THE EFFECT OF TOPICAL ANTIFIBRINOLYTICS AND A NOVEL CHITOSAN GEL ON HAEMOSTASIS AND WOUND HEALING IN ENDOSCOPIC SINUS SURGERY

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By

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Abstract

Introduction:

Endoscopic sinus surgery (ESS) is at present the gold standard therapeutic modality for chronic rhinosinusitis (CRS) resistant to medical therapy. Whilst results from ESS for CRS are generally good, postoperative bleeding and impaired wound healing with adhesion formation remains a concern. Due to patient discomfort and the detrimental effects on wound healing caused by most packing materials, many surgeons no longer routinely use nasal packing. Surgeons have in the past sought agents which would provide post-operative haemostasis without detrimentally affecting wound healing. Antifibrinolytics have been available for many years, however, their topical application has only been explored in the last few years. Recently different forms of chitosan have separately shown significant promise as powerful haemostatic and anti-adhesion agents. The aim of this thesis was to explore the progressive understanding of the interaction between haemostasis and wound healing with possible development of a novel agent.

Methods:

The first step to scientifically assess bleeding after sinus surgery was to develop a standardised method of video endoscopy and grading the surgical field during ESS. This was done as a multinational collaborative trial.

Once this assessment tool was validated a randomised controlled trial evaluating the effect of two antifibrinolytics (epsilon aminocaproic acid and tranexamic acid) was conducted.

Further evaluation was then conducted on other possible hemostatic and anti-adhesion substances. This included various combinations of a novel chitosan gel. These gels were trialled *in vitro* to determine their effect on human nasal fibroblasts

derived from CRS patients. Fibroblast adhesion and proliferation as well as closure of standardised wounds were studied. The most promising of these gels was then used in an in vivo sheep model.

Once effectiveness of the chitosan-dextran gel was shown in the laboratory, this was evaluated against a number of currently available hemostatic and anti-adhesion substances in a standardised model of wound healing in sheep with CRS. This model had been previously extensively validated in our department. Full thickness mucosal injuries were created on the lateral nasal wall and ethmoids of twenty sheep and recombinant tissue factor (rTF), SprayGel or Chitosan-Dextran derivative gel applied topically in a randomized fashion. Adhesion formation and severity as well as microscopic wound healing and ciliary function were analysed at day 28, 56, 84 and 112 post initial surgery.

A further sheep study was conducted applying chitosan dextran gel to standardised mucosal injuries and comparing its effect on the control of bleeding to control. Bleeding time and grade were recorded and wound healing monitored via serial videoendoscopy over two weeks and objectively measured.

Results:

a) Assessment of the bleeding scales showed that inter and intra observer reliability for both scales tested were significantly improved by employing a standardized video-endoscopy technique. The Wormald scale proved to be more reliable and sensitive to changes in the most common surgical fields encountered in ESS.

b) Tranexamic acid showed a modest but clinically significant improvement in the surgical field at 2, 4 and 6 minutes after application. Epsilon aminocaproic acid did not effectively improve the surgical field.

c) Nasal fibroblast adhesion and proliferation were significantly impaired with dextran and chitosan. The most effective ratio that delayed but did not prevent wound closure were 5 % chitosan: 5 % dextran gel.

d) In a standardised sheep model of mucosal wound healing the chitosan gel significantly decreased lateral nasal wall and ethmoidal adhesions at all time points. The chitosan group had a significantly greater percentage of re-epithelialisation and reciliation than control and rTF. In addition the mean cilial grade in the chitosan group was significantly better than control.

e) The chitosan dextran gel was significantly more haemostatic at 2,4, and 6 minutes after injury with no significant difference noted in wound healing.

Conclusions:

Standardised methods of videoendoscopy and grading the surgical field in ESS are valuable tools for further research. Tranexamic acid significantly improved the surgical field to a moderate degree in ESS compared to control. Chitosan gel is a promising new powerful haemostatic bio-polymer which has a mild inhibitory effect on fibroblast attachment and proliferation. This may partially explain the significant improvement in microscopic wound healing and reduction in adhesion formation seen in a sheep model of chronic sinusitis. Future work evaluating this gel in the setting of a human trial is currently underway.

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and that to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I further consent to the thesis being made available for photocopying and loan if applicable, if accepted for the award of the degree.

Theodore Athanasiadis

Preface

A portion of the work described within this thesis has been submitted for publication, as listed below:

- Athanasiadis T, Beule A, Embate J, Steinmeier E, Field J, Wormald PJ. Standardized video-endoscopy and surgical field grading scale for endoscopic sinus surgery: a multi-centre study. *Laryngoscope* 2008;118(2):314-319.

- Athanasiadis T, Beule AG, Wormald PJ. Effects of topical antifibrinolytics in endoscopic sinus surgery: a pilot randomized controlled trial. *Am J Rhinol* 2007;21:737-742.

- Athanasiadis T, Beule AG, Robinson BH, Robinson SR, Shi Z, Wormald PJ. Effects of a novel chitosan gel on mucosal wound healing following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis. *Laryngoscope* 2008;118:1088-1094.

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CHAPTER 1 AIMS

The aims of the study were as follows:

1. Review literature on haemostasis and wound healing following endoscopic sinus surgery (ESS)
2. Develop and validate a standardised system to visualise and grade the surgical field in endoscopic sinus surgery
3. Examine the effect of topical Epsilon Aminocaproic Acid (EACA) and Tranexamic acid in controlling bleeding during ESS and following ESS
4. Develop and evaluate a novel haemostatic agent which reduces adhesions following ESS

CHAPTER 2 INTRODUCTION

Chronic Rhinosinusitis overview

Definition and Disease Burden

Chronic rhinosinusitis (CRS) is a group of disorders which result in mucosal inflammation of the nose and paranasal sinuses lasting at least 12 consecutive weeks[1]. There are four pairs of paranasal sinuses which along with the nasal cavity may be affected by CRS. These pneumatic or air-filled extensions of the respiratory part of the nasal cavity are lined by pseudostratified columnar ciliated (respiratory) epithelium and are named according to the bones in which they are located, namely the frontal, ethmoid, sphenoid and maxillary sinuses[2]. CRS is a very common condition which affects up to 18 % of the adult population[3]. In the USA it affects up to 30 million people and accounts for 2 % of all visits to medical practitioners[4,5]. These patients describe a quality of life which is as debilitating as diabetes or heart failure, they present to their local medical practitioner twice as often and have five times as many pharmacy prescriptions as patients without CRS[6,7]. The cost of CRS to society is difficult to measure, with estimates of US $2.4 billion in 1997 which did not include costs of surgery or radiologic investigation[8]. More recently the total cost of direct healthcare expenditures due to acute and chronic rhinosinusitis has been estimated at US$8 billion[7].

Pathophysiology

CRS has a complex pathophysiology with a number of possible aetiologies postulated. These include bacterial biofilms[9], staphylococcus superantigens[10], fungus[11], abnormal cell-mediated immune responses and abnormal cytokine cascades[12], prolonged sinonasal osteitis[13] as well as anatomical predisposition[14,15].

More rarely other causative factors may include mucociliary dysfunction, aspirin intolerance or cystic fibrosis[15].

These aetiologies are thought to result in mucosal inflammation and increased secretion production which leads to blockage of sinus ostia and drainage outflow tracts. This leads to hypoxia, anaerobic metabolism and an acidic environment which with stagnant mucous is an ideal environment for bacterial growth. The resultant desquamation of ciliated pseudostratified columnar epithelium, squamous metaplasia, fibrosis, goblet cell hyperplasia, and subepithelial thickening propagates the cycle of oedema, thickening mucosa resulting in disruption of the sinonasal mucociliary system[16,17].

Management

Medical Management

Initial management of CRS involves a multifaceted approach. Current therapeutic guidelines for medical treatment describe a combination of saline nasal irrigation[18], oral antibiotics, anti-histamines, nasal decongestants, mucolytics and topical or systemic corticosteroids[19-21]. The role of topical antibiotics and antifungal agents remains controversial[22-25]. More recently the role of leukotriene modifiers has been advocated[21].

Surgical Management

When optimal medical management fails to adequately improve disease progress and patient symptoms, then surgical management is usually indicated[26]. In the USA endoscopic sinus surgery (ESS) is the most common procedure performed by otolaryngologists, accounting for over 50% of procedures performed[27,28]. The annual incidence of ESS in the USA was estimated at 500 000 cases in 2007[29]. In Australia it is the second most common surgical procedure performed by otolaryngologists with 54 392 cases performed in 2007[30].

Historical Perspective

The history of ESS is interesting with ancient Egyptian writings describing the brain being removed through the nose followed by sawdust instilled into the empty skull as part of the mummification process[31]. Hippocrates, considered by many as the father of modern medicine describes a technique for treatment of nasal polyps. A sponge was tied to a piece of string, the string pushed through the nose into the nasopharynx and then pulled through the mouth, pulling the sponge through the nose hopefully also with the polyps[31]. Traditional Chinese medicine dating back to at least 2000 BC involves the use of a herb known as Ma huang for treatment of sinusitis. This herb contains ephedrine which is still used in some nasal decongestant preparations today[32].

Prior to the middle ages, the paranasal sinuses were poorly understood and thought by many to be a system of hollow spaces through which mucous produced by the brain was drained[31]. It was in Germany in 1660 when it was documented that

mucous is not a product of the brain and produced by the mucous lining of the region itself[33].

Early records of surgical management of CRS relate mainly to the maxillary sinus. Jourdain in France and Lamorier in Montpellier opened the maxillary sinus via its natural ostium (the middle meatus) and canine fossa respectively, however Jourdain's method failed to gain widespread acceptance[33]. A technique of entering the maxillary sinus through the anterior face via a sublabial incision was described independently by Caldwell in 1893 and Luc in 1897 and gained widespread acceptance for many decades[33]. Unfortunately this technique involves stripping nasal mucosa resulting in scar formation which can interfere with sinus drainage. This resulted in suboptimal outcomes, however it was several decades before more conservative mucosal sparing techniques were described[34].

Modern ESS is based on the theories of Messerklinger[34] who described the complex system of pathways by which the paranasal sinuses drained into the nasal cavity. Importantly he recognised that mucociliary clearance occurred through the natural ostia even in the presence of alternative surgically created ostia. His technique which was described by Stammberger was directed towards removing diseased tissue, restoring natural drainage pathways and preserving as much normal mucosa as possible[32,35-37]. In the mid 1980's this technique was introduced into the USA by Kennedy who coined the term Functional Endoscopic Sinus Surgery (FESS)[38,39].

Indications for ESS

The majority of ESS cases are performed for treatment of intractable CRS. In recent years, however, with improved technologies in visualisation, instrumentation, and

radiologic techniques, indications for the endonasal approach to various pathologies has expanded considerably[34,35,40,41]. Nasal indications such as fungal sinusitis, resistant vasomotor and allergic rhinitis, nasal polyposis, antrochoanal polyps, mucocoeles or retention cysts of paranasal sinuses, refractory posterior epistaxis, as well as septal or turbinate surgery are now common place[42,43]. Other procedures described using endoscopic techniques include repair of blow out fractures, orbital and optic nerve decompression, dacrocystorhinostomy, treatment of choanal atresia and repair of cerebrospinal fluid leak[44-46]. In addition there are a number of centres now successfully using this approach to treat nasal or paranasal sinus tumours such as inverting papilloma[47,48]. In conjunction with a neurosurgical team this approach is being used to perform hypophysectomy as well as resection of anterior skull base lesions with reduced hospital stay and minimal morbidity compared to conventional techniques[47,49,50].

Short and Long Term Outcomes of Endoscopic Sinus Surgery

A number of well conducted studies have examined the short and long term benefits of ESS for patients[26,51-56]. A significant reduction of medical resource usage after ESS has been reported, in particular less frequent visits to medical practitioners, reduced antibiotic use and more time being spent in productive employment[51]. Excellent subjective quality of life outcomes can be maintained in patients up to at least 8 years following ESS with appropriate post operative managements[52,57]. Interestingly, a long term outcome study noted that endoscopic assessment of the presence of ongoing disease in the sinuses at 18 months correlated with the need for revision surgery, whereas the subjective symptom scores did not[52]. This highlights the importance of complete mucosal healing even in the absence of clinical symptoms and adds weight to the efforts of improving mucosal wound healing[58]. In

summary these studies have demonstrated the vast majority of patients who undergo ESS for CRS are satisfied with the overall result and view ESS as beneficial.

Difficulties With Endoscopic Sinus Surgery

Haemostasis

Bleeding during and following endoscopic sinus surgery (ESS) remains a challenge for sinus surgeons despite a number of techniques and products available for improving the surgical field[59]. ESS is performed in narrow confines and thus even a small amount of blood can adversely affect the intraoperative endoscopic field of view. Regular contamination of the endoscope tip can be frustrating for the surgeon and this can lead to surgical manoeuvres being performed without clear visualisation. This increases the likelihood of complications as well as lengthening the operative procedure and may result in an incomplete surgical procedure[40,59]. Patients who continue to bleed after surgery have increased risk of airway compromise from inhalation of blood clots or from vomiting a stomach full of blood with subsequent aspiration. Therefore, reducing bleeding during and after sinus surgery is seen to be beneficial. Haemostasis in ESS is further discussed in chapter 3.

Complications

Complications of ESS may be arbitrarily divided into major and minor groups. Major immediate complications of ESS include intra-orbital haemorrhage, optic nerve damage and blindness, ocular muscle injury with subsequent intractable

diplopia, dural injury with intracranial penetration, CSF leak with possible meningitis and intracranial haemorrhage. Early series reported these risks being as high as 1-4 % of cases[60,61], however with improved technologies and training, these are now reported as less than 0.5 % of all cases[62]. Surgeons experienced in ESS still have a risk of complications as evidenced by a review of 1500 cases performed by five experienced rhinologists. In this review the highest risk of serious complications was after the surgeons had performed 100 ESS cases[63]. More recently another retrospective review of 421 cases showed that complications were unexpectedly not related to the disease severity or experience of the surgeon[64].

Adhesions

Minor complications of ESS include damage to the lamina papryacea, post operative epistaxis and adhesion formation[61]. The most common of these is adhesion or synechiae formation with randomised controlled trials reporting rates of between 15-30 %[65-71]. Adhesions form as part of the wound healing process, however, the presence of adhesions may interfere with normal mucociliary transport and mucosal function[72]. They frequently narrow or obstruct ostia resulting in the need for revision surgery and thus there is a large body of literature devoted to eliminating or reducing adhesion formation following ESS[65,67,73-75]. This is reviewed in detail in chapter 4.

CHAPTER 3 HAEMOSTASIS IN ENDOSCOPIC SINUS SURGERY

As discussed in chapter 2, bleeding during and following ESS remains a challenge for the endoscopic sinus surgeon. Even small volumes of blood in narrow recesses can obscure the surgical field increasing the difficulty and risk of complications associated with ESS. A number of techniques and materials may be used to improve intraoperative haemostasis and therefore the surgical field, as well as to prevent post operative haemostasis. These will be reviewed below after the topic of coagulation and haemostasis is revisited.

COAGULATION OVERVIEW

Blood coagulation is a physiologic defence mechanism that maintains the integrity of the circulatory system in response to vascular damage. It is a complex system of interrelated mechanisms which maintain the balance between coagulation and anticoagulation.

Following injury to a tissue surface the initial response is constriction of vessels and activation of platelets resulting in their aggregation and formation of a temporary hemostatic plug. Enzymes in the surrounding tissues as well as on the platelets themselves activate a cascade of reactions on the platelet surface involving coagulation proteases which result in thrombin formation. Thrombin is responsible for conversion of the soluble plasma protein fibrinogen into insoluble fibrin which is further cross linked in order to form a more definitive clot[76].

In a simplistic model the coagulation cascade with its proteases (coagulation factors) is activated either through the intrinsic system by exposure of negatively charged surfaces such as collagen fibres or through the extrinsic system by release

of tissue factor (Thromboplastin III) from damaged tissues (Figure 1). In reality there is a dynamic interaction between these systems which remains incompletely understood[76].

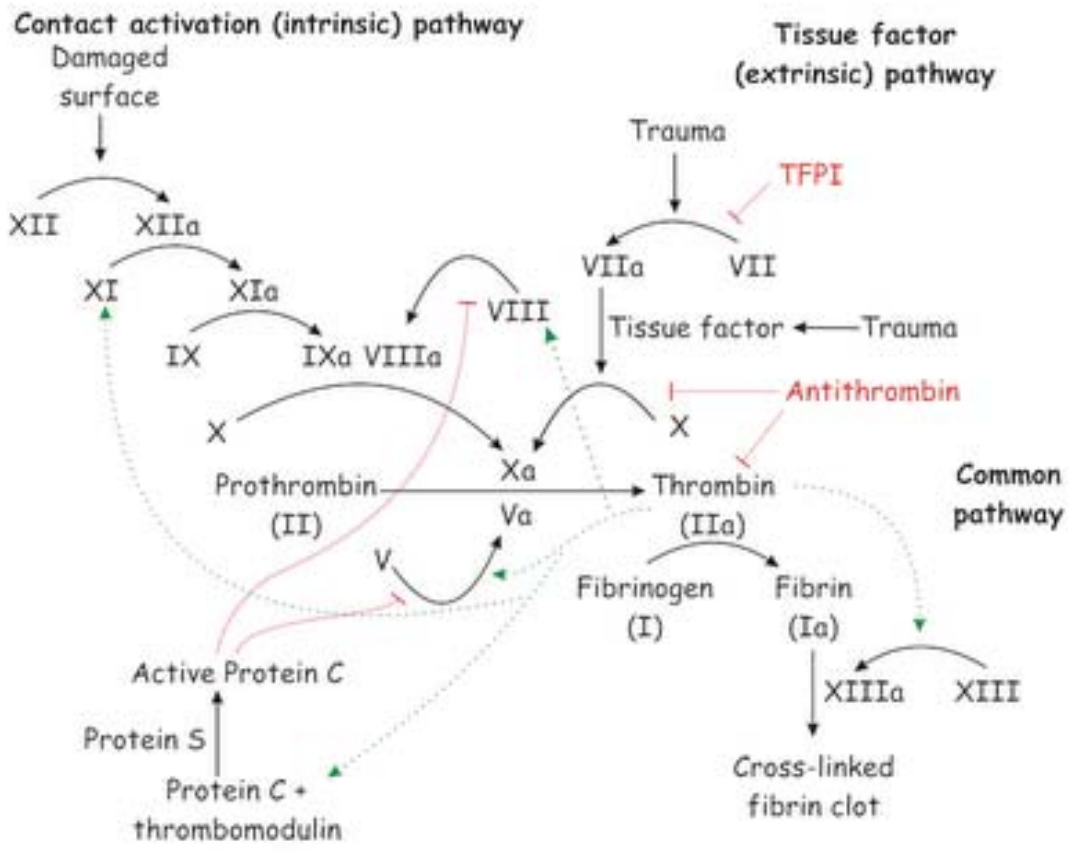

Figure 1: Model of coagulation cascade[77]

The fibrinolytic system shown in figure 1 modulates these reactions in a number of ways. Firstly intact endothelium expresses thrombomodulin which binds thrombin and prevents clot formation. This complex of thrombin-thrombomodulin also activates Protein C which together with Protein S inactivates various coagulation proteases and plasminogen activator inhibitors. This allows the formation of plasmin from plasminogen. Plasmin lyses fibrin and fibrinogen and is activated by tissue Plasminogen Activator (tPA) and urokinase Plasminogen Activator (uPA). In

turn these plasminogen activators are regulated by a Plasminogen Activator Inhibitor (PAI)[76].

