. Sang LY, Liang YX, Li Y, Wong WM, Tay DK, So KF, et al. A self-assembling nanomaterial reduces acute brain injury and enhances functional recovery in a rat model of intracerebral hemorrhage. Nanomedicine. 2015;11(3):611-20.
385. Hsu S-H, Woan K, Sigmund W. Biologically inspired hairy structures for superhydrophobicity. Materials Science and Engineering: R: Reports. 2011;72(10):189-201.
386. Jagota A, Hui C-Y. Adhesion, friction, and compliance of bio-mimetic and bio-inspired structured interfaces. Materials Science and Engineering: R: Reports. 2011.
387. Khadka DB, Haynie DT. Protein- and peptide-based electrospun nanofibers in medical biomaterials. Nanomedicine. 2012;8(8):1242-62.
388. Nagai Y, Unsworth LD, Koutsopoulos S, Zhang S. Slow release of molecules in self-assembling peptide nanofiber scaffold. J Control Release. 2006;115(1):18-25.

389. Ellis-Behnke R. At the nanoscale: nanohemostat, a new class of hemostatic agent. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2011;3(1):70-8.
390. Csukas D, Urbanics R, Moritz A, Ellis-Behnke R. AC5 Surgical Hemostat as an effective hemostatic agent in an anticoagulated rat liver punch biopsy model. Nanomedicine. 2015;11(8):2025-31.
391. Cho Y, Borgens RB. Polymer and nano-technology applications for repair and reconstruction of the central nervous system. Exp Neurol. 2012;233(1):126-44.
392. GhoshMitra S, Diercks DR, Mills NC, Hynds DL, Ghosh S. Role of engineered nanocarriers for axon regeneration and guidance: current status and future trends. Adv Drug Deliv Rev. 2012;64(1):110-25.
393. Ling PC, Sunny; Tay, David; Ellis-Behnke, Rutledge. Using Self-Assembled Nanomaterials to Inhibit the Formation of Metastatic Cancer Stem Cell Colonies In Vitro. Cell Transplantation 2011;20(1):127-31(5).
394. Song H, Zhang L, Zhao X. Hemostatic efficacy of biological self-assembling peptide nanofibers in a rat kidney model. Macromol Biosci. 2010;10(1):33-9.
395. Luo Z, Zhao X, Zhang S. Self-organization of a chiral D-EAK16 designer peptide into a 3D nanofiber scaffold. Macromol Biosci. 2008;8(8):785-91.
396. Arnaud F, Parreno-Sadalan D, Tomori T, Delima MG, Teranishi K, Carr W, et al. Comparison of 10 hemostatic dressings in a groin transection model in swine. J Trauma. 2009;67(4):848-55.
397. Kessler CM, Ortel TL. Recent developments in topical thrombins. Thromb Haemost. 2009;102(1):15-24.
398. Dyer SR, Bathula S, Durvasula P, Madgy D, Haupert M, Dworkin J, et al. Intraoperative use of FloSeal with adenotonsillectomy to prevent adverse postoperative outcomes in pediatric patients. Otolaryngol Head Neck Surg. 2013;149(2):312-7.
399. Guo J, Leung KK, Su H, Yuan Q, Wang L, Chu TH, et al. Self-assembling peptide nanofiber scaffold promotes the reconstruction of acutely injured brain. Nanomedicine. 2009;5(3):345-51.
400. Luo Z, Wang S, Zhang S. Fabrication of self-assembling D-form peptide nanofiber scaffold d-EAK16 for rapid hemostasis. Biomaterials. 2011;32(8):2013-20.
401. Ahmed EM. Hydrogel: Preparation, characterization, and applications: A review. J Adv Res. 2015;6(2):105-21.
402. Ellis-Behnke RG, Tay DKC, Liang YX, Zhang S, Schneider GE, So KF. Crystal clear surgery with self-assembling molecules that act as a bio barrier in the brain and intestine. Nanomedicine: Nanotechnology, Biology and Medicine. 2005;1(3):269-70.
403. Hsu BB, Conway W, Tschabrunn CM, Mehta M, Perez-Cuevas MB, Zhang S, et al. Clotting Mimicry from Robust Hemostatic Bandages Based on Self-Assembling Peptides. ACS Nano. 2015;9(9):9394-406.
