

FACTORS AFFECTING MUCOSAL HEALING, RECILIATION, AND CILIARY FUNCTION AFTER ENDOSCOPIC SINUS SURGERY IN THE SHEEP

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by

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ABSTRACT

The effect of absorbable packing on the healing of nasal respiratory epithelium after endoscopic sinus surgery (ESS) was examined in a diseased sheep model. Full thickness injuries were created on the lateral nasal wall of sheep infested with *Oestrus ovi*. Sites of injury were packed on one side with hyaluronic acid (HA) packing or hyaluronic acid packing impregnated with insulin-like growth factor-1 (HA+IGF1) in a randomized fashion. The opposite side was left unpacked as a control. Biopsies were obtained for light microscopy, scanning electron microscopy, and ciliary beat frequency (CBF) analysis over a period of 16 weeks. Statistical analysis of results was performed in order to determine if any intervention had any impact on healing and to determine if there was any correlation between extent of regeneration as assessed by electron microscopy and CBF. Furthermore assessment of the effect of isotonic and hypertonic saline on ciliary beat frequency was performed in healthy human volunteers.

Reepithelialization was increased in the HA+IGF1 group compared to the HA group and controls at eight weeks after injury but not at later time points. Cilial regeneration was improved in the HA+IGF1 group compared to the HA group and controls at 16 weeks. CBF was noted to be worse at the eight week time point with the HA+IGF1 group compared to the HA group and controls, but no other statistically significant effects on CBF were noted. This most likely represents a spurious finding. Wide distributions of CBF results were noted, reflecting numerous missing data points due to methodological difficulties. There was a trend noted toward increased CBF with improved grades of reciliation, although this correlation

was not statistically significant. However this trend was supported by the finding of statistically significant differences between individual and combined grades of reciliation. Hypertonic saline was found to have a ciliostimulatory effect when compared to normal saline at 5 minutes after administration in healthy human subjects. This effect had disappeared by 60 minutes after administration.

It is suggested that the presence of insulin-like growth factor-1 at the time of mucosal injury improves epithelial regeneration in the short term, but is not sufficient for this effect to be sustained. This improved early epithelial regeneration forms a foundation for ciliary regeneration, as is reflected in an improved grade of reciliation at 16 weeks. Our interventions had no effect on CBF, and various experimental problems made it difficult to provide further comment on CBF results. There is evidence that CBF improves as the grade of ciliary regeneration improves following ESS. Furthermore, hypertonic saline appears to also have a positive impact on CBF, which is likely to reflect changes in the rheological properties of mucous. A number of possible avenues of enquiry are delineated and recommendations for future research are outlined.

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, and that to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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

David Alexander Michael Wabnitz

PREFACE

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

Wabnitz DAM, Wormald PJ (2005). A blinded randomised controlled study on the effect of buffered 0.9% and 3% sodium chloride intranasal sprays on ciliary beat frequency. *Laryngoscope* [in press].

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CHAPTER 1

AIMS

The aims of this thesis were the following:

1. To review the current state of knowledge regarding epithelial healing after endoscopic sinus surgery
2. To examine the effect of nasal packing with hyaluronic acid, with and without insulin-like growth factor-1, on mucosal healing following surgical injury in a diseased sheep model
3. To examine the effect of nasal packing with hyaluronic acid, with and without insulin-like growth factor-1, on ciliary regeneration following surgical injury in a diseased sheep model
4. To examine the effect of nasal packing with hyaluronic acid, with and without insulin-like growth factor-1, on ciliary beat frequency following surgical injury in a diseased sheep model
5. To assess whether a correlation exists between ciliary regeneration and ciliary beat frequency
6. To evaluate the effect of isotonic and hypertonic saline on the sinonasal ciliary beat frequency of healthy human volunteers

CHAPTER 2

INTRODUCTION

2.1 CHRONIC RHINOSINUSITIS

2.1.1 Definition and Disease Burden

Chronic rhinosinusitis (CRS) is defined as a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses of at least 12 consecutive weeks' duration (Benninger et al., 2003). The prevalence of this disease is difficult to ascertain, but one estimate from the United States of America (USA) is that between 18 and 22 million physician consultations per year are due to CRS, with an estimated 545 000 visits to emergency departments per year as the result of this disease (Benninger et al., 2003).