Inflammatory responses are linked to haemostatic activation by a network of humoral and cellular components, including protease factors involved in the clotting and fibrinolytic cascades[78].The bidirectional relationship between haemostasis and inflammation is elegantly reviewed by Levi et al[79]. Cross talk between haemostasis and inflammation occurs with a number of inflammatory cytokines being activated by the coagulation pathway which in turn modulate inflammation through specific cell receptors[79,80]. The precise mechanism by which coagulation proteases activated in the process of haemostasis acts on protease receptors to induce a localised inflammatory response is still incompletely understood[81].

Conversely inflammatory mediators such as endotoxin and tumour necrosis factor alpha (TNFα) elicit the expression of tissue factor (TF) on blood cells[78]. They also inhibit activation of natural anticoagulant mechanisms such as protein C. In addition to shifting the haemostatic system in favour of clot formation, inflammation also elevates the level of plasminogen activator inhibitor (PAI) thereby decreasing fibrinolytic activity[78]. At one extreme this can result in pathologic states such as disseminated intravascular coagulation.

SURGICAL FIELD

Until work from this thesis was recently published there was no standardised and validated method of documenting the quality of the surgical field and in particular bleeding during ESS[82]. Such a tool would enable accurate evaluation and

comparison between multiple centres of techniques and materials used to decrease bleeding and improve the visual field. Additional applications might include investigating patients with recurrent bleeding of unknown origin, teaching and patient education as well as further investigating the recently demonstrated relationship between intra-operative bleeding and post operative healing[83].

Broadly speaking there are two main categories for analysing visualisation of the surgical field due to blood loss. These include quantifying blood loss and assessing visibility of the surgical field.

Objective quantification of blood loss during ESS has previously been used as a surrogate measure of the surgical field[84]. There are various reported methods of measuring total blood loss including estimating blood loss[85], measuring all suctioned contents[86], and slightly more refined methods which give a measurement based on subtracting the amount of rinsing fluids used from the amount of blood suctioned from the surgical field[84]. Estimating blood loss has been shown to underestimate blood loss and be largely inaccurate[87]. Measuring suctioned contents also remains inaccurate as use of "rinsing fluids" results in the suctioned liquid containing saline and tissue as well as blood. Subtracting the volume of rinsing solution used from the total suctioned contents is more accurate, however it does not take into account ingested fluids and is cumbersome to use if multiple time line measurements are to be made intra-operatively. More recently a more complex method of measuring the Haemoglobin (Hb) concentration in the suctioned fluids and comparing this to the patients intra-operative Hb has been used [86].

Unfortunately this is expensive, time consuming and requires specific haematological laboratory expertise which may not be available in all centers.
Both these more refined methods tend to be used to determine total blood loss from an entire procedure. If measured accurately this is a valuable tool, however, due to the dynamic nature of the surgery, volumes of bleeding and the surgical field will change during the procedure often in a matter of minutes. These methods give an overall impression of bleeding and the surgical field, however, are inaccurate to various degrees and unlikely to remain reliable across various centers.

Subjective measurements of bleeding during ESS and of the surgical field have been reported in the literature with tools such as visual analogue scales [86,88] and more commonly the six point "Boezaart" grading scale [59,84,89-91]. Visual analogue scales can be both powerful and sensitive tools with good intra-rater reliability. Their main weakness however, relates to individuals interpreting the scale differently and thus the inability of VAS to provide strong inter-rater reliability[92]. The aversion of graders to extreme ends of the scale also biases the data[93]. The Boezaart grading scale was initially suggested by Fromme et al[94] and then modified by Boezaart et al in 1995[89]. Whilst the Boezaart grading scale is a simple tool which is easy to learn and commonly used in rhinologic literature evaluating haemostatic techniques, it has unfortunately not been validated or standardized. Experience with this scale has shown that most gradings are either a 2 or 3, making it difficult to compare subtle differences in the surgical field[59,91].

There is therefore the need for a more dynamic measurement tool which is easy to use, can be repeated bilaterally multiple times during a procedure perhaps in

response to some surgical or anaesthetic maneuver and is at the same time sensitive enough to measure subtle changes. Development of such a tool is described in chapter 5.

INTRAOPERATIVE HAEMOSTASIS

PREOPERATIVE CORTICOSTEROIDS

There is one randomised trial which supports the use of systemic steroids preoperatively for improved haemostasis[88]. In this trial patients were given either 30mg of prednisolone daily for 5 days preoperatively or no treatment. Total blood loss was only slightly less in the steroid group, however visual conditions of the surgical field improved significantly. A more robust trial examining outcomes in ESS with preoperative corticosteroids supports this finding of a less inflamed mucosal surface making the surgery technically easier, however this study did not comment on haemostasis[76].

OTHER TECHNIQUES

Patient position is critical to the surgical field. Ideally the patient is positioned 30-40 degrees head up in order to prevent venous congestion and lower arterial pressure thereby improving the surgical field[40].

Topical vasoconstriction of the nasal mucosa with an adrenalin and cocaine solution at the commencement of surgery was described as early as 1941[95]. Moffett's solution as it became known also included sodium bicarbonate which dramatically

speeds up and increases the stimulatory effect (and toxicity) of cocaine[96]. Currently the solution is generally applied without sodium bicarbonate to avoid potential toxicity. The addition of adrenalin to cocaine has been elegantly documented to synergistically reduce nasal blood flow[97] and intraoperative blood loss[96]. If there is a contraindication to the use of cocaine then a nasal decongestant such as oxymetazoline may be used[40]. A randomised controlled trial comparing the use of oxymetazoline, phenylephrine and cocaine concluded that in the paediatric population oxymetazoline resulted in less subjective blood loss and thus was the preferred topical vasoconstrictive agent in that centre[98].

Infiltration with a mixture of 2 % lignocaine and 1:80 000 adrenalin in regions such as the lateral nasal wall above the middle turbinate, as well as the anterior and posterior ends of the middle turbinate has been advocated by some expert rhinologists[40]. Additionally infiltrating the pterygopalatine fossa using 2ml of 2 % lignocaine and 1:80 000 adrenaline has been shown to moderately improve the surgical field for around 90 minutes from the time of application[59]. For maximum benefit the needle should be bent 25mm from its tip at an angle of 45 degrees[99].

The use of beta-blockers in patients with a heart rate significantly greater than 60 has been advocated following two well conducted randomised controlled trials. The first study showed that esmolol improved the surgical field with a small reduction in blood pressure[89]. The second study showed that patients given metoprolol prior to surgery had a significantly lower intraoperative heart rate than the placebo group and this translated into an improved surgical field[91]. In this and other studies heart rate has subsequently been shown to correlate with the surgical field independently

of other interventions[59,90]. Some patients undergoing ESS may have asthma or some other condition which contra-indicates the use of β-blockers. In these patients, alternatives such as clonidine may be considered.

Clonidine is a centrally acting α-agonist which primarily acts to reduce sympathetic outflow from the central nervous system and thus reduces blood pressure[100]. It also constricts peripheral blood vessels[101], reduces blood flow to the nasal mucosa (in seals)[102] and has been shown to reduce blood loss in ESS if given pre-operatively[103,104]. Its cautious use is advised as a possible adjuvant agent for maintaining hypotension due to its prolonged action[100].

Total intravenous anaesthesia (TIVA) with the use of propofol and remifentanyl has been shown to significantly reduce bleeding and improve the surgical field compared to sevoflurane and fentanyl[84,90,105]. Interestingly a well conducted study comparing propofol to sevoflurane with fentanyl used in both groups showed no difference in blood loss or the surgical field between the two groups and suggested that perhaps the choice of opioid was more important[86]. A more recent study comparing propofol to sevoflurane using remifentanyl as the opioid in both groups showed that the propofol group had significantly less blood loss and an improved surgical field thus suggesting that propofol is a critical element in this equation[106].

Suction bipolar cautery can also be used to control isolated bleeding points in the surgical field during ESS. The use of cautery however causes tissue necrosis and can impair wound healing, thus its judicious use is suggested.

POST OPERATIVE HAEMOSTASIS

GELATIN/THROMBIN PRODUCTS

Fibrin glue is a topical biological adhesive consisting of concentrated human firbinogen which is activated by the addition of bovine thrombin and calcium chloride[107]. It imitates the final stages of coagulation aiding haemostasis and tissue sealing and has found favour in a number of surgical disciplines including cardiovascular surgery[107,108]. Traditionally, in ESS this glue has been reserved for part of the repair of dural lesions in treatment of cerbrospinal fluid leaks[109,110].

In terms of haemostatic potential, fibrin glues seem to be very potent. In a swine lethal injury model the United States army institue of surgical research compared the American Red Cross (ARC) Dressing to microfibrillar collagen, oxidised cellulose, thrombin, fibrinogen, propyl gallate, alluminium sulfate and fully acetylate glucosamine[111]. They found that only the ARC dressing consisting of fibrinogen and thrombin was significantly better than gauze. Unfortunately this particular dressing consisting of fibrinogen, thrombin, factor XII and calcium is not approved for human use, is expensive, not very durable and is not available in a form easily applicable to ESS[112].

A second generation fibrin glue named Crosseal (Quixil) containing human thrombin, human albumin, human cryoprecipitate and tranexamic acid has been studied in ESS. Two studies from a centre in Israel showed that it significantly improved post operative haemorrhage compared to a traditional pack (Merocel) and was more comfortable for the patient[113].

COLLAGEN

Whilst most studies show that fibrin glue is a more effective haemostatic than microfibrillar collagen such as Avitene or Gelfoam[114,115] it may still be useful due to to its ease of use and relatively lower cost. It has also been used successfully to keep dural repair structures in place and appears to removed by mucociliary clearance[116]

THROMBIN

Topical thrombin has been used as a haemostatic in many surgical disciplines from as early as 1955[117]. Its use alone has now been superceded by its synergistic combination with fibrinogen in fibrin glues. Concerns regarding disease transmission and antibody formation remain and have resulted in the development of a recombinant form of thrombin. A recent phase 3 trial comparing bovine thrombin to recombinant thrombin has found comparable efficacy, a similar safety profile and considerably less immunogenicity with the recombinant form[118]. At present this recombinant form remains unapproved for human use, is expensive and has not been trialled in ESS.

FLOSEAL

FloSeal is a topical agent consisting of bovine thrombin and bovine derived collagen matrix (gelatin). Its haemostatic effects in a number of surgical disciplines are well documented[119-122]. Its beneficial haemostatic effect on controlling acute anterior epistaxis has also been described in a randomised controlled trial[123].

A number of studies have prospectively examined the haemostatic effects of FloSeal following ESS. The first published report of its use following ESS

suggested it was a safe and effective treatment which adequately controlled postoperative bleeding[124]. Baumann et al compared FloSeal to Merocel[125]. They found it equally effective for intra-operative and post-operative haemostasis, however it was significantly more comfortable for patients. More recently, Jameson et al reported that FloSeal resulted in significantly better immediate control of post operative bleeding[126]. It was however no more effective than control (temporarily placed neuropatties) for haemostasis during the first post operative week. Contradictory to previous studies by Chandra et al they reported no difference in adhesion formation compared to control. Chandra et al report on the short and long term effects of FloSeal on wound healing which are discussed in detail in chapter 4 of this thesis[127,128]. In these studies they found FloSeal to be equivalent to thrombin soaked gelatin foam for peri-operative haemostasis[128].

Concerns regarding the adverse effects on wound healing, antibody formation and disease transmission of these fibrin/thrombin products limit the usefulness of these products in ESS.

HYALURONIC ACID/CARBOXYMETHYL CELLULOSE

Both hyaluronic acid packs such as Merogel or Sepragel and more recently carboxymethyl cellulose (CMC) packs such as Sinufoam are marketed for use following ESS as haemostatic and wound healing agents. Although anecdotally the pressure exerted by these packs assists in haemostasis there is limited data to support their use as hemostatics following ESS. In a small retrospective study, Frenkiel et al. report Sepragel as an effective haemostatic[129], however there are no reports of the hemostatic effect of CMC for ESS in the literature.

OXIDISED REGENERATED CELLULOSE

Oxidised regenerated cellulose (ORC) products such as Surgicel have moderate hemostatic potential[112]. Their use as haemostatic agents following nasal procedures was recorded by Huggins in 1969[130]. Despite its extensive history and prolific use in the nose, to date, there are no trials comparing Surgicel to no treatment (control). There is one trial which compares Surgicel Nu-knit (removable pack) to Vaseline gauze and Merocel packs in a prospective randomised fashion[131]. In this study, Shinkwin et al found the Surgicel Nu-Knit pack to be a more effective haemostatic as well as more comfortable for the patient. Unfortunately one of these patients had a fragment of Surgicel which remained in the nose and required a general anaesthetic for removal.

PLATELET GEL

Platelet gel is a fibrin tissue adhesive produced from autologous platelet-rich plasma created from centrifugation of whole blood[132]. Its use had been advocated for healing of chronic cutaneous wounds[133]. Until a recent retrospective review of its use in 16 patients following ESS, it had not been reported in rhinologic literature[134]. This study found the platelet gel to be subjectively more comfortable to the patient, effective as a haemostatic and have no obvious detrimental effect on adhesion formation. The disadvantages of this product are detailed in chapter 4 of this thesis.

ANTIFIBRINOLYTICS

Antifibrinolytics have been in medical use since first described in 1964[135] and in widespread use since the 1970's[136]. Two preparations are available, namely epsilon aminocaproic acid (EACA) and tranexamic acid. They are both synthetic derivates

of lysine and have antifibrinolytics activity in humans[137,138]. Both bind competitively to lysine binding sites on plasminogen thus preventing the binding of plasminogen to fibrin, its subsequent activation and transformation to plasmin[138]. In essence they work by preventing fibrinolysis and stabilising the blood clot[137]. They have a generally safe therapeutic profile with no evidence to suggest increased risk of thrombus formation with major surgery[136,138].

Historically antifibrinolytics have been used in oral or intravenous form for patients with bleeding diatheses such as haemophilia or von Willebrand's disease[137,139]. Their systemic use has found application in the treatment of primary menorrhagia[136], gastrointestinal bleeding[137], urinary tract bleeding[140], joint replacement[141-143], liver transplantation[144], cardiothoracic surgery[145] and patients with thrombocytopaenia[146]. More recently there have been a number of randomised controlled trials suggesting the efficacy of their use as topical agents in cardiac surgery[147,148], major orthopaedic surgery including joint replacement and spinal surgery[149], as well as for treatment of and preventing recurrence of traumatic hyphaema[150,151]. Of particular interest is the increasing use of tranexamic acid over the last decade by oral surgeons in mouthwash form for dental extractions on patients with bleeding diatheses or who remain on oral anticoagulants such as warfarin or coumadin[152,153]. There have also been favourable case reports on the use of systemic and topical tranexamic acid in the management of epistaxis with hereditary haemorrhagic telangiectasia (HHT)[154-156].

Recent work in our department using topical EACA in a sheep model of ESS found a significant reduction in bleeding and equivalent adhesion formation compared to a

saline control[157]. A previous non-blinded and non-randomised study of systemic tranexamic acid showed some haemostatic benefit, however up to 20% of patient experienced minor side effects such as gastrointestinal upset[158]. Based on its safe profile, relatively low cost and a promising animal pilot trial these agents were thought useful to study in a more formal clinical trial in order to evaluate their role in ESS (Chapter 6). Interestingly in work not included in this thesis, tranexamic acid was shown *in vitro* using fibroblasts cultured from CRS patients to improve a marker for nasal wound healing[159].

TERLIPRESSIN

Terlipressin is a gel consisting of trigylcyl lysine-vasopressin in a 3% CMC gel. Its use as a nasal haemostatic was reported in 1990 when it was found that Terlipressin reduced nasal blood flow, however it left ongoing nasal bleeding unaffected[160]. The authors here comment that the vasoconstrictive effect of Terlipressin was not as effective as anticipated and that the gel alone seemed to confer a mild haemostatic benefit.

POLYETHYLENE GLYCOL (PEG)

Products containing substantial amounts of polyethylene glycol (PEG) include SprayGel, CoSeal and Nasopore. SprayGel has not been noted for any significant haemostatic benefit, rather it requires near complete haemostasis prior to application[161]. Haemostatic benefits of CoSeal have been documented in vascular anastomoses. When used on iliac artery grafts one RCT found its haemostatic effects equivalent to a gelatin/thrombin combination product[162]. Similarly a study of its haemostatic effects in aortic anastomoses found it to be superior to a

gelatin/thrombin combination product[163]. To date the use of CoSeal following endoscopic sinus surgery has not been reported.

No literature regarding the haemostatic effects of Nasopore have been published. In unpublished trials provided by the manufacturer, its efficacy for haemostasis is supported in general terms[164,165].

CHITOSAN

Chitosan is a natural polymer obtained from chitin, a native polymer present in shellfish which has recently been shown in a number of animal and human abdominal and pelvic studies to have anti-adhesion properties[166-172]. Additionally chitosan has come under intense study for its hemostatic properties. A number of controlled animal and pre-clinical human trials have demonstrated potent haemostatic abilities[112,173-181]. These studies found chitosan to be superior to alternative dressings such as collagen, oxidized regenerated cellulose, thrombin, fibrin/thrombin sealants or bandages, QuickClot (a granular zeolite powder) as well as gauze in large venous and arterial injury models. Additional evidence of its life saving haemostatic benefits from its use in the battle fields of Iraq and Afghanistan - resulted in every US soldier in active duty being rationed a chitosan haemostatic patch[182].