404. Arch Therapeutics Website.
405. Kumar VA, A; Aster, J. Robbins & Cotran Pathologic Basis of Disease. 9 ed: Elsevier; 2015.
406. Karl F. Kothbauer MD, George I. Jallo, M.D., Joao Siffert, M.D., Elpidio Jimenez, M.D., Jeffrey C. Allen, M.D., and Fred J. Epstein, M.D. Foreign body reaction to hemostatic materials mimicking recurrent brain tumor. J Neurosurg. 2001;95(3):503-6.
407. Gyoneva S, Ransohoff RM. Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell-cell communication by chemokines. Trends Pharmacol Sci. 2015;36(7):471-80.

408. Arvin BN, L; Barone, F; Feuerstein. The role of inflammation and cytokines in brain injury. Neuroscience and biobehavioural reviews. 1996;20(3):445-52.
409. Agabegi SS, McClung HL. Open lumbar laminectomy: Indications, surgical techniques, and outcomes. Seminars in Spine Surgery. 2013;25(4):246-50.
410. Komotar RJ, Mocco J, Kaiser MG. Surgical management of cervical myelopathy: indications and techniques for laminectomy and fusion. Spine J. 2006;6(6 Suppl):252S-67S.
411. Whaley C. Decompressive lumbar laminectomy: Indications and discussion. Techniques in Regional Anesthesia and Pain Management. 2013;17(2):39-42.
412. Hulen CA. A Review of the Significance, Indications, Techniques, and Outcomes of Revision Lumbar Laminectomy Surgery. Seminars in Spine Surgery. 2008;20(4):270-6.
413. Al Kaisy A, Pang D, Desai MJ, Pries P, North R, Taylor RS, et al. Failed back surgery syndrome: who has failed? Neurochirurgie. 2015;61 Suppl 1:S6-S14.
414. Baptiste DC, Fehlings MG. Pathophysiology of cervical myelopathy. Spine J. 2006;6(6 Suppl):190S-7S.
415. Kim PK, Alexander JT. Indications for circumferential surgery for cervical spondylotic myelopathy. Spine J. 2006;6(6 Suppl):299S-307S.
416. Wood KB, Fritzell P, Dettori JR, Hashimoto R, Lund T, Shaffrey C. Effectiveness of spinal fusion versus structured rehabilitation in chronic low back pain patients with and without isthmic spondylolisthesis: a systematic review. Spine (Phila Pa 1976). 2011;36(21 Suppl):S110-9.
417. Rabb CH. Failed back syndrome and epidural fibrosis. Spine J. 2010;10(5):454-5.
418. Schuenke MS, Erik; Schumacher, Udo. Thieme Atlas of Anatomy - Head and Neuroanatomy 2ed. Ross LL, Edward; Taub, Ethan, editor. Stuttgart: Thieme; 2010 2010.
419. Garcia R, Jr., Yue JJ, Blumenthal S, Coric D, Patel VV, Leary SP, et al. Lumbar Total Disc Replacement for Discogenic Low Back Pain: Two-year Outcomes of the activL Multicenter Randomized Controlled IDE Clinical Trial. Spine (Phila Pa 1976). 2015;40(24):1873-81.
420. Hsu E, Atanelov L, Plunkett AR, Chai N, Chen Y, Cohen SP. Epidural lysis of adhesions for failed back surgery and spinal stenosis: factors associated with treatment outcome. Anesth Analg. 2014;118(1):215-24.
421. Lv C, Zhou Z, Song Y, Liu L, Liu H, Gong Q, et al. Novel biodegradable lamina for lamina repair and reconstruction. Spine J. 2013;13(12):1912-20.
422. <post op arachnoiditis.pdf>.
423. Mulleman D, Mammou S, Griffoul I, Watier H, Goupille P. Pathophysiology of disk-related low back pain and sciatica. II. Evidence supporting treatment with TNF-alpha antagonists. Joint Bone Spine. 2006;73(3):270-7.
424. Zhao CQ, Wang LM, Jiang LS, Dai LY. The cell biology of intervertebral disc aging and degeneration. Ageing Res Rev. 2007;6(3):247-61.
425. Jones TR, Rao R. Pathophysiology of Axial Low Back Pain. Seminars in Spine Surgery. 2008;20(2):78-86.
426. Massie JB, Schimizzi AL, Huang B, Kim CW, Garfin SR, Akeson WH. Topical high molecular weight hyaluronan reduces radicular pain post laminectomy in a rat model. Spine J. 2005;5(5):494-502.