The total cost of treating patients with primary diagnosis of sinusitis (both acute and/or chronic) was estimated to be at least \$3.4 billion in the USA for the year 1996 (Ray et al., 1999). Over the past two decades, there has been an increasing trend for physician consultations for this disease, as well as an increasing rate of antibiotic prescriptions (Kaliner et al., 1997; Benninger et al., 2003). As Benninger et al. (2003) points out, the cost of antimicrobial therapy is expected to escalate given the move toward newer-generation antibiotics and antifungals which have increased cost, as well as using treatment agents for longer periods of time.

2.1.2 Pathophysiology of Chronic Rhinosinusitis

Many different factors have been implicated in the aetiology of CRS. These include extrinsic factors such as infection (viral, bacterial, fungal, parasitic), allergy,

hypersensitivity, irritants, trauma, and surgery, as well as intrinsic factors such as mucociliary abnormalities, immunodeficiency, autonomic dysregulation, hormonal imbalance, structural abnormalities, and autoimmune diseases (Benninger et al., 2003).

The above aetiologies are ultimately thought to result in blockage of sinus ostia, which in turn results in sinusitis (Kennedy et al., 1995). Kennedy et al. (1995) state that this leads to stagnation and thickening of sinus secretions. Intrasinus pH and mucosal gas metabolism change resulting in damage to cilia and epithelium. Retained secretions and changes in host milieu lead to tissue inflammation and bacterial growth in a closed cavity. Oedema and thickening of the mucosa worsens and the cycle continues, leading to the symptoms and signs of chronic sinusitis.

2.1.3 *Microscopic Features of Chronic Rhinosinusitis*

The normal lining of the sinonasal complex is that of pseudostratified columnar epithelium composed of four major types of cells – ciliated cells, nonciliated cells, goblet cells, and basal cells. This thin epithelium rests on a delicate basement membrane that sits on the submucosa which is made up of loose connective tissue and seromucinous glands (Forsgren et al., 1995; Watelet et al., 2002).

Stammberger (1986a) has documented the findings of chronic rhinosinusitis based on histological examination of mucosa from over 3000 patients. Mucous is found to be thicker and hence can block ducts and tips of cilia, leading to expanded ducts and flattening of the ciliated epithelium. Goblet cell metaplasia is also seen, and mucous

cysts may result. Extravasation of mucous into surrounding tissue can lead to a granulomatous reaction and permanent thickening of the mucous membrane. An inflammatory cell infiltrate, with squamous cell metaplasia and epithelial desquamation may also be a feature (Forsgren et al., 1995). The inflammatory cell infiltrate varies from patient to patient, but lymphocytes, eosinophils, and neutrophils typically predominate.

Abnormalities of ultrastructure have been demonstrated on electron micrography, and include cytoplasmic protrusions, mitochondrial and endoplasmic reticulum swellings, separation of cellular junctions, metaplasia, frequent goblet cells, compound cilia, ciliary disorientation, microvilli, central and peripheral microtubule alterations, absence of dynein arms, absence of radial spokes, and ciliary membrane alterations (Ohashi and Nakai, 1983; Maurizi et al., 1984; Jorissen 1989; Keleş et al., 2001; Toskala and Rautiainen, 2003). However, the most pronounced finding is that of a decreased number of cilia (Keleş et al., 2001), which is particularly marked in the region of the sinus ostium compared to other portions of the sinus (Guo et al., 1997)