Mechanism

In vitro studies using thrombin generation and scanning electron microscopy techniques describe the mechanism of action as aggregation of platelets and erythrocytes via cross-linking, independent of the coagulation cascade[174,175,180,181]. There is one *in vitro* study which suggests that β-chitosan is far superior to α-

chitosan in this regard[183] and another that highly modified poly-N-acetyl-chitosan induces more marked erythrocyte aggregation than chitin or chitosan[184].

Chitosan is the most important biocompatible, biodegradable, mucoadhesive product formed from the second most abundant biopolymer (after cellulose) called chitin. It is used extensively in agriculture for frost protective coatings and protective coating of fruits and vegetables during transport[185]. Additional uses include as a flocculent in water and waste treatment[186], as a component of cosmetic products such as toothpaste, shampoos and moisturisers[185], as an over the counter medication marketed for weight loss[187], as well as a variety of biomedical applications[188,189].

Although it has been used successfully in the nose as an agent to deliver topical medications including prednisolone, vaccines, growth hormones, anti-inflammatory agents, antibiotics and insulin, there are no reports of its use following nasal surgery[190-197].

CYANOACRYLATE

Tissue adhesives such as cyanoacrylate have been used for a number of years to successfully close lacerations and surgical incisions with some reports of their haemostatic properties on skin[198,199]. One animal study has investigated the use of cyanoacrylate glue as a haemostatic agent in the nose[200]. Using a porcine epistaxis model Singer et al found the glue to be effective in controlling epistaxis even in heparinised animals. There are no reports of its use following ESS, however it is known to cause chronic inflammation, fibrosis and some connective tissue

necrosis[201]. Given these serious concerns regarding its adverse impact on wound healing cyanoacrylate glue is unlikely to be recommended for use following ESS.

TISSUE FACTOR

Tissue factor (TF) is the major cellular initiator of the blood coagulation cascade with a primary role of maintaining haemostasis[202]. As discussed above, release of TF from injured surfaces results in its binding to factor VII activation of the coagulation cascade and subsequent fibrin deposition. TF is also thought to play an important (but undefined) role in the crossroad between coagulation and inflammation[203]. In fact inhibition of TF may have some benefit in systemic inflammatory diseases such as disseminated intravascular coagulation in which the combination of coagulation and inflammation play a role[204].

While there are no reports of the systemic or topical use of TF as a haemostatic agent there is an atypical emerging role for its use in bone graft material in combination with ground bone and platelet gel for maxillary sinus floor augmentation[205-207].

Given its potent haemostatic potential, reports of improved bony wound healing in the maxillary sinus as well as the recent availability of inexpensive recombinant TF it was thought an ideal agent for further investigation as a topical agent in ESS.

OTHERS

A number of other rare topical haemostatic agents are reported in the literature with varying degrees of success, however none of them have been used in ESS. These include guava leaf extract[208], crushed geranium leaf[209], *Thalassophryne nattereri* (toadfish) venom[210], tripeptide copper complex[211] and amylose succinate[212,213].

SUMMARY

The ideal haemostatic agent for ESS would display a number of properties. These include:

1. the ability to achieve immediate and prolonged haemostasis;
2. having no detrimental effect on and preferably improve wound healing compared to the current "gold standard" of no packing;
3. being comfortable for the patient, and
4. having no risk of disease transmission or allergic reaction.

Other properties that would be useful include it being an inexpensive product which is stable, requires minimal preparation and is simple to use. The ability for it to be used as a carrier for other medication such as corticosteroids would also be potentially valuable. Ideally the haemostatic agent would not stimulate the coagulation cascade as this simultaneously stimulates the inflammatory cascade potentially worsening wound healing.

In this thesis, the need for a valid and standardised system to grade bleeding in ESS is explored and developed, a new role for established antifibrinolytics is described and new roles for a novel chitosan gel, polyethylene glycol and tissue factor are further investigated.

CHAPTER 4 WOUND HEALING IN ENDOSCOPIC SINUS SURGERY

NORMAL WOUND HEALING

Wound healing is an attempt by the organism to repair traumatised tissues in an effort to maintain homeostasis. The purpose of repair is to protect against a repeated injury, prevent the loss of important substances and to replace or repair damaged anatomical structures[214]. Importantly, wound healing addresses both anatomic structure and function[215].

Normal wound healing is characterized by a highly organised complex sequence of physiologic processes following some injury to the organism[216]. These processes of inflammation, cell proliferation, matrix deposition, and remodelling are finely regulated by a wide variety of sequentially released or secreted Growth Factors (GF's) and cytokines. The outcome of these processes lies along a continuum between complete replacement of injured tissue with newly regenerated cells or with scar tissue formation[217]. The ultimate healing pathway depends on the ability of the injured tissue to be replicated, the rate of cell proliferation and migration of regenerated tissue, and cell-matrix interactions[218]. Key to this process is the presence of an intact basement membrane layer, just superficial to the lamina propria, which can direct cell migration, proliferation and polarity[217] (Figure 2).

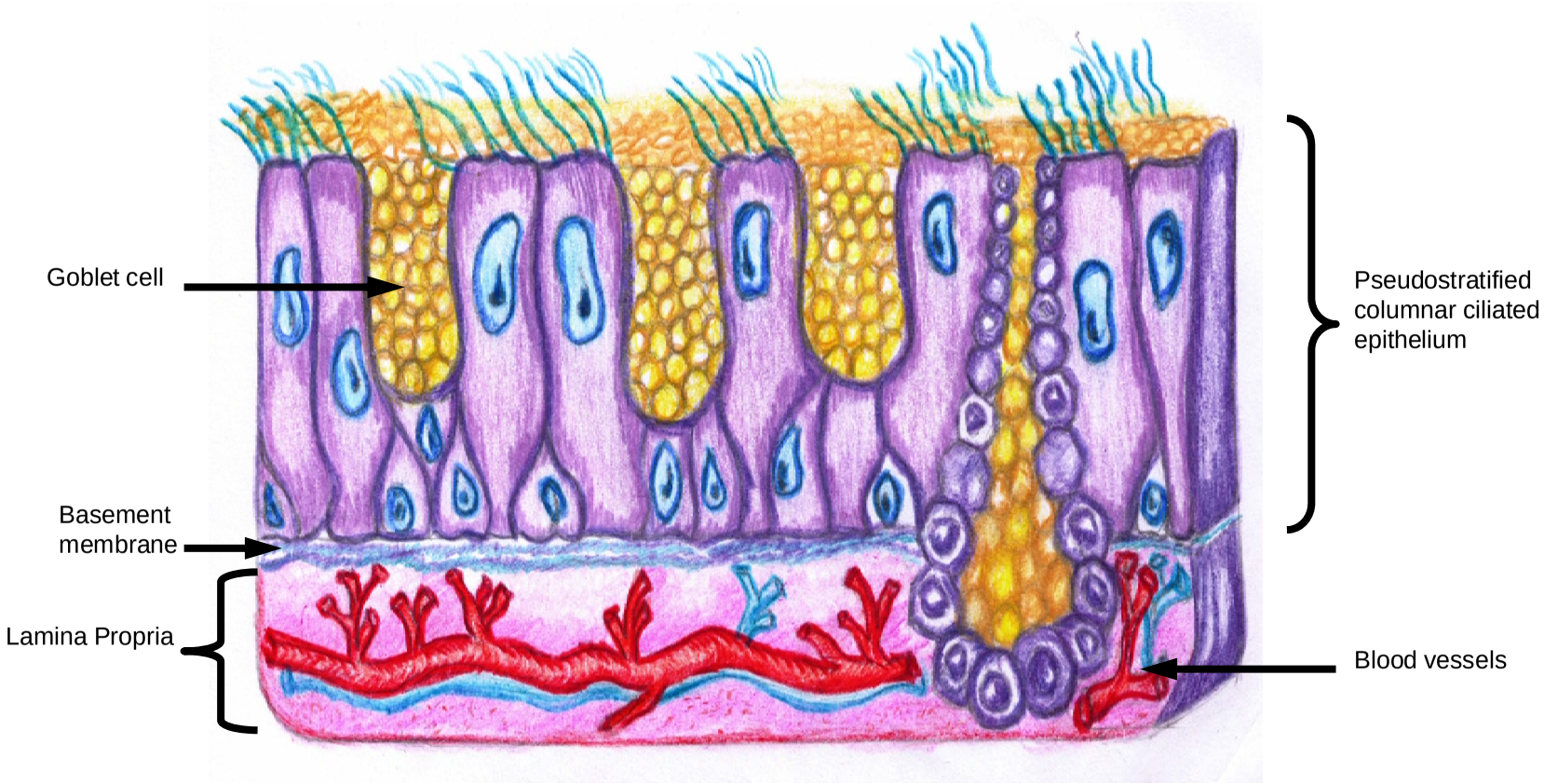

Fig 2. Cellular structures in sinonasal epithelium

Knowledge of microscopic nasal and paranasal sinus wound healing remains somewhat limited with most histopathologic studies based on cutaneous or gingival tissues. There are however four overlapping stages common to all wound healing (Figure 3). These are: Coagulation phase (<24 hours), Inflammatory phase (1-4 days), Proliferative phase (day 3-16) and the Remodelling phase (6-12 months)[214,219].

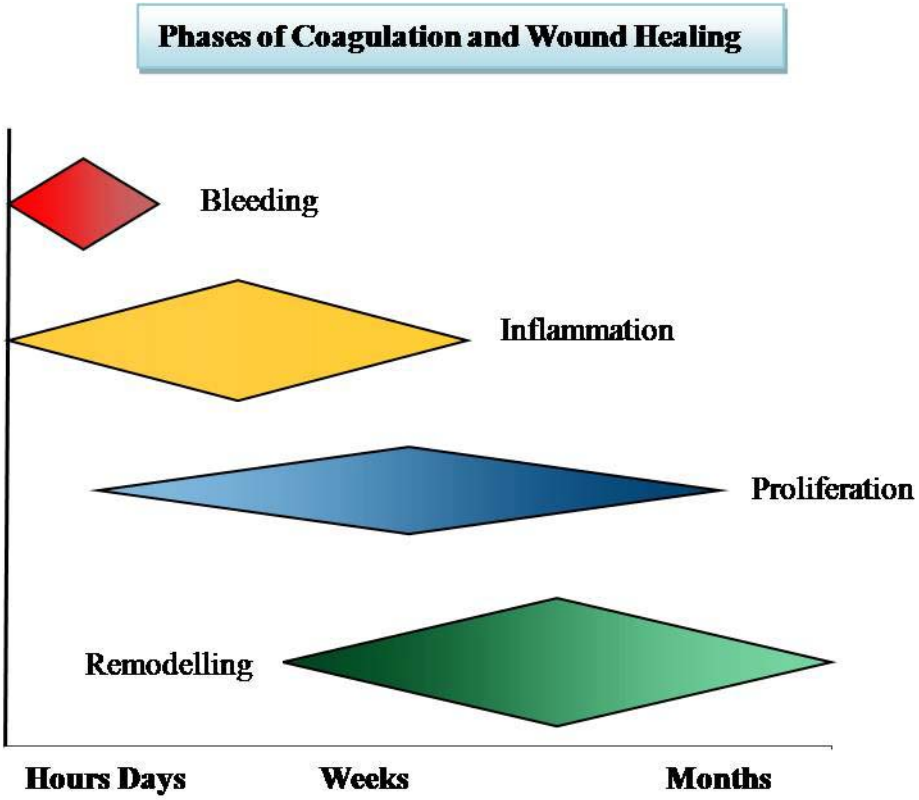

Figure 3 – Phases of wound healing

Coagulation

Injury to the nasal epithelium results in rupture and damage to vessels with subsequent exposure of platelets to subendothelial connective tissue. This activates the platelets causing them to aggregate and form a haemostatic plug whilst releasing numerous vasoactive substances such as serotonin, bradykinin, and histamine[219]. A transient vasoconstriction occurs over the next 5-10 minutes which assists in preventing blood loss as the haemostatic plug develops. While the platelets are aggregating, the intrinsic and extrinsic coagulation cascades are also activated forming a fibrin network within the wound[214]. Damaged cells release a number of GF's including Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-alpha (TGFα) and Transforming GF-beta (TGFβ) which regulate early repair. Fibrin within the clot also stimulates platelet release of PDGF, Epidermal GF (EGF), Insulin like GF-I (IGF-I), TGFβ, and Fibroblast GF (FGF) from the platelet α-granules[217]. This fibrinous clot at the end of the coagulation cascade acts as scaffolding for migrating cells which are the hallmark of the inflammatory phase.

Inflammation

The inflammatory phase is characterised by increased vascular permeability, chemotaxis of cells from the circulation into the wound and the local release of cytokines and growth factors[219]. Within the lamina propria an intense inflammatory reaction commences simultaneously with the coagulation phase resulting in leukocyte infiltration. During the first 24-48 hours these are predominantly polymorphonuclear neutrophils that assist cell penetration into the extracellular matrix (ECM) by stimulating release of collagenases and elastase. Neutrophils reach

their greatest number by 24-48 hours and begin to disappear after 72 hours[214]. They are replaced by monocytes over the next 3-5 days with activated monocytes becoming macrophages. These have an essential role to play in continuation of wound healing including debridement, matrix synthesis, angiogenesis and fibroplasia[217,220]. These roles are achieved by secreting a number of cytokines including TGF-β, basic FGF, EGF, TGFα and PDGF[217]. A prolonged inflammatory phase may persist if microbial infection occurs which is thought to result in an excited phase of fibroplasia[214]. This gives some weight to the surgical principle of removing dead and devitalised tissue as well as all remnants of disease.

Proliferation

Lasting between 3-21 days the proliferative phase is characterised by the multiplication of fibroblasts, endothelial cells and epithelial cells to regenerate new tissue[219]. Fibroblasts appear 48-72 hours after injury, however, a lag of approximately 48 hours occurs before significant collagen synthesis occurs. This corresponds to angiogenesis and the increased blood flow to the healing tissue[214]. Angiogenic GF's are released from injured nasal cells, platelets and the ECM with endothelial cell migration relying on continuing collagen synthesis. Collagen synthesis reaches its maximum in the first 2 weeks and wound collagen levels are at a maximum 3 weeks following injury[217]. The final proliferative stage commences within a few hours of injury. Epithelial regeneration begins with the migration of new epithelial cells from adjacent undamaged areas. The steps of proliferation of undifferentiated respiratory basal cells, reorientation and subsequent differentiation complete the epithelial healing process[217]. In the sinuses this epithelial migration occurs at an estimated 4-20 μm/h with undifferentiated respiratory basal cells from adjacent non-traumatized areas as the main source of cells[216,221]. Ciliogenesis and

differentiation however can take several months as documented by several scanning electron microscopy studies[222-225].

Maturation and Remodelling

Nasal ECM remodelling continues for much longer than dermal wound healing. It has been histologically documented for at least 6 months after ESS and is to some degree dependent on the injuries caused[217]. A superficial wound will heal with complete maturation within 6 months, however a full thickness mucosal injury may not completely mature for more than 18 months[226]. Remodelling is both upregulated and prolonged in the presence of inflammation[215]. As remodelling occurs, the levels of various collagen types alter with the large quantities of type III collagen replaced with type I collagen[227]. Other changes include formation of larger bundles of fibres, altered cross-linking and reduced levels of water and hyaluronic acid[219]. Ultimately maturation of the wound is dependent on the dynamic balance between collagen synthesis and lysis with increasing tensile strength displayed over time[217]. In dermal tissue when collagen levels reach a critical level, synthesis and secretion slow and fibroblasts transform into myofibroblasts which are able to contract the wound, however this has not been demonstrated in nasal fibroblasts[217].

ANIMAL MODELS OF NASAL WOUND HEALING

One of the first pioneers in the field of research into the effect of surgical interference of sinonasal epithelium was Hilding[228]. In a canine model he described how removing bands of epithelium resulted in scar formation which impaired mucociliary transport[229]. As described earlier in the section on maturation and re-

modelling, a superficial injury with intact underlying basement membrane and lamina propria will recover as quickly as 3 days, however when the basement membrane is damaged or removed the regeneration process is less ordered and prolonged[217,230]

A summary of animal studies on this topic reveals that normal ciliated respiratory mucosa, acute and chronic inflammatory changes, fibrosis, ulcerations, and granulation tissue are some of the possible histological patterns following ESS[69]. In a rabbit maxillary sinus wound healing model Benninger found that even though the mucosa regenerates, this did not necessarily translate into functional regeneration as cilia were absent[69]. The lamina propria which is critical to normal wound healing may be replaced by dense connective tissue and subepithelial glands do not always regenerate[69]. In addition, bone remodelling with neo-osteogenesis can occur, particularly when periosteum is removed[217,229].

HUMAN MODELS OF NASAL WOUND HEALING

Endoscopic features of nasal wound healing are elegantly described by Hosemann et al[231]. In this landmark paper he describes four sequential phases of endoscopic appearance following ESS correlating them with simultaneously obtained histologic features. Phase 1 involves massive clot and crust formation which is observed for the first few days. Phase 2 sees granulation tissue covering the wound and this is gradually replaced in phase 3 at around 4 weeks by an oedematous appearing wound. The final phase of normalization of the paranasal lining is seen between weeks 6-8. A more recent similar study by Weber et al supported this early research,

however, Weber describes a much longer oedematous phase than Hosemann. This could not entirely be explained by the inflammatory response to injury and was possibly related to the tendency of diseased mucosa to be oedematous prior to surgery as well as the more extensive ESS now being routinely performed[214].

The impact of a number of factors such as intraoperative technique and post operative protocols on wound healing following ESS are discussed below.

IMPACT OF SURGICAL TECHNIQUE ON WOUND HEALING

Surgery itself results in tissue injury in addition to the trauma created by CRS. Surgical wound healing depends not only on the surgical technique and instruments used but also the disease process and its extent.

Weber states that the most important requirement for successful surgery is achievement of early regeneration of normal mucosa composed of many ciliated cells[226]. Following full thickness mucosal injuries, a number of studies have shown a decreased number of cilia with resultant poor mucosal function[69,226]. Similarly, other studies have shown impaired mucociliary transport even with normal numbers of cilia[232]. Mucosal preservation is thus essential for rapid post operative recovery of mucociliary function.