427. Ragab A, Deshazo RD. Management of back pain in patients with previous back surgery. Am J Med. 2008;121(4):272-8.
428. Taylor RS, Taylor RJ. The economic impact of failed back surgery syndrome. Br J Pain. 2012;6(4):174-81.

429. Schofferman J, Reynolds J, Herzog R, Covington E, Dreyfuss P, O'Neill C. Failed back surgery: etiology and diagnostic evaluation. The Spine Journal. 2003;3(5):400-3.
430. Boccard SG, Pereira EA, Aziz TZ. Deep brain stimulation for chronic pain. J Clin Neurosci. 2015;22(10):1537-43.
431. Nagel SJ, Lempka SF, Machado AG. Percutaneous spinal cord stimulation for chronic pain: indications and patient selection. Neurosurg Clin N Am. 2014;25(4):723-33.
432. Rajiv S, Harding M, Bassiouni A, Jardeleza C, Drilling A, James C, et al. The efficacy and safety of chitosan dextran gel in a burr hole neurosurgical sheep model. Acta Neurochirurgica. 2013;155(7):1361-6.
433. Richards PT, S; Gisler, S; Kraft, S; Nuss, K; Mark, S; Seim, H; Schense, J. Reduction in Postlaminectomy Epidural Adhesions in Sheep Using a Fibrin Sealant-Based Medicated Adhesion Barrier. Journal of Biomedical Materials Research Part B- Applied Biomaterials. 2010(92B):439-46.
434. Gunzburg R, Szpalski M, Passuti N, Aebi M. Biomaterials: the new frontiers in spine surgery. Eur Spine J. 2001;10 Suppl 2:S85.
435. <fixation nerve sheath.pdf>.
436. <sheath infiltration.pdf>.
437. Zeinalizadeh MMM, S; Azmoodeh Ardalan, F; Maleki, F; Zakeri, M; Aghajanzadeh, E; Habibi, Z. Reduction of epidural fibrosis and dural adhesions after lamina reconstruction by absorbable cement: an experimental study. The Spine Journal. 2014;14:113-8.
438. El Ahmadieh TY, Adel JG, El Tecle NE, Daou MR, Aoun SG, Nanney AD, 3rd, et al. Surgical treatment of elevated intracranial pressure: decompressive craniectomy and intracranial pressure monitoring. Neurosurg Clin N Am. 2013;24(3):375-91.
439. Takeuchi S, Takasato Y, Masaoka H, Hayakawa T, Yatsushige H, Nagatani K, et al. Hydrocephalus after decompressive craniectomy for hemispheric cerebral infarction. J Clin Neurosci. 2013;20(3):377-82.
440. Tan LA, Lopes DK. Surgical management of malignant cerebral edema secondary to immune reconstitution inflammatory syndrome from natalizumab-associated progressive multifocal encephalopathy. J Clin Neurosci. 2015;22(10):1669-71.
441. Vahedi K, Hofmeijer J, Juettler E, Vicaut E, George B, Algra A, et al. Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials. The Lancet Neurology. 2007;6(3):215-22.
442. Huang YH, Lee TC, Chen WF, Wang YM. Safety of the nonabsorbable dural substitute in decompressive craniectomy for severe traumatic brain injury. J Trauma. 2011;71(3):533-7.
443. Preul MC, Patrick; Garlick, David; Spetzler, Robert. Application of a new hydrogel dural sealant that reduces
epidural adhesion formation: evaluation in a large animal
laminectomy model. Journal of Neurosurgery Spine. 2010;12:381-90.
444. Chaudhary H, Kohli K, Kumar V. A novel nano-carrier transdermal gel against inflammation. Int J Pharm. 2014;465(1-2):175-86.
445. Feng Chen ZZ, Kai Wang, Chengdong Zhang, Haifeng Gong, Fagang Ye, Aiyu Ji and Hao Tao. Study on salvianolic acid B in the reduction of
epidural fibrosis in laminectomy rats. BMC Musculoskeletal Disorders. 2014;15:337-42.
446. Holtz G. Prevention of postoperative adhesions. Journal of reproductive medicine. 1980;24:141.
447. <Floseal pituitary surg.pdf>.