Due to the benefits of mucosal preservation some authors propose the minimally invasive sinus technique (MIST) in which structures covering ostia (eg uncinate process, medial wall of the bulla ethmoidalis, and perhaps the posterior wall of the agger nasi cell, as well as any gross polyps) are removed, however, diseased ostia

are not enlarged and diseased mucosa is preserved[233]. The theory is that the pathology of diseased mucosa is reversible if the sinus is ventilated and this theory was initially supported by Stammberger and others[35,234]. However, several concerns with this method exist. Firstly, intrasinus hypoxia is not the sole aetiologic factor in CRS and thus its correction will not completely address other potential aetiologies, particularly osteitis[235]. Other disadvantages include the inability to remove thick mucin intraoperatively and impaired application of topical treatments post operatively[235]. The influence of retained infected secretions and diseased tissue on wound healing following ESS is not definitively known, however, first principles of wound healing would suggest that this is an important aspect of any treatment. This is supported by a sheep ESS model where ongoing inflammation was noted to adversely affect wound healing[58]

Perhaps a more important aspect of ESS is the care taken by the surgeon to avoid critical structures and unnecessary mucosal trauma[27,35]. This is particularly relevant to areas such as the leading edge of the middle turbinate and the frontal ostium[236,237].

Aside from surgical technique, other factors discussed in this chapter such as inflammation, ongoing infection, nasal packing and perhaps post surgical therapies will also influence the rate and completeness of mucosal and cilial regeneration[226].

Initial extent of disease as reflected by poor endoscopic findings, requirement of a more extensive surgical procedure as well as a history of previous sinus surgery are the strongest prognostic factors for poor outcome and poor wound healing following ESS[57,216,238,239]. More recently an objective marker for poor wound

healing in the form of elevated levels of nasal secretion and connective tissue Matrix Metalloproteinase 9 (MMP9) has also been documented[240].

PATHOPHYSIOLOGY OF ADHESION FORMATION

An adhesion is a band of scar tissue that binds together two or more anatomic surfaces which are normally separated from each other. Iatrogenic damage to at least one surface is the most common initiator of the development of these pathological fibrotic bands.

Relevance of Adhesions to Sinus Surgery

The most common complication of nasal and sinus surgery is the development of post operative nasal adhesions[35]. Rates of between 15-30% are reported in randomised controlled trials and between 3-36% in retrospective reviews[65-71,241]. Adhesions are known to interfere with the normal mucociliary transport resulting in pooled mucous which is an ideal growth medium for a variety of microbial pathogens[35]. Where they narrow or obstruct ostia, adhesions may cause pain from pressure of retained secretions in addition to predisposing to recurrent disease[35,217].

Management of adhesions in revision surgery is of primary concern. In those patients requiring revision surgery some authors describe adhesions as being a causative factor of surgical failure in up to 60% of these cases[242]. In recalcitrant frontal sinus disease revision surgery may involve a modified endoscopic lothrop procedure[243]. The neo-ostium created in this procedure will on average stenose by 33% at 12 months postoperatively[244]. Interestingly, all patients in this prospective

study who were to develop clinically relevant stenosis requiring revision (>60%), developed this stenosis within 12 months of surgery[244]. This may well reflect the fibrotic nature of wound healing which commences during the inflammatory phase and continues for several months in this patient group. On the other hand not all adhesions require revision surgery and some are intentionally created between the middle turbinate and septum in order to prevent lateralisation of the middle turbinate[245].

Abdominal Adhesions

Pathogenesis of adhesion formation is extensively studied in the abdomen and pelvis where adhesions occur in 55% of males and 97% of females undergoing abdominal surgery[246]. The incidence of adhesion following ovarian surgery remains in the order of 85-90% regardless of the type of surgery being performed[246]. The sequelae of these adhesions in the abdomen and pelvis are lifelong and multifaceted with a large UK trial reporting that women undergoing an initial abdominal surgery had a 5% likelihood of being hospitalised in the next 10 years as a direct result of adhesions formed during the first surgery[247]. Whilst abdominal adhesions are the most common cause of bowel obstruction, adhesions in the pelvis are a well recognised cause pelvic pain and infertility[248].

It has been estimated that the presence of pelvic or abdominal adhesions may prolong subsequent abdomino-pelvic surgeries by an average of 24 minutes, having been reported to extend operating theatre time by as much as 17 hours [249]. Adhesions may also necessitate any subsequent procedures to convert from

laparoscopic to open, and have been associated with inadvertent enterotomy, resulting in higher complication rates associated with bowel perforation[246,250-253].

Epidemiological studies have shown that 30-35% of all hospital readmissions are associated with adhesion related complications[247,254]. Direct costs of adhesions in the pelvis were estimated at $US1.3 billion in 1994 for the USA alone [255] and €40 million for Sweden in 2006[256]. Given the extensive nature of adhesion formation in the abdomen and pelvis described above much of our current understanding of adhesions comes from peritoneal research.

The intraperitoneal organs are covered by parietal and visceral layers of the peritoneum which has a layer of squamous epithelial cells called the mesothelium. Trauma of the mesothelial layer results in combined stimulation of the coagulation and inflammatory cascades with subsequent fibroplasia[257]. Molecules produced by inflammatory, immune, mesothelial and fibroblast cells during the coagulation and inflammatory phases regulates fibrinolytic activity, tissue remodelling, angiogenesis as well as the deposition of ECM which are central to adhesion formation [258]. The formation of adhesions in the abdomen depends to a large degree on the dynamic balance between fibrinolysis of this clot and appropriate epithelial recovery versus failure of fibrinolysis followed by over exuberant proliferation, migration and differentiation of fibroblasts[257]. Thus the process of adhesion formation can be summarised as epithelial trauma, inflammatory response, fibrin deposition, cell proliferation, differentiation, migration and apoptosis, angiogenesis, ECM remodelling regulated by cytokines, hypoxia and genetic factors.

It has been shown that fibroblasts from chronic wounds migrate into the fibrinous clot deposit, secrete ECM proteins and initiate adhesion formation[259]. Additional cytokines and hypoxic conditions at the site of injury may also influence these peritoneal fibroblasts to alter their phenotype[260]. Peritoneal fibroblasts are thus the main cause of abdomino-pelvic adhesions. A study examining 4325 randomly selected known genes has convincingly shown that fibroblasts involved in peritoneal adhesions have a different phenotype to those fibroblasts which do not form adhesions[261]. Additionally, in abnormal wound healing with adhesion formation, fibroblasts are more resistant to apoptotic signals and have a higher proliferative rate[262]. This altered phenotype of adhesion fibroblasts may lead to accumulation of extra fibroblasts at the wound site resulting in fibrosis and adhesion formation[261] . How to maintain apoptosis at a desired level for normal wound healing however remains unanswered.

Specific phenotypic changes include reduced ratio of tissue plasminogen activator to plasminogen activator inhibitor-1, reduced apoptosis in hypoxic conditions, greater ability to produce TGFβ (inflammatory cytokine) and ECM molecules than normal peritoneal fibroblasts. *In vitro* these phenotypic changes can be induced by hypoxia, however are irreversible even following the restoration of normoxia[263] .

TGFβ regulates the inflammatory response and ECM production and thus is a critical factor in the development of adhesions[258]. In cutaneous wounds addition of TGFβ increases scarring while neutralising antibodies to TGFβ decreases collagen deposition at the healing site resulting in a more ordered dermis with normal tissue architecture[264]. In addition to adhesion formation and skin scarring, over expression

of TGFβ is implicated in the pathogenesis of several fibrotic disorders including pulmonary fibrosis, glomerulonephritis, and liver cirrhosis[265,266].

In animal models, Nitric Oxide (NO) has been shown to inhibit excessive collagen deposition and increased levels of adhesions demonstrated in the inducible nitric oxide synthase (iNOS) knockout mice[267-269]. Studies have also shown the reduced levels of NO produced by adhesion fibroblasts[263]. Addition of NO to adhesion fibroblasts caused a marked increase in the level of apoptosis, however it had no effect on normal fibroblasts[263]

Sinonasal Adhesions

Similar to abdominal adhesions, sinonasal adhesions form where mucosal surfaces are traumatised, usually iatrogenically[215]. The subsequent coagulative and inflammatory phases of wound healing result in a fibrinous clot forming between two injured mucosal surfaces[217]. Where this bridge fibrinous clot remains and is not removed by cleaning of the nasal cavity or where local fibrinolytic activity is inefficient, fibroblasts are likely to form adhesions[217]. From our knowledge of abdominal adhesions it would seem logical that the presence of blood clot, ongoing inflammation, presence of diseased tissue and hypoxia all contribute to increased adhesion formation.

To date there are no studies which examine the role of genetic influences on adhesion formation in the nose. There is however one study which has shown that patients with CRS express significantly higher levels of TGFβ (at both mRNA and protein levels). This correlated with increased fibrosis in the same regions of these

patients suggesting a role for TGFβ in nasal fibroblasts similar to that seen in the peritoneum[270].

The role of nitric oxide in nasal sinus wound healing remains unknown, however decreased levels of NO have been demonstrated in patients with CRS[271]. Higher levels of NO are known to improve peritoneal wound healing[267]. It is also thought to have a beneficial effect on mucociliary transport in the upper respiratory tract and to have significant antibacterial properties[272]. Future studies examining nitric oxide and iNOS levels following ESS and relating this to wound healing outcomes are outside the scope of this project but would add to the knowledge of sinonasal wound healing.

ADHESION PREVENTION

Peritoneum

There is a large volume of scientific literature and ongoing research dedicated to adhesion prevention in the peritoneal cavity. A summary of the more commonly used methods is shown Table 1 classed according to their proposed mechanism of aetiology.

Table 1: Classes of adhesion reduction agents (Adapted from Diaa and Diamond 2005[273])

Fibrinolytic Agents (fibrinolysis, stimulation of plasminogen activators)	Fibrinolysin Streptokinase Urokinase

	Pepsin Trypsin Plasminogen activators
Anticoagulants (prevent clot and fibrin formation)	Heparin Citrates Oxalates
Anti-inflammatory agents (reduce vascular permeability, reduce histamine release and stabilise lysosomes)	Corticosteroids Non-steroidal anti-inflammatory agents Anti-histamines Calcium channel blockers
Antibiotics (prevent infection)	Tetracyclines Cephalosporins
Mechanical Separation *A.* ***Intra-abdominal instillates*** (hydroflotation) ***B. Physical Barriers***	Crystalloid solutions Dextran Carboxymethylcellulose (CMC) Hyaluronic acid *Endogenous tissues*: bladder strips, omental grafts, peritoneal grafts, foetal membranes *Exogenous biomaterials*: Rubber sheets, Silicone sheets, Metal foil Fibrin glue Oxidised regenerated cellulose (ORC)

	Oxidised cellulose Gore-Tex (polytetrafluorotheylene) Polyethylene glycol (PEG) Chitosan Combination products eg sodium hyaluronate/CMC (Seprafilm)

Fibrinolytic Agents

The use of fibrinolytic agents in humans awaits investigation. Results of animal trials are controversial with most trials suggesting that any possible adhesion reduction effect is negated by the significant impairment of the early phase of wound healing[274]. The levels of fibrinolytics required to prevent adhesion formation resulted in haemorrhagic complications and reduced wound strength[275,276].

Anticoagulants

Heparin is the most commonly applied anticoagulant for the prevention of adhesions[273]. Several well conducted animal trials have failed to show efficacy of heparin when delivered by intra-peritoneal lavage, intravenous injection, or intra-abdominal installation[277]. It has however been shown to be effective in a rabbit model when using Interceed, an ORC as a carrier agent[277]. Unfortunately this did not translate into success in a human trial[278].

Anti-inflammatory agents

Anti-inflammatory agents have been widely studied for their potential adhesion reduction in the peritoneum[273]. Animal studies have failed to show any benefit with topical or systemic corticosteroids. Non Steroidal Anti-Inflammatory Drugs (NSAIDS) decrease secretion of plasminogen inhibitors and thus increase fibrinolysis[273]. They have been shown to be effective in small animal models in reducing adhesions, however no clinical trials with NSAIDS have been published at present, although several are reported to have been conducted[273]. Use of antihistamines for adhesion prevention has been shown not to be effective in human studies[279]. Initial promising reports with the use of subcutaneous calcium channel blockers in animal models have not been repeated[273].

Antibiotics

Systemic antibiotics are widely used in abdominal surgery for prophylaxis against infection and hence the inflammatory response that leads to adhesion development. There are no studies supporting the use of antibiotics for adhesion prevention in the peritoneum and in fact intra-peritoneal instillation of antibiotics in rats has been shown to increase adhesion formation[273].

Mechanical Separation

Mechanical separation of injured surfaces is the most widely used and effective method of adhesion prevention. This can be either by intra-abdominal instillates which cause hydroflotation or by physical barriers.

Intra-abdominal Instillates

Crystalloid solutions are absorbed from the peritoneal cavity at a rate of approximately 35ml/hr and thus were replaced by high molecular weight dextran (Hyskon) in an attempt to prolong the period of instillate persistence[280,281]. Four clinical trials evaluating Hyskon have been conducted with two supporting its use and two failing to detect any benefit[273].

CMC is a high molecular weight polysaccharide. In liquid form it has been found to reduce adhesion formation if applied prior to injury but not following injury[282]. Similar results have been found with a variety of hyaluronic acid polymers[283]. A more persistent gel-like combination of CMC with hyaluronic acid has been shown in rat and rabbit models to be effective in reducing adhesions when applied after surgery[284,285].

Physical Barriers

Endogenous physical barriers have failed to have the preventive qualities initially expected and are no longer commonly used[273].

Exogenous physical barriers such as rubber or silicone sheets and metal foils have been used in the past, however, have been abandoned in the abdomen due to the requirement for a second procedure to remove them[273].

There are many reports of the benefits of fibrin glue when used as a tissue sealant, and for haemostasis (when containing thrombin), however studies examining its adhesion prevention role in the abdomen are contradictory[273]. It may be that the

actual constituents of the glue are critical with fibrin glue sourced from cryoprecipitate being moderately effective and from fresh frozen plasma being ineffective in adhesion prevention[286]. The use of fibrin flue in general has also been limited due to the use of pooled human blood in its production[273].

Early oxidised regenerated cellulose (ORC) products such as Surgicel were introduced mainly for their haemostatic effects[273]. Most animal and clinical trials examining Surgicel have failed to demonstrate an adhesion prevention benefit[273]. A newer form of ORC called Interceed has been shown to be effective in a number of animal and clinical trials[287-289]. It forms a gel around 8 hours after application, is metabolised into glucose and glucuronic acid within a few days and is usually degraded without any evidence of a foreign body reaction[290].

Gore-Tex is a permanent non-toxic, non-reactive and anti-thrombogenic substance not affected by tissue enzymes[291]. This material has been shown to be effective in reducing abdominal and pelvic adhesions in clinical trials, although it can become encased in a soft tissue membrane[292,293]. It has even been shown to be superior to Interceed[294]. The major complication of its use is the possibility of infection which requires its surgical removal and increases adhesion formation[273].

More recently a polyethylene glycol based gel (SprayGel) which dissolves around 8 days following application and is renally excreted has found favour for its role in abdominal and adhesion prevention[295]. A number of animal and clinical trials as well as a large randomised controlled multicentre study support its safe use for adhesion prevention[161,296-299].

Recently there has been some interest in a pseudo-natural cationic polymer named chitosan for a number of its biologic effects[189]. N-O carboxymethyl chitosan (NOCC) is the most studied type of chitosan with regards to adhesion prevention. *In vitro* studies mainly concerned with murine fibroblasts have shown a significant impairment of cell adhesion, migration and proliferation when exposed to chitosan[166,172,300]. Generally the more highly de-acetylated forms of chitosan seem to impair fibroblastic activity[301].

A well designed study by Yeo et al found promising effects of chitosan on fibroblasts *in vitro*, however the same chitosan was found to cause a granulomatous reaction in rat abdomens lasting up to 4 weeks after exposure. This study highlights the value of animal studies, suggesting that while *in vitro* studies are useful, they should be interpreted with caution[302]. Most animal trials to date have used the NOCC form of chitosan and show a significant reduction in the incidence, severity and percentage area covered by post operative peritoneal adhesions as well as the ability to prevent adhesion recurrence[168-172]. Zhang et al, have also shown that a highly deacetylated form of chitosan (structurally different to NOCC) prevents peritoneal adhesions in rats, however a chitosan film seemed to exacerbate peritoneal adhesion formation[303]. Combinations of chitosan with gelatine, polyethylene glycol or hyaluronic acid have also shown poor outcomes with regard to adhesion prevention[304-306].

One prospective RCT has examined the role of NOCC in a clinical trial with 34 patients. This study showed the safety and effectiveness of NOCC when applied in the peritoneal cavity and is currently being followed up by a much larger multi-centre trial[167].

Combination products such as the bioresorbable Seprafilm membrane (sodium hyaluronate/CMC) have been shown to be effective and safe for the prevention of pelvic adhesions[248,307,308]. In a meta-analysis of its use in the abdomen by Zeng et al, it was also found to be effective in adhesion prevention, however, it did not reduce post operative intestinal obstruction and unfortunately increased rates of abdominal abscess formation and anastomotic leaks[309].

Peritoneal Adhesion Prevention: Summary

There are obvious concerns with application of most fibrinolytic, anticoagulant and anti-inflammatory agents in the surgical patient due to the increased risk of haemorrhage and demonstrated impairment of wound healing.

Intraperitoneal solutions are generally poor adhesion prevention agents unless they persist for at least 6 days and coat the damaged surfaces. In order to do this, a gel or film is preferred to a liquid[273]. Gels which are metabolised and excreted by the body without toxicity are preferred to more solid films which tend to result in foreign body reactions and remain as a nidus for potential infection. SprayGel and chitosan fit these criteria, with chitosan discussed in more detail in chapter 3.

Sinuses

A well known authority on respiratory wound healing suggests that adhesion prevention is best achieved by minimizing surgical trauma, long term packing with an occlusive nonadherent material such as rubber finger packing and the use of topical steroids[217]. It should be noted, however, that despite these recommendations

the a uthors r eveal t hat s tenosis, pa rticularly o f t he f rontal s inus c ould not be prevented[310].

Table 2: Methods of adhesion prevention in Endoscopic Sinus Surgery

Surgical Technique	Mucosal Preservation Powered Instrumentation
Medication	Corticosteroids (topical/systemic) Antibiotics Mitomycin C
Packing ***1. Non-absorbable***	Gauze *Polyvinyl Acetate*: Merocel Polyurethane Beschitin
2. Absorbable	*Hyaluronic Acid:* Merogel, Sepragel *Oxidised Regenerated Cellulose:* Surgicel, Surgicel Fibrillar Interceed *Carboxy-Methyl Cellulose (CMC):* Stammberger Foam, Sinufoam *Topical Haemostatics:* FloSeal, SurgiFlo, Crosseal *Polyethylene Glycol*: SprayGel, Nasopore, CoSeal Platelet Gel

	Chitosan
Crust/Clot Removal	Debridement Irrigation

Surgical Technique

Surgical technique and in particular the critical importance of handling tissues with care in order to preserve mucosa have been covered previously in this chapter. With the introduction of powered instrumentation such as the microdebrider there were initial reports of reduced rates of adhesion formation[311,312]. Since then a number of studies have shown no difference in adhesion formation or early postoperative outcome between powered instruments, through-cutting forceps, and non-through cutting forceps[313-315].