448. Lewis KM, Atlee HD, Mannone AJ, Dwyer J, Lin L, Goppelt A, et al. Comparison of two gelatin and thrombin combination hemostats in a porcine liver abrasion model. J Invest Surg. 2013;26(3):141-8.
449. A novel collagen-based composite offers effective hemostasis for multiple surgical indications: Results of a randomized controlled trial. Surgery. 2001;129(4):445-50.
450. <collagen matrix.pdf>.
451. Hoell T, Hohaus C, Huschak G, Beier A, Meisel HJ. Total dura substitute in the spine: double layer dural substitute made from polylactide layer and bovine pericardium. Acta Neurochir (Wien). 2007;149(12):1259-62; discussion 62.
452. Pradeep K. Narotam M, ChB, MMED, FCS(SA), FRCSC, FACS, Sunil Jose´, MBBS,, Narendra Nathoo M, ChB, PhD, FCS, Charles Taylon, MD, and Yashail Vora, MD, FRCSC. Collagen Matrix (DuraGen) in Dural Repair: Analysis
of a New Modified Technique. Spine 2004;29(24):2861-7.
453. Narotam P, Jose S, Nathoo N, Taylon C, Vora Y. P63. Collagen matrix (Duragen) in spinal durotomy: technique appraisal and clinical results. The Spine Journal. 2003;3(5):147-8.
454. Raffa SJ, Benglis DM, Levi AD. Treatment of a persistent iatrogenic cerebrospinal fluid-pleural fistula with a cadaveric dural-pleural graft. Spine J. 2009;9(4):e25-9.
455. Grigoryants V, Jane JA, Jr., Lin KY. Salvage of a complicated myelomeningocele using collagen (Duragen) and dermal (Alloderm) matrix substitutes. Case report and review of the literature. Pediatr Neurosurg. 2007;43(6):512-5.
456. Neulen A, Gutenberg A, Takacs I, Weber G, Wegmann J, Schulz-Schaeffer W, et al. Evaluation of efficacy and biocompatibility of a novel semisynthetic collagen matrix as a dural onlay graft in a large animal model. Acta Neurochir (Wien). 2011;153(11):2241-50.
457. Zerris VA, James KS, Roberts JB, Bell E, Heilman CB. Repair of the dura mater with processed collagen devices. J Biomed Mater Res B Appl Biomater. 2007;83(2):580-8.
458. Esposito F, Cappabianca P, Fusco M, Cavallo LM, Bani GG, Biroli F, et al. Collagen-only biomatrix as a novel dural substitute. Examination of the efficacy, safety and outcome: clinical experience on a series of 208 patients. Clin Neurol Neurosurg. 2008;110(4):343-51.
459. McCall TD, Fults DW, Schmidt RH. Use of resorbable collagen dural substitutes in the presence of cranial and spinal infections-report of 3 cases. Surg Neurol. 2008;70(1):92-6; discussion 6-7.
460. Fransen P. Reduction of postoperative pain after lumbar microdiscectomy with DuraSeal Xact Adhesion Barrier and Sealant System. Spine J. 2010;10(9):751-61.
461. He YR, M; Loty, B. A quantitative model of post-laminectomy scar formation - effects of a nonsteroidal anti-inflammatory drug. Spine. 1995;20(5):557-63.
462. Moore JT, Collins JL, Pearce KH. The nuclear receptor superfamily and drug discovery. ChemMedChem. 2006;1(5):504-23.
463. Fang Q, Schulte NA, Kim H, Kobayashi T, Wang X, Miller-Larsson A, et al. Effect of budesonide on fibroblast-mediated collagen gel contraction and degradation. J Inflamm Res. 2013;6:25-33.
464. Tliba O, Cidlowski JA, Amrani Y. CD38 expression is insensitive to steroid action in cells treated with tumor necrosis factor-alpha and interferon-gamma by a mechanism involving the up-regulation of the glucocorticoid receptor beta isoform. Mol Pharmacol. 2006;69(2):588-96.

465. Chen LL, X; Cao, L; Li, X; Meng, J; Dong, J; Ding, J. An injectable hydrogel with or without drugs for prevention of epidural scar adhesion after laminectomy in rats. Chinese Journal of Polymer Science. 2016;34(2):147-63.