Medication

A review of postoperative protocols later in this chapter details the effects of medication and crust/clot removal on adhesion formation and wound healing.

With regards to mitomycin C, *in vitro* fibroblast studies suggest a suppression of activity with which is further supported by animal studies showing a reduced rate of maxillary ostium restenosis following its application[74]. At present, however no human trial has shown a statistically significant benefit to topical mitomycin C application on wound healing or adhesion formation following ESS[316]. In addition, due to its alkylating cytotoxic nature real concerns regarding its potential

carcinogenicity have been demonstrated in other applications and as such its cautious use is suggested in treatment of benign disease[317].

Packing

The use of nasal packing as a means of keeping mucosal surfaces separate from each other in order to prevent adhesions has been advocated by a number of authors[35,65,68,241]. They may also be used as a haemostatic aid as well as providing some support and structure to the nose after some sinonasal surgeries[318]. Risks described with packs include local or systemic infection and possible toxic shock syndrome, alar necrosis, dislodgement with possible aspiration, iatrogenic obstructive sleep apnoea, septal perforation and finally triggering the naso-vagal reflex with subsequent hypotension and bradycardia[319].

Below is a review of the packs available as documented in sinonasal literature with particular reference to wound healing and adhesion formation:

Non-Absorbable Packs

A number of non-absorbable physical barriers have been tried following ESS. These include paraffin gauze[320], polyurethane packs[65], a combination pack of plastic film and cotton[318], and expandable polyvinyl acetate packs[241]. A chitosan based pack named Beschitin has also been in common use in Japan since the early 1980's for use following ESS. There is some evidence for its haemostatic benefit as well as comfort compared to other non-absorbable packs[321]. The use of these packs and particularly gauze packing is strongly advised against by most authors for a number of reasons[40]. The nature of these packs requires their removal at some stage which has been noted to be detrimental to wound healing. During removal of such packs,

the surface tissue is peeled away, leading to renewed trauma, bleeding, initiation of inflammatory processes, fibrin exudation and consequent scar formation[214]. This process is said to be desirable only when debriding necrotic cutaneous wounds and not in wound care following ESS[214].

As described above, non-absorbable packs have been largely superseded by absorbable packs due in part to concerns regarding their negative impact on wound healing. Additionally patients report the significant pain and discomfort on pack removal as the worst part of the peri-operative experience[320]. For the paediatric patient this may necessitate a further general anaesthetic to remove the pack[322]

Absorbable Packs

Absorbable packs were initially (and continue) to be promoted for their supposed benefits on wound healing as well as the well established benefits for patient comfort.

Two separate studies have examined wound healing and adhesion formation with the most commonly used non-absorbable pack (Merocel) to the most commonly used absorbable pack (Merogel) as well as a newer absorbable packing (FloSeal)[125,323]. Both of these clinical studies showed equivalent wound healing and adhesion results, however patient comfort was improved with the use of absorbable packing.

Hyaluronic Acid

The effect of hyaluronic acid packs on healing of the sinonasal mucosa and adhesion formation following ESS has been studied in animal and clinical trials. McIntosh et al, found that Merogel improved epithelial regeneration at 12 weeks following full thickness mucosal injuries in a healthy sheep model[225]. Subsequent work however showed that this effect was limited to healthy mucosa and in a sheep model of sinusitis the dissolvable hyaluronic acid packs did not have any significant effect on mucosal healing or adhesion formation[224]. In a similar sheep model, hyaluronic acid packs impregnated with IGF-1 were shown to improve microscopic wound healing in healthy sheep, however, in sheep with CRS these packs had a detrimental effect[324]. In a rabbit maxillary sinus model, Procter et al, found the fibrotic and granulomatous reaction depended on the formulation.[325] When testing 54 different variations of hyaluronic acid packs *in vitro* as well as in a rabbit maxillary model, Orlandi et al found that subtle chemical changes can have dramatic biologic effects[326]. In this study, those stents that caused the least acute inflammation were most effective in maintaining the size of the neo-ostium. More recently a rabbit maxillary sinus study by Stumpe et al comparing two types of absorbable hyaluronic acid dressings found control (no packing) to give superior wound healing[327].

As mentioned above, Miller et al found no significant difference between Merogel or a non-absorbable pack (Merocel) on wound healing and adhesion rates in a small clinical trial[323]. When used with minimally invasive ESS, Merogel was found to have less adhesion formation than gelfilm, however, this may be due to the shorter retention time of gelfilm stents in the nose (5 versus 8 days)[73]. Interestingly, a well

conducted RCT found no difference in wound healing and adhesion formation between Merogel and no packing[328].

Oxidised Regenerated Cellulose:

Products containing oxidised regenerated cellulose (ORC), such as Surgicel, have been in use since at least 1965[329]. In the otorhinolaryngologic literature their use for haemostasis was described as early as 1969[330]. Whilst there are some recommendations for the use of Surgicel as a dissolvable pack following ESS[331], no animal or clinical trials examining its effects on wound healing have been conducted.

Surgicel Nu-knit is a more dense form of ORC which if applied after ESS needs to be removed during the healing phase. It has been evaluated as comfortable, and efficacious with regards to haemostasis, however no data are available on its wound healing properties following ESS. Of note, one patient in this study of 60 patients required a second general anaesthetic to remove a retained fragment of Surgicel Nu-knit[131].

A newer form of ORC named Surgicel Fibrillar has recently been introduced to the Australian market and is approved for use following ESS. To date no data on its haemostatic or wound healing properties are published. Anecdotal experience at a tertiary rhinology centre suggests it may have a role to play with exposure of large bony defects such as following an endoscopic medial maxillectomy[332].

Carboxy-Methyl Cellulose (CMC):

Although carboxy-methyl cellulose products such as "SinuFoam" or "Stammberger foam" are marketed for use following ESS, there is as yet no published data to support their use.

Products combining CMC with hyaluronic acid in a non-absorbable Merocel pack, however, have been advocated for improved patient comfort compared to standard Merocel packs[333].

Topical Haemostatic Gels/Pastes

As discussed in chapter 3, topical haemostatic agents can be quite useful in obtaining haemostasis peri-operatively, particularly after ESS.

Thrombin-gelatin combination products have been studied in ESS with informative results. Gelatin consists mainly of hydrolysed collagen typically obtained from bovine or porcine sources[29]. Thrombin may be added to gelatin in order to improve its haemostatic properties. Gelatin film (Gelfilm) alone has been shown to increase adhesion formation and granulation tissue in both paediatric and adult populations[73,334].

Chandra et al have reported the short and long term outcomes of FloSeal a bovine collagen derived matrix and human thrombin, to a thrombin soaked gelatin foam[127,128]. At 6-8 weeks post operatively they found a significant increase in granulation tissue and adhesion formation in the FloSeal group[128]. Reviewing the same patients at 12-24 months later they found a significant increase in adhesion formation (56% versus 11%), an increased requirement for adhesiolysis in the

FloSeal group (28% versus 0%) and histologic analysis showed that FloSeal became incorporated into the healing mucosa[127]. In a review article, these same authors suggest avoiding use of topical haemostatic packs such as FloSeal or Avitene (microfibrillar collagen) due to their detrimental effect on wound healing[335]. Although FloSeal is said to be completely resorbed within 6-8 weeks[336], a histologic study of its use in ESS found it to cause a reactionary fibrosis, be incompletely resorbed and grossly incorporated into the healing epithelium[337]. In support of Chandra's work a retrospective review of patients with and without FloSeal found that the placement of FloSeal with middle turbinate medialization resulted in a higher incidence of adhesion formation[338].

Another thrombin-gelatin paste named Surgi-Flo has recently been approved for use following ESS in Australia. It differs to FloSeal in that the gelatin is of porcine and not bovine origin[339]. To date there is no published literature to support its use, however, given its nature would be expected to have similar adverse effects on wound healing and adhesion formation.

Crosseal or (Quixil) is a fibrin glue consisting of human thrombin, human albumin, human cryoprecipitate and tranexamic acid. Its beneficial effects on haemostasis following ESS have been documented, however no studies have examined its effect on wound healing[113,340].

Most topical haemostatic agents will stimulate the coagulation cascade and in so doing simultaneously stimulate the inflammatory cascade. Aside from increased fibrosis associated with their use there are a number of other concerns[335]. Topical

application of thrombin has been demonstrated in many studies to result in antibody formation to elements of the coagulation pathway such as thrombin, factors V and X as well as fibrinogen[341,342]. The severity of the subsequent coagulopathy experienced by the patient correlates to the amount of thrombin exposure and increases with each application during the patient's lifetime[341,343]. Whilst these risks are mainly for bovine thrombin, there are case reports of antibody formation with human thrombin[344]. In addition there is the theoretical risk of infectious disease transmission with infections of Human Immunodeficiency Virus, Hepatitis and Creutzfeldt-Jakob disease possible with blood products[345]. To counteract some of these risks, the haemostatic efficacy of autologous fibrin glue in ESS has been reported, however its effects on wound healing have not been studied[346].

Polyethylene Glycol:

Products consisting mainly of inert polyethylene glycol (PEG) such as the fragmentable Nasopore pack and SprayGel topical spray are approved for use following ESS. Unfortunately there is no published data to support their use in this application.

An unpublished pilot trial sponsored by the manufacturers of Nasopore involving 30 patients found Nasopore to be more comfortable than non-absorbable packs such as Merocel and to have equivalent macroscopic wound healing outcomes[164]. Anecdotal experience from a tertiary rhinology centre suggests that some fragments of Nasopore may remain in the nasal cavity for several weeks requiring removal with suction. In the same centre, application of SprayGel to large bony defects such as

following an endoscopic Lothrop procedure has resulted in less re-stenosis, however there are concerns regarding possible increased infection rates in these patients[332].

Platelet Gel

A small trial involving 16 patients retrospectively evaluated the use of platelet gel following ESS[134]. They concluded that platelet gel is an effective haemostatic with no obvious detrimental effects on wound healing. It is however expensive, takes time and special personnel to prepare and has a short half life (6 hours) in which it must be used.

Chitosan

No animal or clinical trial data is available on the use of chitosan following ESS. There is however a review of the use of chitin coated sponges in Japanese literature which suggests that these sponges have benefits for haemostasis, prevention of infection, wound healing and patient comfort[321].

Summary of Nasal Packing:

At present many available biomaterials represent a compromise by excelling in one area (eg. haemostasis) while possibly worsening another (eg. adhesions, impairing wound healing or infection)[29]. In order to avoid co-stimulation of the coagulation and inflammatory cascades it would appear that haemostatic agents used in ESS should exert their haemostatic potential outside of the coagulation cascade. Products containing PEG for example are thought to induce platelet aggregation independent of the coagulation cascade[164]. Similarly products containing chitosan induce

coagulation by sealing vessels and stimulating erythrocyte aggregation, again independent of the coagulation cascade[174,347].

Several authors advocate avoiding nasal packing altogether on most occasions following ESS[348-350]. Orlandi found that nasal packing did not improve post operative haemostasis in a review of 165 patients and advised avoiding packing in the majority of patients due to the risks, costs and discomforts associated with them[237].

In general therefore, it seems that due to the risks, patient discomfort and pain, as well as the lack of evidence for any improvements in wound healing or reduction in adhesion formation nasal packing should be avoided in most cases. If however there was a pack available that was comfortable, haemostatic, improved wound healing and reduced adhesion formation, then this would be most beneficial to ESS.

Prior to widespread clinical use of such a biomaterial its effects should be objectively and prospectively evaluated[326]. This testing should include microscopic and macroscopic analysis with standardised and validated measurement methods.

Stents

In order to maintain the ostial size and patency achieved during ESS a number of authors recommend the use of stents and silicon tubes[351,352]. This is particularly true for the frontal sinus which has a tendency to re-stenose in a circular fashion (cicatrize) following ESS resulting in disease recurrence and the need for revision surgery. A number of stents and drainage tubes have been designed to address this need with equivocal results. Examining the pathophysiologic processes it would seem that the stents need to be in place for at least 3-6 months to obtain optimal

outcomes[214]. If removed earlier the wound healing is unlikely to be complete and thus remodelling will not be optimised. Additionally, removal in the inflammatory or proliferative phases (1-3 weeks) post operatively can result in localised haemorrhage and reinitiate the inflammatory cascade[214]. On review of the literature definitive recommendations regarding stents are lacking. The inadequate data to support their use as well as increased patient discomfort and possibility of a foreign body reaction would on balance suggest that they are not routinely used, rather reserved for specific patient groups.

POST OPERATIVE CARE

Debridement

Although most of the standard literature on ESS includes a program of post operative debridement, the role of debridement remains controversial[352]. Initial management by pioneers of the techniques such as Stammberger and Kennedy suggested that aggressive debridement of the nasal cavity was required post operatively to remove crust, blood clot and mucous in order to improve outcomes[35,239]. Stammberger recommends cleaning the ethmoid cavity 2 to 4 days post operatively, and then every 3 to 5 days for the following 10 days[35]. Kennedy recommends debridement on days 1, 3 or 4 post operatively and then weekly till the cavity has macroscopically re-epithelialized[239]. Lund advises debridement at day 3-4 post operatively and then weekly until the surgical area has healed[353]. Similarly Kuhn and Citardi in their early experience recommended reviewing the patients on 1-2 days post operatively, then every 3-4 days for 2 weeks and weekly till the 6th post operative week[354]. Several centres attribute their excellent long term results to

an inclusion of careful post operative management including regular post operative debridement[52,311]. In a recent randomised controlled trial, Bugten et al, report a significant reduction in bilateral adhesions (1:30 versus 9:30) with "gentle" debridement on 2 occasions at 6 and 12 days post operatively, although the patients in the debridement group experienced more pain[355]. They do however recommend that most patients only require 2 debridement procedures and that a third or further debridements are difficult to justify without any scientific evidence[355]. Thaler suggests the rationale for intensive post operative debridement to remove clot and granulation tissue and lyse synechiae is 3-fold[356]:

1. Large crusting and clot may trap mucous which can reinfect the sinuses as well as act as a nutrient medium for bacterial growth.
2. Crusts can act as a bridge across which adhesions may occur, particularly between the middle turbinate and lateral nasal wall.
3. Any denuded bone or retained bony fragments are potential sites of infection and should be removed.

Others recommend minimal post operative intervention and claim equivalent results to centres performing intensive post operative debridement. In a retrospective review conducted in the UK, 120 patients were seen 2 weeks post operatively and the cavity minimally suctioned if required. This study reported equivalent short term outcomes to other centres[357]. Interestingly following paediatric ESS, post operative debridement is often not possible and yet success rates are similar to those seen in adults who undergo intensive post operative debridement[322,356,358]. Fernandes has reported two prospective studies on cohorts of 55 and 102 patients with minimal post operative care who had excellent subjective outcomes and similar rates of

adhesion formation to patients with intense post operative debridement[359,360]. He suggests that intense post operative debridement may not be necessary and likens it to removing a scab from a healing cutaneous wound for which there is some histological evidence in other regions of the upper respiratory tract[359,361]. Nilssen et al, in 2002 performed a randomised controlled trial on 32 sinus cavities which received either no post operative debridement or intensive post operative debridement and found no difference between the two groups for adhesion rates, post operative symptom scores or endoscopic appearance scores[362]. Some authors recognise the inflammatory potential of re-bleeding which can stimulate adhesion formation and stress the importance of avoiding re-bleeding where possible[363].

In a landmark histological study examining the crusts debrided from patients as well as biopsies under these crusts, Kuhnel et al found that debridement of crusts during the first post operative week avulsed epithelium in 23% of cases. Similar debridement in the second week however revealed no epithelial crusts, therefore they advise debridement be delayed till the second post operative week[364]. The hours of patient care required to perform post operative debridement as well as the significant pain which can be caused to the patient are additional factors which should be considered[356].

In summary then, there is some evidence for gentle debridement on 2 occasions post operatively and given the histological evidence this is best commenced in the second post operative week.

Saline Irrigation

Eight randomised controlled trials have compared saline irrigation to placebo, no treatment or as an adjunct to other treatments. A recent Cochrane review examined these studies and concluded that there is evidence that saline irrigation is useful in the treatment of CRS, particularly for symptom control[18]. It seems that hypertonic saline is superior to isotonic saline in increasing mucociliary clearance, however how this translates into treatment for CRS is not clear[365-367].

The evidence for saline irrigation use post operatively is scarce however it is advocated by many leading rhinologists as an adjunct to post operative wound healing[354,356,367-369]. Recent work in our department has shown the efficacy of nasal douching with a squeeze bottle in reaching the post operative frontal ostium, particularly in a "vertex to floor" position[370]. Proponents of post operative saline irrigation suggest that hypertonic saline may improve mucociliary clearance as it does in healthy volunteers[367]. However, the main function of post operative saline irrigation is to reduce crusting and oedema, clear nasal mucous, pus and debris and possibly reduce the risk of adhesion formation by removing bridges of clot and crust[354,367]. Weber suggests that the irrigation solution be at body temperature as cool or cold douches act as inflammatory agents and induce nasal mucosal trauma[214].

Antibiotics

Although many rhinologists support the use of routine perioperative antibiotics, studies examining their role in this situation are few[239]. One prospective randomised controlled trial found no benefit on a range of subjective symptoms, endoscopic

appearance, or incidence of infections in the early post operative phase following ESS in patients who received a low dose of oral cefuroxime (500 mg/day)[371]. A novel method of prescribing antibiotics via nebulized route following ESS in patients with acute infection has been described[372]. In this open label pilot trial the nebulised group experienced a significantly longer infection free period compared to the oral antibiotic group (17 versus 6 weeks)[372]. Some authors suggest prescribing antibiotics only if infection is noticed at the time of surgery[354]. Where pus is present intraoperatively Orlandi suggests giving antibiotics targeted to the gram stain or culture results[369]. If this is not possible then he suggests a broad spectrum antibiotic against common sinus pathogens[369]

Some evidence that macrolides such as clarithromycin may down regulate key inflammatory transcription factors such as NF-kB and inflammatory markers such as IL-5, IL-8 and GM-CSF exists[373-375]. Thus it is postulated that they may offer anti-inflammatory benefits in addition to antibiotic effects. Long term, low dose macrolides may improve symptoms of CRS, endoscopic appearance and mucociliary clearance in the patient with persistent symptoms following ESS[376].