466. Barman Balfour JF, R. Deferiprone: A Review of its Clinical Potential in Iron Overload in β-Thalassaemia Major and Other Transfusion-Dependent Diseases. Drugs. 1999;58(3):553-78.
467. Piga A, Roggero S, Salussolia I, Massano D, Serra M, Longo F. Deferiprone. Ann N Y Acad Sci. 2010;1202:75-8.
468. De Sanctis V. Growth and Puberty and Its Management in Thalassaemia. Horm Res. 2002;58(supp)(1):72-9.
469. Browne PV, Shalev O, Kuypers FA, Brugnara C, Solovey A, Mohandas N, et al. Removal of erythrocyte membrane iron in vivo ameliorates the pathobiology of murine thalassemia. J Clin Invest. 1997;100(6):1459-64.
470. Kartikasari AE, Georgiou NA, Visseren FL, van Kats-Renaud H, van Asbeck BS, Marx JJ. Intracellular labile iron modulates adhesion of human monocytes to human endothelial cells. Arterioscler Thromb Vasc Biol. 2004;24(12):2257-62.
471. Kartikasari AE, Visseren FL, Marx JJ, van Mullekom S, Kats-Renaud JH, Asbeck BS, et al. Intracellular labile iron promotes firm adhesion of human monocytes to endothelium under flow and transendothelial migration: Iron and monocyte-endothelial cell interactions. Atherosclerosis. 2009;205(2):369-75.
472. Cragg lH, R; Miller, W; Solovey, A; Selby, S; Enright, H. The Iron Chelator L1 Potentiates Oxidative DNA Damage in Iron-Loaded Liver Cells. Blood. 1998;92(2):632-8.
473. Mattews AV, G; Menchaca, H; Bloch, P; Michalek, V; Marker, P; Murar, J; Buchwald, H. Iron and Atherosclerosis: Inhibition by the Iron Chelator Deferiprone (L1. Journal of Surgical Research. 1997;73:35-40.
474. Ernst E. Chelation therapy for coronary heart disease: An overview of all clinical investigations. Am Heart J. 2000;140(1):139-41.
475. Anderson TJ, Hubacek J, Wyse DG, Knudtson ML. Effect of chelation therapy on endothelial function in patients with coronary artery disease: PATCH substudy. Journal of the American College of Cardiology. 2003;41(3):420-5.
476. Richter K, Ramezanpour M, Thomas N, Prestidge CA, Wormald PJ, Vreugde S. Mind "De GaPP": in vitro efficacy of deferiprone and gallium-protoporphyrin against Staphylococcus aureus biofilms. Int Forum Allergy Rhinol. 2016;6(7):737-43.
477. Mohammadapour MB, M; Fassihi, A. Wound healing by topical application of antioxidant iron chelators; kojic acid and deferiprone. international Wound Journal. 2013;10:260-4.
478. Sharma OP, Bhat TK. DPPH antioxidant assay revisited. Food Chemistry. 2009;113(4):1202-5.
479. Arora S, Sevdalis N, Nestel D, Woloshynowych M, Darzi A, Kneebone R. The impact of stress on surgical performance: a systematic review of the literature. Surgery. 2010;147(3):318-30, 30 e1-6.
480. Hull L, Arora S, Kassab E, Kneebone R, Sevdalis N. Assessment of stress and teamwork in the operating room: an exploratory study. Am J Surg. 2011;201(1):24-30.
481. Pinto A, Faiz O, Bicknell C, Vincent C. Acute traumatic stress among surgeons after major surgical complications. Am J Surg. 2014;208(4):642-7.

482. Muller MP, Hansel M, Fichtner A, Hardt F, Weber S, Kirschbaum C, et al. Excellence in performance and stress reduction during two different full scale simulator training courses: a pilot study. Resuscitation. 2009;80(8):919-24.
483. Sivathasan N, Ramamurthy NK, Knight MJ. Letter to editor: "Comparisons between the responsibilities and roles of airline pilots and surgeons". Int J Surg. 2011;9(3):273.
484. Arora S, Aggarwal R, Moran A, Sirimanna P, Crochet P, Darzi A, et al. Mental practice: effective stress management training for novice surgeons. J Am Coll Surg. 2011;212(2):225-33.
485. Campbell J, Ehlert U. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? Psychoneuroendocrinology. 2012;37(8):1111-34.
486. Gillespie BM, Withers TK, Lavin J, Gardiner T, Marshall AP. Factors that drive team participation in surgical safety checks: a prospective study. Patient Saf Surg. 2016;10:3.
487. Goransson K, Lundberg J, Ljungqvist O, Ohlsson E, Sandblom G. Safety hazards in abdominal surgery related to communication between surgical and anesthesia unit personnel found in a Swedish nationwide survey. Patient Saf Surg. 2016;10:2.
488. Thorpe P. Training and patient safety. Br J Surg. 2004;91(4):391-2.
489. Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR. The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. Neural Plast. 2007;2007:60803.
490. Stokes AF, Kite K. On grasping a nettle and becoming emotional. In: Desmond PAHPA, editor. Stress, workload, and fatigue. New Jersey: Mahwah, NJ: L. Erlbaum; 2001.
491. Allen AP, Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci Biobehav Rev. 2014;38:94-124.
492. Kidd SA, Corbett BA, Granger DA, Boyce WT, Anders TF, Tager IB. Daytime secretion of salivary cortisol and alpha-amylase in preschool-aged children with autism and typically developing children. J Autism Dev Disord. 2012;42(12):2648-58.
493. Wolf JM, Nicholls E, Chen E. Chronic stress, salivary cortisol, and alpha-amylase in children with asthma and healthy children. Biol Psychol. 2008;78(1):20-8.
494. Bergen AW, Mallick A, Nishita D, Wei X, Michel M, Wacholder A, et al. Chronic psychosocial stressors and salivary biomarkers in emerging adults. Psychoneuroendocrinology. 2012;37(8):1158-70.
495. Dantzer R, Kalin N. Salivary biomarkers of stress: cortisol and alpha-amylase. Psychoneuroendocrinology. 2009;34(1):1.
496. Diaz Gomez MM, Bocanegra Jaramillo OL, Teixeira RR, Espindola FS. Salivary surrogates of plasma nitrite and catecholamines during a 21-week training season in swimmers. PLoS One. 2013;8(5):e64043.
497. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology. 2009;34(4):486-96.
498. Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, et al. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. Int J Psychophysiol. 2005;55(3):333-42.
499. Payne JD, Jackson ED, Hoscheidt S, Ryan L, Jacobs WJ, Nadel L. Stress administered prior to encoding impairs neutral but enhances emotional long-term episodic memories. Learn Mem. 2007;14(12):861-8.

500. Rai B, Kaur J. Mental and physical workload, salivary stress biomarkers and taste perception: Mars desert research station expedition. N Am J Med Sci. 2012;4(11):577-81.
501. Valentin B, Grottke O, Skorning M, Bergrath S, Fischermann H, Rortgen D, et al. Cortisol and alpha-amylase as stress response indicators during pre-hospital emergency medicine training with repetitive high-fidelity simulation and scenarios with standardized patients. Scand J Trauma Resusc Emerg Med. 2015;23:31.
502. van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. Psychoneuroendocrinology. 2006;31(1):137-41.
503. van Stegeren AH, Wolf OT, Kindt M. Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. Int J Psychophysiol. 2008;69(1):33-40.
504. Koh D. 0334 Use of salivary biomarkers to evaluate response to a stress management intervention. Occupational and Environmental Medicine. 2014;71(Suppl_1):A109-A.
505. McGrath JE. Stress and behavior in organizations. In: Dunnette MD, editor. Handbook of
industrial and organizational psychology. Chicago: Rand MCNally; 1976. p. 1351-95.
506. Craig C, Klein MI, Griswold J, Gaitonde K, McGill T, Halldorsson A. Using Cognitive Task Analysis to Identify Critical Decisions in the Laparoscopic Environment. Human Factors: The Journal of the Human Factors and Ergonomics Society. 2012;54(6):1025-39.
507. Goon SS, Stamatakis EA, Adapa RM, Kasahara M, Bishop S, Wood DF, et al. Clinical decision-making augmented by simulation training: neural correlates demonstrated by functional imaging: a pilot study. Br J Anaesth. 2014;112(1):124-32.
508. Mason V, Balloo S, Upton D, Heer K, Higton P, Shiralkar U. Surgeons' experience of learning psychological skills: a preliminary evaluation of a psychological skills training course. Ann R Coll Surg Engl. 2009;91(4):321-5.