Corticosteroids

Historically a number of authors have supported the use of systemic and topical corticosteroids pre and post ESS[239,354].Generally if there is severe nasal polyposis then systemic corticosteroids are advocated in a tapered fashion and topical corticosteroids commenced in most cases during the first postoperative week[369]. The rationale behind steroid use is to reduce excessive inflammation and subsequent poor wound healing as well as potentially decreasing intraoperative bleeding when given preoperatively[239].

Three randomised controlled trials have examined the role of topical corticosteroids following ESS. Rowe-Jones report a prospectively conducted RCT with five year follow up examining the effects of post operative fluticasone propionate nasal spray versus placebo. The treatment group had significantly better endoscopic scores of mucosal oedema and polyps at five years and overall visual analogue endoscopic scores at 4 years than the placebo group. Also, significantly more prednisolone rescue medication was used in the placebo group[377]. In a follow on study from the same group it was found that CT and polyp scores were the strongest predictors of the need for post operative systemic corticosteroids[377,378].

Bross-Soriano performed a RCT in which patients were allocated to one of three treatment arms and followed up for 12 months. They were saline irrigation alone versus saline irrigation + fluticasone or saline irrigation + beclomethasone. Polyp recurrence was found to be highest in the irrigation alone group (44%), then in the beclomethasone group (26%) and lowest in the fluticasone group (15%)[379].

Djikstra et al performed a placebo controlled RCT with topical fluticasone and reported that at 12 months their was no difference in recurrence or persistence of disease between the two groups[380].

Interestingly both Djikstra et al and Rowe-Jones et al commenced topical steroid use after 6 weeks which is not the usual commencement time advocated by most authors. Therefore, the question as to whether early topical corticosteroid use, particularly during the inflammatory phase of wound healing remains inadequately answered.

Histologic studies are difficult to perform in ESS patients, however there is one study by Hosemann on human subjects which examines the macroscopic and microscopic pattern of nasal wound healing. This study also showed that topical application of budesonide improved wound healing[310].

Animal studies are much better suited to histologic examination. There are a number of these studies in rabbits which advocate the use of systemic and local corticosteroids[231,381]. There is also a well conducted sheep study from Adelaide which showed no difference in microscopic parameters of wound healing following ESS in nasal cavities packed with hyaluronic acid packs versus hyaluronic packs soaked in prednisolone[382].

CHAPTER 5: STANDARDIZED VIDEO-ENDOSCOPY AND SURGICAL FIELD GRADING SCALE FOR ENDOSCOPIC SINUS SURGERY: A MULTI-CENTRE STUDY

Statement of authorship

Standardized Video-Endoscopy and Surgical Field Grading Scale for Endoscopic Sinus Surgery: A Multi-Centre Study

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CHAPTER 6: EFFECTS OF TOPICAL ANTIFIBRINOLYTIC AGENTS IN ENDOSCOPIC SINUS SURGERY: A PILOT RANDOMIZED CONTROLLED TRIAL

Statement of authorship

Effects of topical antifibrinolytics in endoscopic sinus surgery: A pilot randomized controlled trial

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CHAPTER 7: EFFECTS OF A NOVEL CHITOSAN GEL ON NASAL FIBROBLASTS IN VITRO

Statement of authorship

Effect of a novel chitosan dextran gel on in vitro nasal fibroblast wound healing

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Introduction

Endoscopic sinus surgery (ESS) is the gold standard surgical treatment for chronic rhinosinusitis resistant to medical therapy[1]. In addition the role of ESS over the last decade has extended to include repair of CSF leak, nasolacrimal duct repair, orbital and optic nerve decompression, resection of sinonasal lesions as well as tumours extending into the skull base and intracranially[2]. Generally this surgical approach has good outcomes with most studies reporting an 80-90% success rate, however, post operative bleeding and formation of scar tissue (adhesions) remain a concern[3]. Use of post operative nasal packing to address haemostasis, whilst effective, remains controversial due to patient discomfort and its detrimental effect on wound healing[4].

Adhesions are bands of scar tissue binding together two or more anatomic surfaces and form following 15-30% of ESS procedures[5,6]. They are most commonly located between the middle turbinate and lateral nasal wall. Their tendency to obstruct the normal mucociliary transport system results in them being one of the most common causes of surgical failure and subsequent need for revision surgery[7].

The pathogenesis of adhesion formation in the paranasal sinuses has not been well studied, although recent work by Watelet et al on upper respiratory tract wound healing has added considerably to the literature described in the early 1990's by

Hosemann et al[7-12]. These two groups have shown that wound healing in the paranasal sinuses follows a systematic pattern of coagulation, inflammation and oedema, cell proliferation, matrix deposition and remodelling regulated by a wide variety of cytokines and growth factors. The outcome of these processes lies along a continuum between complete replacement of injured tissue with newly regenerated cells or with scar tissue formation. In the upper respiratory tract, fibroblasts are the most important cells with regards to adhesion formation as they are the major and almost exclusive producers of ECM[13].

Over the years a large volume of research has been dedicated to the prevention and treatment of adhesion formation both in the sinuses as well as other anatomic regions[14-20]. Recently there has been significant interest in the use of chitosan for its adhesion prevention effects in the abdomen and pelvis. A number of animal trials examining chitosan have shown significant reductions in adhesion incidence, severity and percentage area covered by post operative peritoneal adhesions as well as the ability to prevent adhesion recurrence[21-25]. A deacetylated form of chitosan has also been developed as the main agent in a powerful haemostatic dressing (Hemcon) employed by the United States military[26,27]. The data for chitosan as a biomaterial are promising, however, contradictory *in vitro* reports in the literature exist on its role in adhesion prevention which may well be due to variations in the molecular characteristics of the chitosan studied[28-32].

A review of the *in vitro* and clinical literature suggests that in general, for adhesion prevention a higher concentration of chitosan[25] which is highly deacetylated[32,33] and cross linked with a di- or multi aldehyde[34,35] would be appropriate. Additionally,

chitosan in a film form[36] or solution form[24] appears less effective than in gel preparation.

The aim of this study was to determine the effect of a novel chitosan-dextran (CD) gel and its separate components at various concentrations on fibroblast attachment and proliferation as well as in a standardised wound injury model. The secondary aim of this study was to compare CD gel to SprayGel (Confluent Surgical, Waltham, MA) which is a dissolvable adhesion barrier used extensively in the abdomen for the prevention of adhesions.

Given the well documented variety in response of different fibroblast strains to biomaterials[30], fibroblasts from human nasal specimens with chronic rhinosinusitis were chosen as the most representative sample.

Materials and Methods

This study was approved by the Institutional Ethics Committee.

Chitosan and dextran were both kindly provided by the Department of Chemistry, University of Otago. The succinylchitosan is derived from commercial chitosan by reaction with succinic anhydride in DMF. It contains both succinyl groups for solubility under neutral conditions and some residual amine groups for cross-linking. The dextran aldehyde is produced from the periodate oxidation of dextran, and contains 130 mol% aldehyde groups per sugar residue. SprayGel was purchased from Confluent Surgical (Waltham, MA).

Cell lines and culture conditions

Sinonasal biopsies were obtained from 3 patients undergoing ESS who had given informed consent. Primary fibroblast lines were generated as previously described[37]. Fibroblastic cells were separated from epithelial cells by immunomagnetic bead separation and allowed to proliferate. The separation process was then repeated to ensure a pure population of fibroblast cells. Only cells between the third and fifth passage were used for this study and all experiments were conducted in triplicate except for the wound model which was conducted in quadruplicate on two separate occasions. Cells were cultured at 37ºC with 5% CO_2 in "airway medium" with constituents per litre below: Dulbecco's Modified Eagle's Medium 1:1 nutrient mixture F12 HAM (15.6 g), sodium bicarbonate (1.1 g), ITS+1 liquid media supplement x100 (3 mL), bovine pituitary extract (25 mg), sodium pyruvate (0.11 g), retinoic acid ($2x10^{-8}$ moles), endothelial cell growth supplement (15 mg), hydrocortisone (2.5 mg), epidermal growth factor (10 mg), and 10% fetal calf serum (FCS). All media constituents were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO), except bovine pituitary extract (Invitrogen, Carlsbad, CA).

Fibroblast Attachment Model

Using 96 well plates (Nunc, Roskilde, Denmark), wells were coated in triplicate with 50µl of the test agent suspended at a 5% concentration in airway media. Control consisted of standard "airway media", while the other 3 test agents included a 5% chitosan solution, a 5% dextran solution and a 5% chitosan-dextran gel. Fibroblasts from each of the three cell lines were seeded at a concentration of $2x10^4$ cells per well and allowed to set for four hours prior to washing twice with

phosphate buffered saline (PBS) containing penicillin at 500U/ml, streptomycin and amphotericin B both at 0.5mg/ml (Sigma-Aldrich, St. Louis, MO). (Pilot studies had shown $2x10^4$ cells to be the optimal concentration for examining fibroblast attachment and proliferation). Airway media with a 10% solution of Alamar Blue (Serotec, Australian Laboratory Sevices, Mel AUS) was then added to the cells and the relative reduction of Alamar Blue spectrophotometrically measured every 12 hours for 24 hours and then every 24 hours for 4 days. Alamar Blue (AB) was used as a measure of relative fibroblast growth, their metabolic activity resulting in a reduction of the indicator dye from blue to red to determine relative cell growth[38,39].

Fibroblast Proliferation Model

Each of the 3 cell lines were seeded in triplicate on 96 well plates (Nunc, Roskilde, Denmark) at $2x10^4$ cells per well in airway medium and allowed to enter the log phase of growth over 24 hours. Cells were then washed twice with PBS and exposed to chitosan or dextran at serial dilutions of 5%, 2.5%, 1.25%, 0.63% and 0.32% in airway media + 10% AB. Positive controls consisted of the cells + airway media + AB whilst negative controls consisted of the various chitosan and dextran concentrations + airway media + AB. Four hours later and then every 24 hours for 4 days the relative reduction of AB over time was determined by spectrophotometry.

In vitro wound model

Fibroblasts were seeded at 10^5 cells per well in 12 well tissue culture plates (Nunc, Roskilde, Denmark) and cultured in airway medium. Media was changed every 24 hours until cells were confluent. On reaching confluence, the monolayer was starved for 48 hours by reducing the airway media concentration of FCS to 1%. In each

well, media was removed and 3 standardised injuries were created using a 2mm punch biopsy under 2x loop magnification (Carl Zeiss, North Ryde, Australia). There were 4 replicates for each of the three treatments for each of the 3 individual fibroblast cell lines. Treatments were as follows: control (airway media), chitosan-dextran gel and SprayGel (Confluent Surgical, Waltham, MA). Following the injuries, fresh airway media was added which was changed every 48 hours. The wound areas were digitally recorded and measured every 24 hours using SPOT, Version 3.0 (Silicon Graphics, Mountain View, CA) image analysis software by two blinded independent observers. This study was twice repeated.

Descriptive fibroblast growth study

Test agents of airway media (control), chitosan-dextran gel and SprayGel were placed in triplicate in the centre of a 12 well plate (Nunc, Roskilde, Denmark) and allowed to settle for 4 hours. Fibroblasts from each of the three cell lines were then seeded at $2x10^4$ cells per well, their growth was digitally recorded every 24 hours for 7 days and subjectively characterised with regards to cell attachment, cell proliferation, evidence of cytotoxicity and presence of an inhibitory zone.

Statistical Analysis

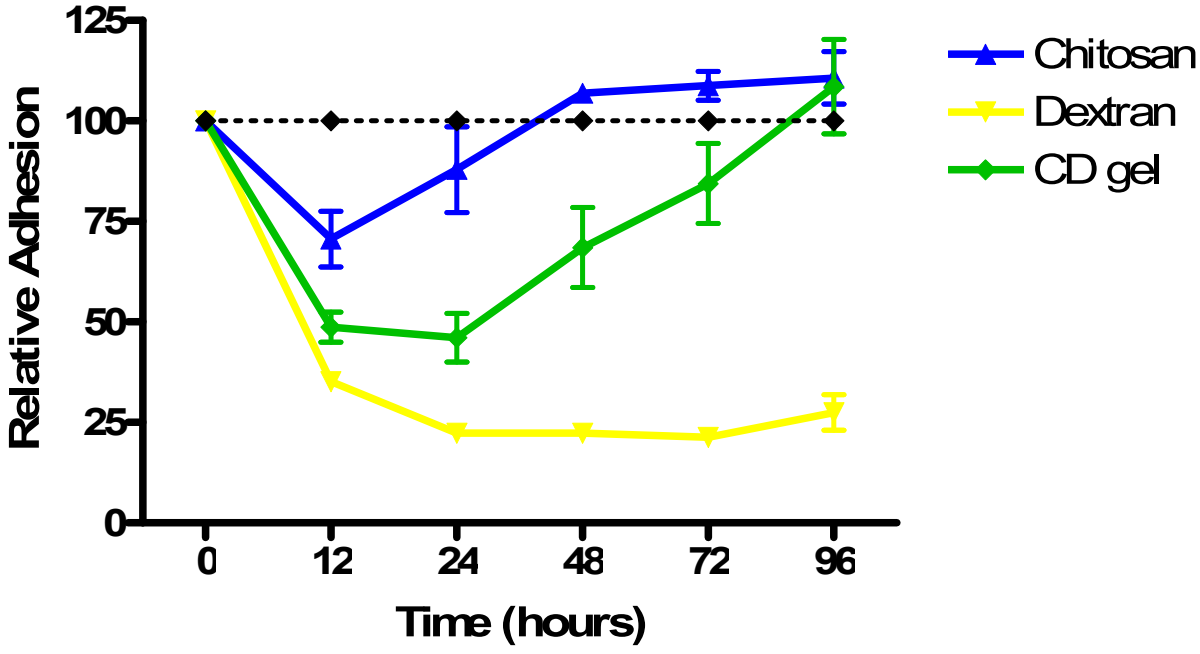
Data were analysed using two way repeated measures ANOVAs with Bonferroni correction for multiple testing. Computations were performed using GraphPad Prism 4.0 (San Diego, CA). Significance was set as $p<0.05$.

Results

Fibroblast attachment

Relative reduction of AB over time in the fibroblast attachment model is shown in figure 1. At each time point measured after baseline, fibroblast attachment was significantly impaired in the dextran treated group as was subsequent proliferation compared to the control group ($p<0.001$). Chitosan significantly impaired fibroblast adhesion (70.66% vs 100%, $p<0.01$) at 12 hours, however this difference was not significant at time points after this. CD gel showed a significant reduction compared to control from 12 hours till 72 hours post application, however at 96 hours the difference was no longer significant.

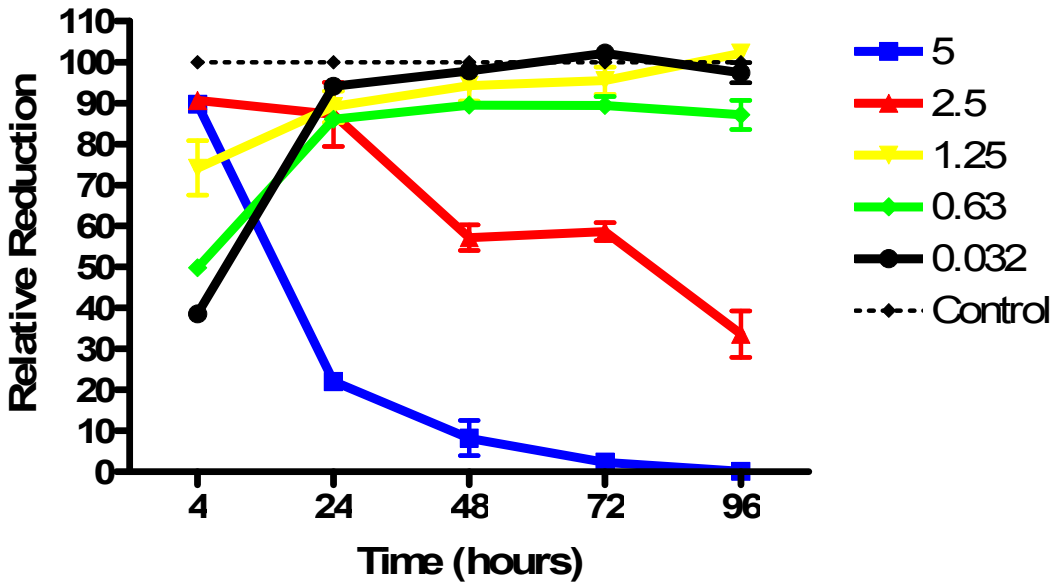
Figure 1. Relative attachment of fibroblasts over time in various treatment groups



Fibroblast Proliferation

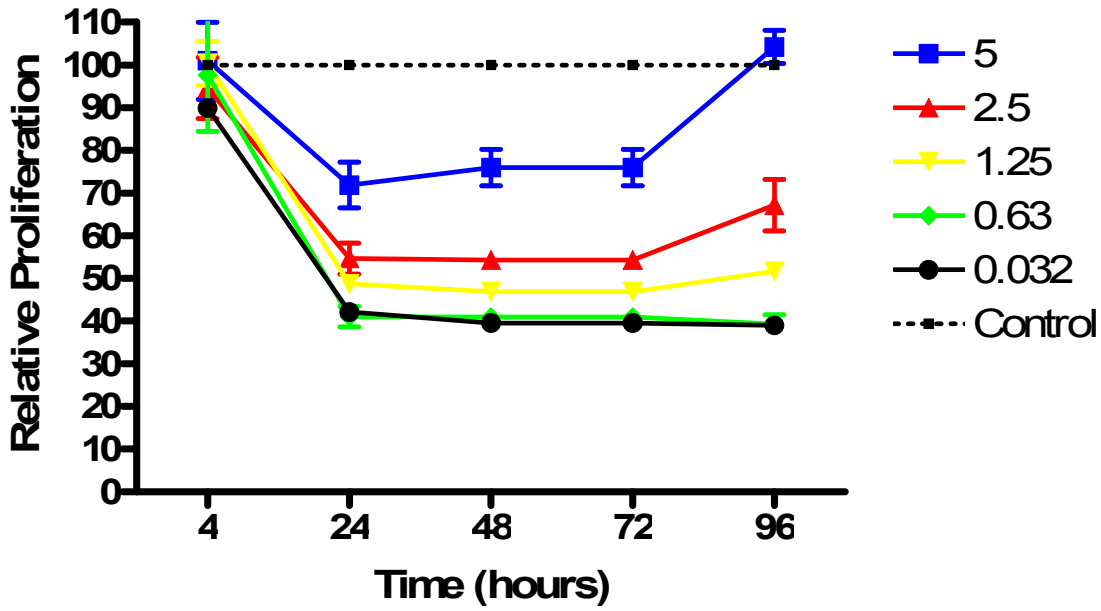
Results of fibroblasts exposed to dextran, chitosan and CD gel are summarised in figures 2a, 2b and 2c respectively. Low concentrations of dextran (1.25%, 0.63% and 0.032%) significantly impaired fibroblast proliferation when measured at 4 hours post application of dextran ($p<0.001$). This initial effect was short lived however, and after this time point the lower concentrations of dextran did not significantly impair fibroblast proliferation. In contrast, after 4 hours, dextran at concentrations of 2.5% and 5%, significantly impaired fibroblast proliferation ($p<0.001$).