509. Lifchez SD, Redett RJ, 3rd. A standardized patient model to teach and assess professionalism and communication skills: the effect of personality type on performance. J Surg Educ. 2014;71(3):297-301.
510. Sturm LP, Windsor JA, Cosman PH, Cregan P, Hewett PJ, Maddern GJ. A systematic review of skills transfer after surgical simulation training. Ann Surg. 2008;248(2):166-79.
511. Arora S, Aggarwal R, Sirimanna P, Moran A, Grantcharov T, Kneebone R, et al. Mental practice enhances surgical technical skills: a randomized controlled study. Ann Surg. 2011;253(2):265-70.
512. Marcus HJ, Hughes-Hallett A, Cundy TP, Di Marco A, Pratt P, Nandi D, et al. Comparative effectiveness of 3-dimensional vs 2-dimensional and high-definition vs standard-definition neuroendoscopy: a preclinical randomized crossover study. Neurosurgery. 2014;74(4):375-80; discussion 80-1.
513. Cundy TP, Gattas NE, Yang GZ, Darzi A, Najmaldin AS. Experience related factors compensate for haptic loss in robot-assisted laparoscopic surgery. J Endourol. 2014;28(5):532-8.
514. Retrosi G, Cundy T, Haddad M, Clarke S. Motion Analysis-Based Skills Training and Assessment in Pediatric Laparoscopy: Construct, Concurrent, and Content Validity for the eoSim Simulator. J Laparoendosc Adv Surg Tech A. 2015;25(11):944-50.
515. Beyer-Berjot L, Berdah S, Hashimoto DA, Darzi A, Aggarwal R. A Virtual Reality Training Curriculum for Laparoscopic Colorectal Surgery. J Surg Educ. 2016.

516. Choudhury N, Gelinas-Phaneuf N, Delorme S, Del Maestro R. Fundamentals of neurosurgery: virtual reality tasks for training and evaluation of technical skills. World Neurosurg. 2013;80(5):e9-19.
517. Kockro RA. Neurosurgery simulators--beyond the experiment. World Neurosurg. 2013;80(5):e101-2.
518. Hull L, Arora S, Kassab E, Kneebone R, Sevdalis N. Observational teamwork assessment for surgery: content validation and tool refinement. J Am Coll Surg. 2011;212(2):234-43 e1-5.
519. Spanager L, Beier-Holgersen R, Dieckmann P, Konge L, Rosenberg J, Oestergaard D. Reliable assessment of general surgeons' non-technical skills based on video-recordings of patient simulated scenarios. Am J Surg. 2013;206(5):810-7.
520. Larkin AC, Cahan MA, Whalen G, Hatem D, Starr S, Haley HL, et al. Human Emotion and Response in Surgery (HEARS): a simulation-based curriculum for communication skills, systems-based practice, and professionalism in surgical residency training. J Am Coll Surg. 2010;211(2):285-92.
521. Solheim O. Is aneurysm surgery too exciting for our own good? Acta Neurochir (Wien). 2016;158(2):217-9.
522. Hirst AR, Escuder B, Miravet JF, Smith DK. High-tech applications of self-assembling supramolecular nanostructured gel-phase materials: from regenerative medicine to electronic devices. Angew Chem Int Ed Engl. 2008;47(42):8002-18.
523. Geczy CL. Cellular Mechanisms for the Activation of Blood Coagulation. 1994;152:49-108.
524. Biswas D, Daudia A, Jones NS, McConachie NS. Profuse epistaxis following sphenoid surgery: a ruptured carotid artery pseudoaneurysm and its management. J Laryngol Otol. 2009;123(6):692-4.
525. Houkin K. Iatrogenic carotid artery injury in neurosurgery. Neurosurgical Review. 2005;28(4):248-.
526. Paradís-Bas M, Tulla-Puche J, Zompra AA, Albericio F. RADA-16: A Tough Peptide - Strategies for Synthesis and Purification. European Journal of Organic Chemistry. 2013;2013(26):5871-8.
527. Ellis-Behnke RG, Liang YX, Tay DK, Kau PW, Schneider GE, Zhang S, et al. Nano hemostat solution: immediate hemostasis at the nanoscale. Nanomedicine. 2006;2(4):207-15.