Figure 2a – Relative proliferation of fibroblasts over time exposed to various concentrations of dextran



Initially there was no significant difference between fibroblast proliferation of control and any of the chitosan concentrations studied. After this however, there was a significant impairment of proliferation compared to control ($p<0.001$). This was true for all concentrations, except for 5% chitosan which at 96 hours was not significantly different to control. The lower concentrations of chitosan significantly impaired fibroblast proliferation more than the higher concentrations.

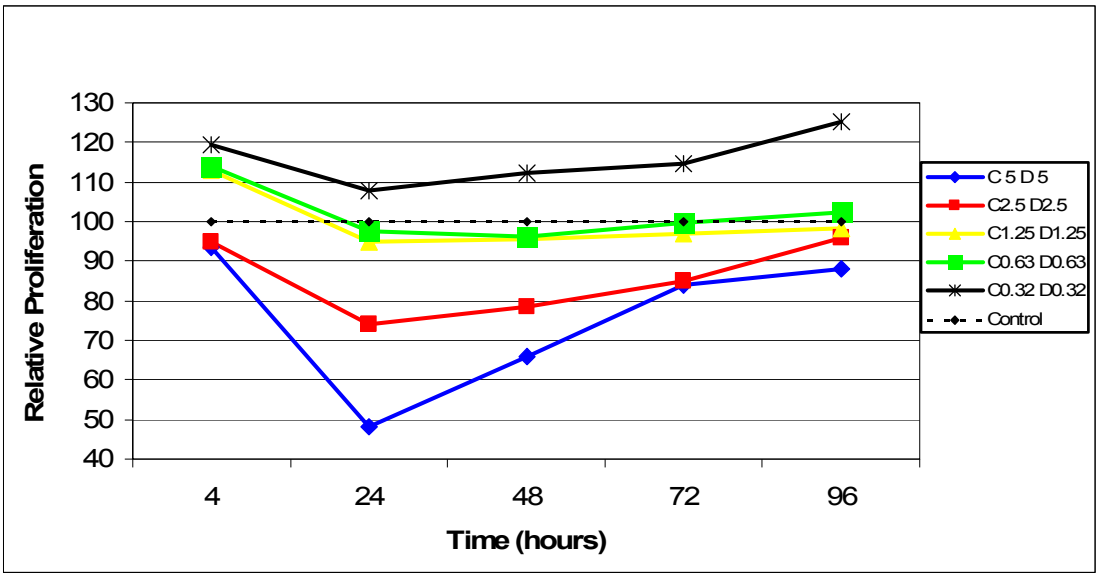
Figure 2b – Relative proliferation of fibroblasts over time exposed to various concentrations of chitosan



In general, higher concentrations of chitosan and dextran (2.5% and 5%) in gel form significantly impaired fibroblast proliferation until 96 hours at which time there was no significant difference compared to control. In contrast, lower concentrations of 1.25% and 0.63% made no impact on fibroblast proliferation while the lowest

concentration tested of 0.32% significantly improved fibroblast proliferation by 10-15% at the time points tested ($p<0.05$).

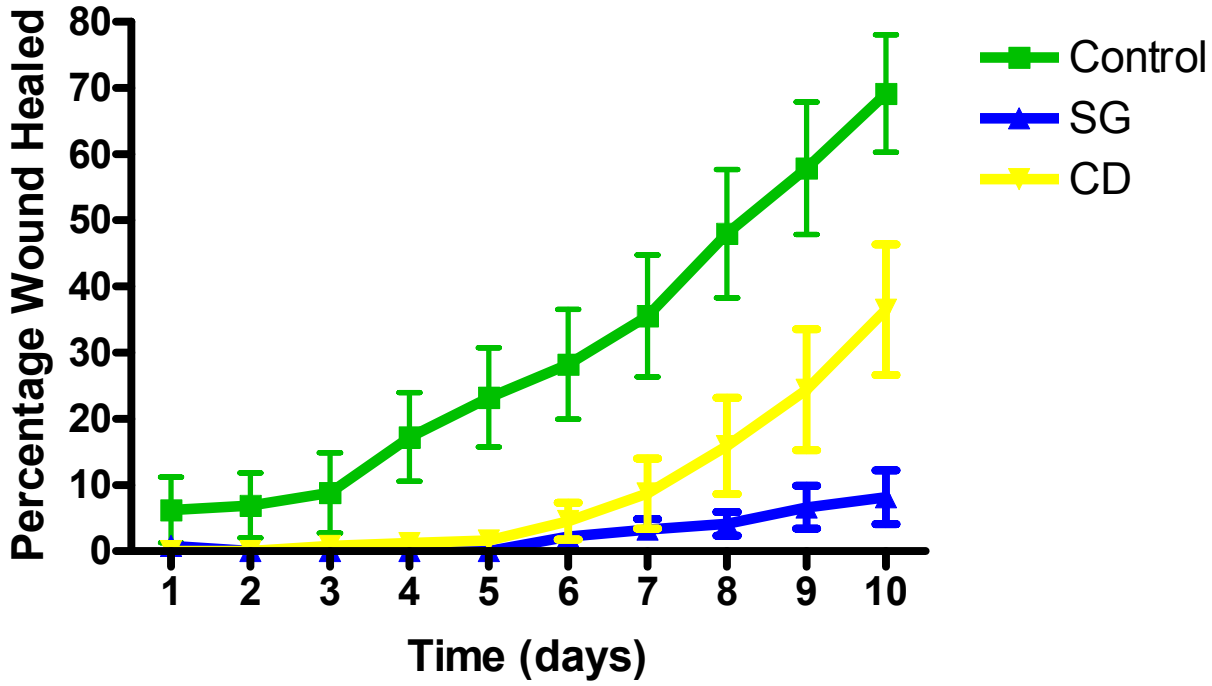
Figure 2c – Relative proliferation of fibroblasts over time exposed to various concentrations of CD gel



Fibroblast Wound Healing

Results of the fibroblast wound healing model are summarised in figure 3. Compared to control, the CD gel and SG groups both significantly impaired fibroblast growth and wound healing ($p<0.001$). Wound healing in the CD gel group lagged behind control on average by 3-5 days. Microscopic examination of the SG group revealed that the addition of SG resulted in lysis of most fibroblasts within 24 hours. Subsequently only fibroblasts in 3 wells (1 well from each of the 3 fibroblast lines) survived and began to heal the wound.

Figure 3 – Percentage of wound healed in each treatment group over time



Descriptive fibroblast growth study

In the SG group, cells exposed to SG did not attach to the well (Figure 4a) and within 24 hours were all lysed with no further fibroblast growth occurring (Figure 4b). Fibroblasts in the CD gel groups had adhered within several hours of seeding and formed a blanket of proliferating fibroblasts outside and "inhibitory zone" surrounding the CD gel. On day 5 however, bridges of fibroblasts were seen to cross over this inhibitory zone.

Figure 4a – Fibroblasts at 4 hours following exposure to SG (2x magnification)

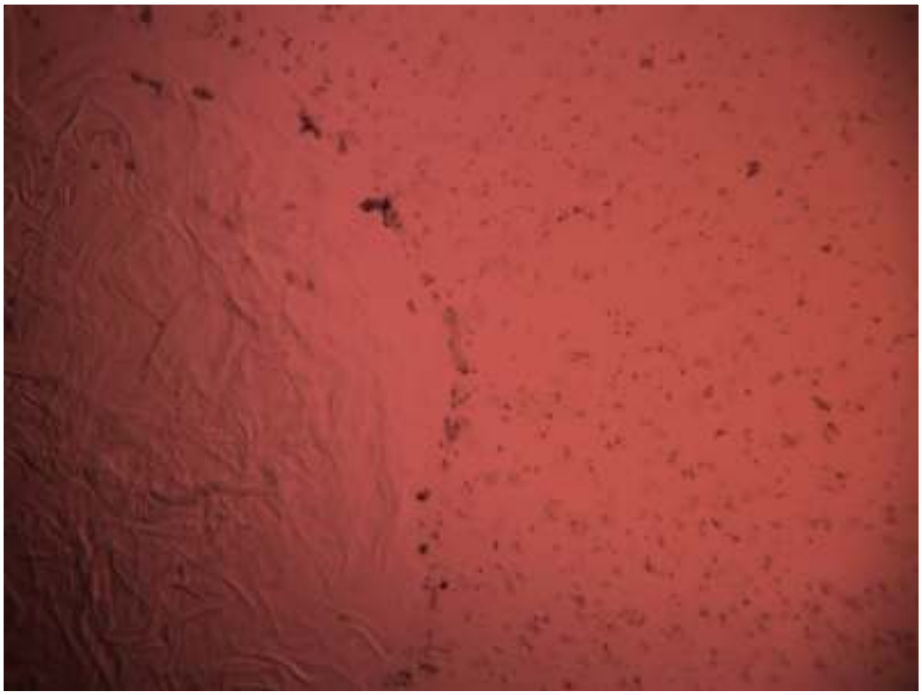


Figure 4b – Lysed fibroblasts 24 hours following exposure to SG (2x magnification)

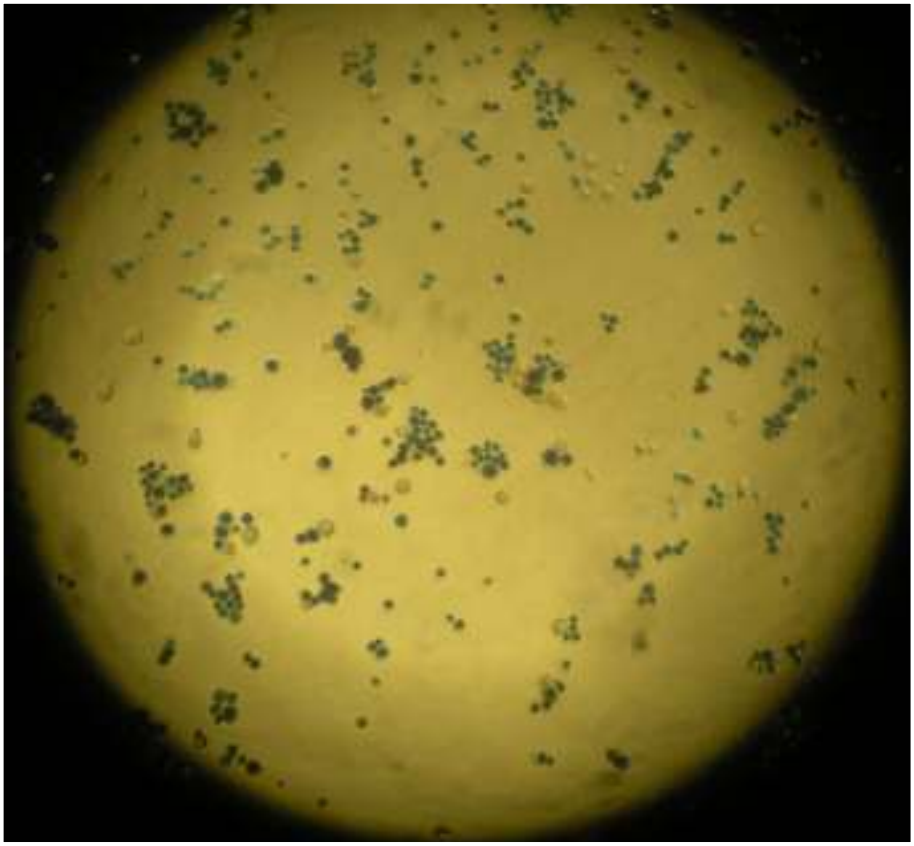


Figure 5a – Low magnification view of CD gel with zone of inhibition from surrounding fibroblasts at 3 days (1x magnification)

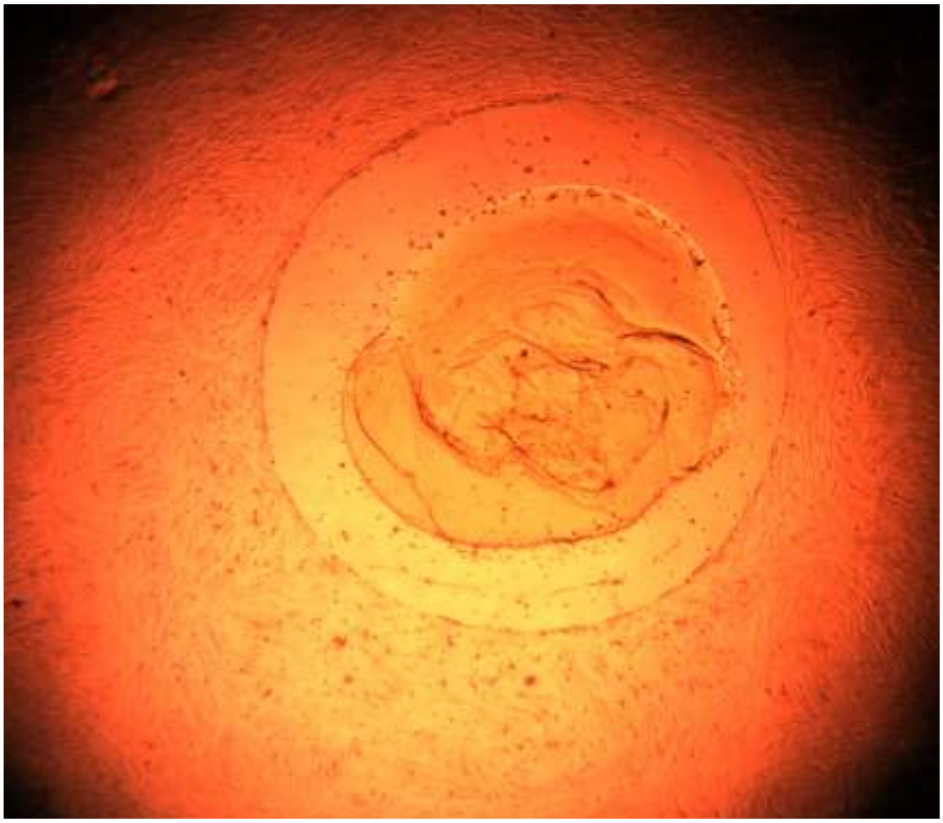


Figure 5b – High magnification view of CD gel with zone of inhibition from surrounding fibroblasts at 3 days (2x magnification)

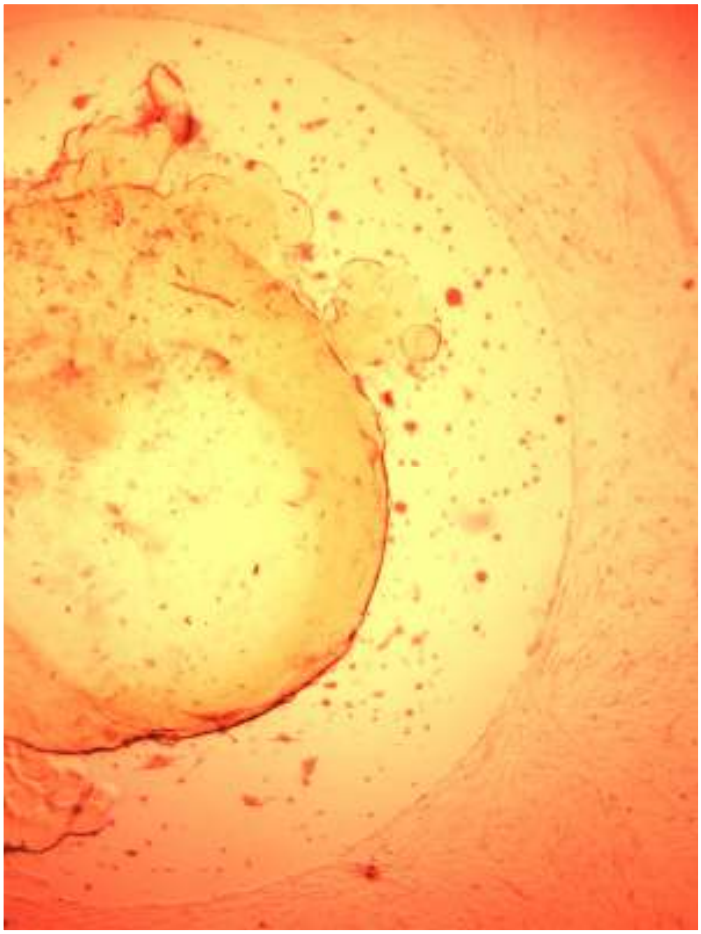
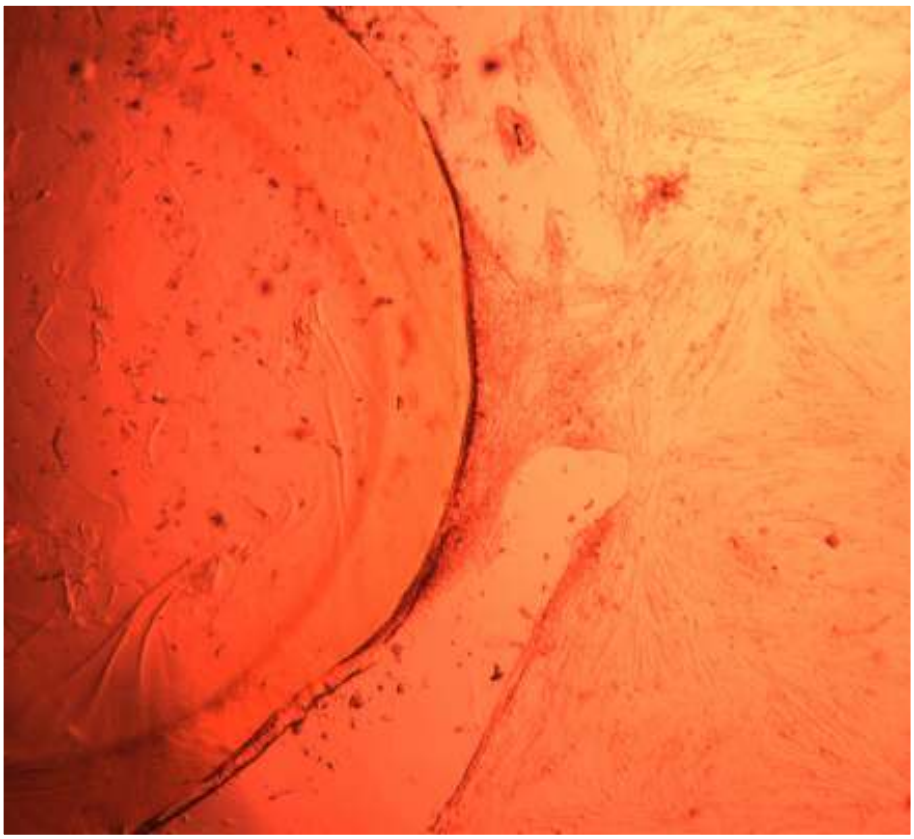


Figure 5c – High magnification view of CD gel with fibroblast bridges crossing the zone of inhibition (4x magnification)



Discussion

This study has shown that dextran at 5% concentration significantly reduces nasal fibroblast attachment and proliferation. Lower concentrations of dextran do not seem to have this inhibitory effect. Chitosan at a 5% concentration also significantly reduces fibroblast attachment and proliferation for up to 72 hours. Interestingly lower concentrations of chitosan seem to have a more marked inhibitory effect. In gel form the combination of 5% chitosan and 5% dextran moderately impairs fibroblast attachment by 50% and subsequently has an inhibitory effect on

proliferation which lasts for a further 72-96 hours. This translates into slowed wound healing of between 3-5 days compared to control.