528. Heller M, Wei C. Self-assembly peptide prevents blood loss. Nanomedicine. 2006;2(4):216.
529. Vaz-Guimaraes F, Su SY, Fernandez-Miranda JC, Wang EW, Snyderman CH, Gardner PA. Hemostasis in Endoscopic Endonasal Skull Base Surgery. J Neurol Surg B Skull Base. 2015;76(4):296-302.
530. Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H. Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv. 2011;29(3):322-37.
531. Whang HS, Kirsch W, Zhu YH, Yang CZ, Hudson SM. Hemostatic Agents Derived from Chitin and Chitosan. Journal of Macromolecular Science, Part C: Polymer Reviews. 2005;45(4):309-23.
532. Koitschev A, Simon C, Lowenheim H, Naegele T, Ernemann U. Management and outcome after internal carotid artery laceration during surgery of the paranasal sinuses. Acta Otolaryngol. 2006;126(7):730-8.

533. Fyrmpas G, Constantinidis J, Foroglou N, Selviaridis P. Pituitary apoplexy following endoscopic sinus surgery. J Laryngol Otol. 2010;124(6):677-9.
534. Harrold C, Randeva H. Conservative Versus Surgical Decompression for Pituitary Apoplexy. 2014:13-7.
535. Takeda N, Fujita K, Katayama S, Akutu N, Hayashi S, Kohmura E. Effect of transsphenoidal surgery on decreased visual acuity caused by pituitary apoplexy. Pituitary. 2010;13(2):154-9.
536. Zhang X, Zhang W, Fu LA, Cheng JX, Liu BL, Cao WD, et al. Hemorrhagic pituitary macroadenoma: characteristics, endoscopic endonasal transsphenoidal surgery, and outcomes. Ann Surg Oncol. 2011;18(1):246-52.
537. Herbstreit FP, J. Spinal anaesthesia despite combined clopidogrel and aspirin therapy in a patient awaiting lung transplantation: effects of platelet transfusion on clotting tests. Anaesthesia. 2005;60:85-7.
538. Wetzel CM, Kneebone RL, Woloshynowych M, Nestel D, Moorthy K, Kidd J, et al. The effects of stress on surgical performance. Am J Surg. 2006;191(1):5-10.
539. Hindmarsh J, Hyland L, Banerjee A. Work to make simulation work: 'Realism', instructional correction and the body in training. Discourse Studies 2014;16(2):247-69.
540. Reinhardt T, Schmahl C, Wust S, Bohus M. Salivary cortisol, heart rate, electrodermal activity and subjective stress responses to the Mannheim Multicomponent Stress Test (MMST). Psychiatry Res. 2012;198(1):106-11.
541. Lane RD, McRae K, Reiman EM, Chen K, Ahern GL, Thayer JF. Neural correlates of heart rate variability during emotion. Neuroimage. 2009;44(1):213-22.
542. Jang JH, Kim KH, Lee YM, Kim JS, Kim YZ. Surgical Results of Pure Endoscopic Endonasal Transsphenoidal Surgery for 331 Pituitary Adenomas: A 15-Year Experience from a Single Institution. World Neurosurg. 2016;96:545-55.
543. Agrawal A, Kato Y, Sano H, Kanno T. The incorporation of neuroendoscopy in neurosurgical training programs. World Neurosurg. 2013;79(2 Suppl):S15 e1-3.
544. Koc K, Anik I, Ozdamar D, Cabuk B, Keskin G, Ceylan S. The learning curve in endoscopic pituitary surgery and our experience. Neurosurg Rev. 2006;29(4):298-305; discussion
545. Kshettry VR, Do H, Elshazly K, Farrell CJ, Nyquist G, Rosen M, et al. The learning curve in endoscopic endonasal resection of craniopharyngiomas. Neurosurg Focus. 2016;41(6):E9.
546. Jukes A, Murphy J, Vreugde S, Psaltis A, Wormald P. Nano-hemostats and a Pilot Study of Their Use in a Large Animal Model of Major Vessel Hemorrhage in Endoscopic Skull Base Surgery. Journal of Neurological Surgery Part B: Skull Base. 2016;e-first(Dec).
547. Jukes A, Miljkovic D, Wormald PJ, Psaltis AJ. Platelet activation by crushed and uncrushed muscle: a flow cytometry analysis. Int Forum Allergy Rhinol. 2017.