This delay of 3-5 days can be explained by the "zone of inhibition" seen around the CD gel on the descriptive study and may be due to certain unknown inhibitory elements leaking from the gel. This effect is temporary and with a 5% gel lasts only 3-5 days. This may be particularly important in prevention of sinonasal adhesions as these are thought to be formed mainly by fibroblast migration and proliferation at sites of mucosal injury up to 7 days after injury. The prevention of fibroblast attachment and proliferation during this time period may simply slow wound healing. However, if the formation of adhesions is considered as an exuberant extreme of the wound healing continuum then this may well be beneficial. In addition, from a simply mechanical standpoint, the delay of 3-5 days may allow the resorption of some crusts and blood in the nasal cavity delivering a reduced scaffold on which fibroblasts can migrate.

A number of recent in vitro studies have demonstrated the inhibitory effects of chitosan on fibroblast proliferation[23,40-42]. Most work in this area is with N-O Carboxymethyl Chitosan (NOCC) which has been shown to be an effective inhibitor at concentrations as low as 0.1%[40] and to be significantly more effective *in vitro* than hyaluronic acid gels[43]. It remains unclear as to exactly how this effect is mediated however one hypothesis is that the negatively charged chitosan strongly results in its strong attachment to the underlying injured surface and prevents usual cell adhesion molecules such as the negatively charged fibronectin and vitronectin

from attaching[40]. This is supported by work which suggests that chitosan remains effective even in the presence of serum[40,44]. Importantly for wound healing, some work has suggested that chitosan selectively inhibits fibroblast growth while supporting growth of epithelial cells[45].

As early as 1973 there have been reports of the cell attachment inhibition of dextran sulphate[46]. This is thought to be related to its blocking of the glycosaminoglycan binding site of fibronectin[47-49] and has also been shown to limit fibroblast migration[50,51]. Early promising reports[52] of the adhesion preventing results from instilling dextran in the peritoneal cavity following peritoneal surgery have subsequently been discredited by larger more thorough trials[53,54]. To our knowledge there are no reports of chitosan and dextran combination gels to date in the literature.

Studies of adhesion formation at an *in vitro* level require a targeted approach in order to maximise the applicability of results. Adhesion formation has been well documented in dermal and peritoneal studies, however, there are significant differences between wound healing in the skin and peritoneum compared to the upper respiratory tract. Respiratory wound healing is in the order of months (at least 6 months), whilst skin and peritoneum occurs over several weeks. Nasal mucosa is also known to respond differently to various cytokines such as epidermal growth factor which slows wound closure in the nose[9] whereas it speeds it up in skin[55].

Apart from location specific variations there are also variations in wound healing in various disease states compared to normal. Fibroblasts involved in peritoneal

adhesion formation for example have a different phenotype to those which do not form adhesions[56]. Likewise nasal fibroblasts from patients with CRS produce different growth factors under various conditions and are sensitive to different cytokines[13]

While ATCC fibroblast lines are easily accessible and allow international verification and replication of studies they may be less relevant to the study of sinonasal wound healing. Wound healing and adhesion formation has been shown in clinical trials to be very different between patients with chronic mucosal inflammation such as in chronic rhinosinusitis and normal controls[7,57]. Additionally, previous studies have shown the response to chitosan to vary between different fibroblast lines[30]. Therefore we would recommend the use of using human nasal fibroblasts to study sinonasal wound healing and with regards to adhesion formation they would ideally be from patients with CRS.

The main limitation of *in vitro* studies is extrapolating their results to real world clinical scenarios. A well designed study by Yeo et al for example found promising effects of chitosan on fibroblasts *in vitro*, however the same chitosan was found to cause a granulomatous reaction in rat abdomens lasting up to 4 weeks after exposure. This study highlights the limitation of *in vitro* work and the value of animal studies, suggesting that while in vitro studies are useful, they should be interpreted with caution[58]. In this light the most promising concentration of the CD gel will be chosen for future work in a standardised animal nasal wound healing model.

The major limitation of this study is the absence of duplication of studies. This is true for all aspects except the wound healing model which was repeated on two occasions. Given the novel nature of the compounds and the unique properties they possess, repeating the studies is essential to validate the results. Having said this, all aspects of this study in attachment, proliferation, wound healing and even the descriptive aspects tend to support each other. Future studies would in addition to repeating this work to determine its validity, ideally use a variety of different types of chitosan from various sources with different molecular weights and levels of deacetylation. This would add significantly to the volume of work on chitosan and to adhesion prevention agents especially given Orlandi's findings of how small changes in molecular structure can have a wide variety of clinical effects[59].

Conclusion

Independently both chitosan and dextran have some inhibitory effect on fibroblast attachment and proliferation. Small changes in concentration have been shown to have marked differences in cellular response. CD gel has a significant temporary inhibitory effect on human sinonasal fibroblasts cultured from patients with CRS. The most promising combination is a 5% chitosan and 5% dextran gel. On the other hand, SG seems to have a significant cytotoxic effect on human sinonasal fibroblasts. The role of CD gel in clinical wound healing remains to be evaluated.

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CHAPTER 8: EFFECTS OF A NOVEL CHITOSAN GEL ON MUCOSAL WOUND HEALING FOLLOWING ENDOSCOPIC SINUS SURGERY IN A SHEEP MODEL OF CHRONIC RHINOSINUSITIS

Statement of authorship

Effects of a novel chitosan gel on mucosal wound healing following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis

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CHAPTER 9: THE EFFICACY OF A NOVEL CHITOSAN GEL ON HAEMOSTASIS FOLLOWING ENDOSCOPIC SINUS SURGERY IN A SHEEP MODEL OF CHRONIC RHINOSINUSITIS

Statement of authorship

The efficacy of a novel chitosan gel on hemostasis following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis.

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SUMMARY AND CONCLUSION

Review of ESS and ongoing concerns

Chronic rhinosinusitis (CRS) encompasses a very common group of disorders with its debilitating effects placing a large burden on society[3,6,7]. Endoscopic sinus surgery (ESS) remains the gold standard treatment for those cases refractory to maximal medical therapy and is one of the most commonly performed procedures by otolaryngologists worldwide[29,30].

Ongoing concerns with ESS include

a) bleeding during and following surgery requiring nasal packing which is painful, uncomfortable and causes mucosal trauma, and
b) excess scar tissue (adhesion) formation with subsequent need for revision surgery.

Current techniques and products developed to manage post operative bleeding are inadequate as they have either suboptimal or no haemostatic effect, or if they are haemostatic they impede wound healing and increase adhesion formation[127,128,328]. It is hypothesised that the mechanism for impaired wound healing with haemostatic agents is related to the simultaneous co-stimulation of coagulation and inflammatory pathways[79,80]. This leads to increased granulation tissue, increased organised blood clot and adhesion formation.

The ideal agent would then be haemostatic without stimulating the coagulation and inflammatory cascades, improve wound healing with decreased adhesion formation, safe, inexpensive, simple, stable and able to incorporate other promising biomedical agents.

Standardised Videoendoscopy and Surgical Field Grading

Prior to analysing the efficacy of current nasal dressings or developing a new agent there needs to be a standardised method of examining the nasal cavity as well as a standardised validated method of grading bleeding in ESS. This has been established with the development of a standardised video-endoscopy method shown to improve inter- and intra-rater reliability in a multi-centre and multi-national setting[81]. Similarly the new "Wormald" grading scale has been demonstrated to be a simple, effective and reliable method of grading bleeding during ESS[81]. It has the added advantage of being more sensitive to subtle changes at the most common gradings in ESS.

Topical Antifibrinolytics

Epsilon aminocaproic acid (EACA) and its more potent relation, tranexamic acid are the two main antifibrinolytics which have been in widespread medical use since the early 1970's[136,138]. They have a safe therapeutic profile, are cheap and essentially act by preventing fibrinolysis and stabilising the blood clot[138]. While the systemic use of antifibrinolytics for treatment of patients with bleeding diatheses as well as for primary menorrhagia, gastrointestinal bleeding, joint replacement surgery, liver transplantation and cardiothoracic surgery is well established[137,139-143], their role in topical applications has only recently been trialled. The data from randomised controlled trials of topical antifibrinolytics in major orthopaedic[149] and cardiac surgery[147,148] are promising as is the use of tranexamic mouth wash following oral surgery in patients who remain on anticoagulant medication[152,153]. This study was the first to examine the effect of topical antifibrinolytics in ESS. The significant improvement in surgical field compared to saline with tranexamic acid

was moderate, however, it is felt that this is still a useful agent in the approach to improved haemostasis in ESS. Perhaps the most interesting finding was the blinded surgeon assessing the treatment side as most improved in 80% of cases, suggesting a very real role for this drug.

The benefits of topical application include rapid onset of action as well as lower risk of any possible side effects. Conflicting reports regarding antifibrinolytics and adhesion formation exist in the literature[383,384] and whilst wound healing was not specifically examined in this study, there were no subjective differences between treatment groups. Some *in vitro* work examining the effect of tranexamic acid on human nasal fibroblasts in which the author was involved has shown that it improves a marker for the quality of wound healing (MMP-9) and mildly decreases fibroblast proliferation[159]. This suggests a possible positive role for wound healing and adhesion prevention following ESS. Future studies investigating antifibrinolytics should further evaluate their effect on wound healing as well as the possibility of introducing them in a mucoadhesive form in order to increase contact time with the clot surface.

Nasal fibroblast interactions with Chitosan

Chitosan is a natural polymer obtained from chitin and has extensive applications in agriculture, water and waste treatment, cosmetics, some foods as well as a variety of biomedical applications[185]. Its haemostatic properties in particular are well documented[112] and there are some studies to suggest that some forms of chitosan have anti-adhesion properties[166-172]. Contradictory *in vitro* reports on the anti-adhesion properties of chitosan exist and may well be due to variations in the molecular characteristics of the chitosan studied[385-389].

Wound healing is a complex process with significant variations between different tissues and organs as well as between various disease states. Previous work has shown how some materials are beneficial for wound healing under normal healthy conditions while detrimental under different disease conditions[324]. Given the well documented variety in response of different fibroblast strains when exposed to a selection of biomaterials, it is important that the cell lines chosen reflect as accurately as possible the specific application for which the studied products will be used[387]. In this study, fibroblasts from human nasal specimens with chronic rhinosinusitis were chosen as the most representative sample.

Both components of the chitosan-dextran gel influence fibroblast attachment, migration and proliferation. Studies of dextran in the 1970's revealed its anti-fibroblastic activity[390], however this did not translate into anti-adhesion effects when instilled in the abdomen[276,277]. While this may have been due to the easily absorbable liquid form in which it was instilled, its use fell out of favour by the 1980's. The anti-fibroblast properties of chitosan are not well understood, however, they may be due to the negative charge allowing it to strongly attach to the damaged surface and thus prevent usual cell adhesion molecules from attaching[391]. When used in combination, the 5% chitosan + 5% dextran gel resulted in a moderate fibroblast inhibition which translated into a slowing of their activity by some 3-5 days. Given that fibroblasts are mainly responsible for ECM production and adhesion formation in the upper airway this temporary effect maybe beneficial in sinonasal healing of CRS patients. In particular it may prevent fibroblasts from migrating across blood clot and allow fibrinolysis to proceed without organisation and subsequent adhesion formation.

Previous research has shown how some forms of chitosan shown to be effective at reducing fibroblast attachment and proliferation *in vitro* have caused significant fibrosis when used in animal models[302]. This supports the need for animal trials with the 5% gel, given it was the most effective combination.

Sheep wound healing and chitosan

Using an animal model to investigate the effects of new and established agents on wound healing is a useful exercise. It may help in the understanding of how various agents work as well as provide evidence to support their use in patient care. A well established protocol for investigating wound healing in the sinonasal cavities of sheep has many advantages[223,348]. Sheep are readily available animals that are easy to care for and tolerate general anaesthesia well. They have large sinuses oriented in the same fashion as humans which can be easily accessed with endoscopic equipment. Their mucosa is histologically similar to human sinonasal mucosa on haematoxylin and eosin staining[392]. In addition, an elegant disease state model whereby the sheep are naturally infected with *Oestrus ovis* (bot fly) infection resulting in an eosinophilic inflammation mirrors the eosinophilic inflammation seen in many CRS patients[393]. This model has previously shown the neutral effects of hyaluronic acid products and Insulin-like growth factor 1 (IGF-1) on healthy sheep mucosa and their detrimental effect on microscopic wound healing and adhesion formation in sheep with diseased mucosa[224,324].

In this sheep model, CD gel significantly reduced adhesion formation in the anterior ethmoid and lateral nasal wall regions. The mode of action of CD remains incompletely understood with the previous *in vitro* study demonstrating some anti-

fibroblast properties. It is also possible that the gel acts as a physical barrier separating injured mucosal surfaces until re-epithelialisation occurs.

Improved microscopic wound healing in the CD gel group was profound. In particular rates of re-epithelialisation and re-ciliation were significantly more rapid than control and tissue factor. Cilial maturity in the CD group was also more rapid, with a significant improvement compared to tissue factor and control at 3 and 4 months post injury. It is possible that an early reduction in fibroblast activity and their associated inflammatory mediators assists in the earlier recovery and more rapid maturation observed in the chitosan group.

SprayGel also had some beneficial effects on adhesion prevention and microscopic wound healing. These effects were not as marked as those of CD gel and rarely statistically significant. Importantly however, this is the first study examining the effect of SprayGel on nasal mucosal wound healing in a product already licensed in Australia and the United States for use following ESS.

As was expected from the wound healing paradigm of simultaneous stimulation of inflammatory and coagulation cascades, tissue factor resulted in increased adhesion formation and had a negative impact on microscopic wound healing. While it is cheaply and readily available in recombinant form (avoiding potential infectious disease transmission), and subjectively seemed to have very potent haemostatic ability, its usefulness in ESS is limited by these findings.

Sheep haemostasis and early wound healing with chitosan

The haemostatic potential of chitosan continues to be studied in the clinical and pre-clinical literature[112]. Its potent haemostatic effects have been well documented in lethal animal trials[173] and described in traumatic battlefield injuries[182]. Using thrombin generation techniques as well as scanning and transmission electron microscopy its mechanism of action has been postulated as aggregating platelets and erythrocytes via cross-linking, independent of the coagulation cascade[174,175,180,181,184]. This ability to cause topical haemostasis whilst avoiding stimulation of the coagulation cascade and associated inflammatory stimulation is of great potential in situations where minimising inflammation and adhesion is preferred.

Following on from the above study, the effects of the CD gel on haemostasis and early wound healing required more formal evaluation. Similarities between coagulation in sheep and humans exists with evidence to suggest that in readily available large animal models, apart from swine, sheep coagulation most closely reflects that of humans[394,395]. The sheep model then remains appropriate for this specific analysis.

Our results of significantly improved surgical field grade at 2, 4 and 6 mins following injury as well as significantly improved time to complete haemostasis in the CD gel group were not unexpected given our earlier subjective experience. Although the earlier sheep study showed improved microscopic wound healing and decreased adhesion formation at one month post injury, no difference was found in this current study between crusting in the treatment group and control groups up

to14 days post injury. This is an important finding which should be closely examined in any future human trial.

The Future

The work presented in this thesis potentially represents a significant advance in haemostasis and wound healing following endoscopic sinus surgery. It may also translate into advances in other surgical disciplines such as abdominal and pelvic surgery. There are however, several key steps needed to further this research.

In order to add to the understanding of the molecular mechanism of action of chitosan, dextran and the combination gel on nasal and perhaps other types of fibroblasts, the *in vitro* study should ideally be replicated analysing a broader range of gels with the protocols now established from this early work. This research could incorporate antifibrinolytics agents and more completely analyse their effect on various markers such as matrix metalloproteinases known to affect wound healing in sinonasal tissue.

Chitosan has been reported as having a wide spectrum of antibacterial[396], antiviral[397], antifungal[398,399] and even antibiofilm[400-402] activity. Use of chitosan has also been shown to prevent post operative infection developing in highly contaminated wounds in mice[403]. Given the potential risk of infection from novel foreign materials as well as the emerging role of biofilms in CRS, an evaluation of the gel on known and potential nasal pathogens warrants investigation.

Perhaps the most interesting step in further evaluating this gel is a human trial. A pilot trial assessing its safety ESS in 9 patients proved successful and at present a randomised controlled trial involving over 30 patients nears completion. The results of this trial are due to be published early next year. Further work could evaluate the role of the gel in more extensive sinus surgery such as its effect on long term outcomes in the modified Lothrop procedure.

In a coordinated multi-disciplinary research setting, the gel is also currently being evaluated for its potential role in prevention of adhesions following abdominal surgery. An early pilot rodent trial has shown some success (results not shown) and further research in this area promises to be very exciting.

With our increasing knowledge of the intricacies of haemostasis and wound healing and the interrelationship between these two physiologic systems there remains the potential to modulate both processes. Additives to the gel such as corticosteroids, antibiotics, antifungals, immune modulators and other growth factors may well prove to be beneficial in the years ahead.

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