2.2 SURGICAL TREATMENT

2.2.1 *Functional Endoscopic Sinus Surgery*

The foundation of endoscopic sinus surgery is essentially the opening of natural sinus ostia and removal of diseased mucosa. This is based on the work of

Messerklinger who in 1978 reported on his observations of mucociliary clearance in healthy and diseased mucosa (Kennedy, 1985). One of the conclusions drawn from his work was that mucociliary drainage pathways within the sinuses continue to be directed towards the natural sinus openings even in the presence of any other surgically created openings into the sinuses. Kennedy and Shaalan (1989) demonstrated that the mucociliary clearance pattern of the sinuses remains directed towards the natural ostium in an animal model, regardless of what surgery is performed on that sinus. It is expected that the act of clearing drainage pathways restores aeration and mucociliary clearance, and therefore health, to the paranasal sinuses. Further evidence for this concept comes from patients who have patent accessory sinus ostia or patent inferior meatal antrostomies, yet continue to suffer from maxillary sinusitis (Salman, 1992).

Surgical enlargement of the ostiomeatal complex was probably first proposed in the 18th century, but appeared to fall out of favour due to difficulties visualizing the middle meatus region combined with complications of surgery (Lund, 2002). However, with the advent and development of rigid endoscopes, examination and treatment of this region could be performed with increasing degrees of precision and safety.

In addition to improvements in our understanding of the pathophysiology of this disease, a number of relatively recent technological advances have contributed to the development and acceptance of functional endoscopic sinus surgery (FESS) as the surgical treatment of choice for CRS. Computed tomography (CT) of the sinuses provides important detailed information regarding sinonasal anatomy and disease

affecting this area, and has therefore guided surgeons and increased the safety margin of the FESS procedure. Finally the use of rigid endoscopes has revolutionized the assessment and treatment of the paranasal sinuses, allowing a magnified panoramic view of the operative field (Kennedy et al., 1985; Rice, 1989; Smith and Brindley, 1993).

Although there was widespread acceptance in Europe and Japan soon after Messerklinger's work was published, it was not until Kennedy and colleagues published their papers that the endoscopic technique was accepted into the English-speaking world (Kennedy et al., 1985; Kennedy 1985). In fact, it was Kennedy et al. (1985) that first used the term Functional Endoscopic Sinus Surgery. This was reinforced by the work of Stammberger who published his papers in the following year (Stammberger, 1986a; Stammberger, 1986b).

The introduction of FESS into Australia can be traced back to the mid-1980's. It may be that the first presentation of this technique was at a conference in Adelaide by Wolfgang Draf of Germany in 1984 (D. Close, personal communication). According to the literature, the first surgeon to perform FESS in Australia was Kevin Kane, who began in October 1986 at the Royal Victorian Eye and Ear Hospital, Melbourne (Kane, 1992). Heinz Stammberger of Austria was an invited guest at the 37th General Scientific Meeting of the Otolaryngological Society of Australia held in Brisbane in May 1988 (Willis, 1988). This was followed by an instructional course by Stammberger in Melbourne. Soon after, a further instructional course was held in Sydney, with faculty including David Kennedy of USA and Stammberger (D. Close, personal communication). International visiting

speakers with instructional courses focusing on FESS have continued over the years that have followed.

Although endoscopic sinus surgery has been accepted by most otolaryngologists as the surgical treatment of choice for sinusitis, clinical guidelines and scientifically valid support for its wide acceptance is scarce and has been slow in coming. Indeed, some have questioned the scientific validity of the functional endoscopic approach. Fairley (1991) points to previous beliefs regarding treatment of the sinuses that had a “rational” pathophysiological basis and that were widely accepted by the medical community of the time. Ultimately, these treatments were discredited. The suggestion is that functional endoscopic sinus surgery may follow the same path if there is no careful analysis of effects and outcomes. Other researchers have certainly suggested alternative mechanisms of sinus dysfunction apart from sinus ostial occlusion (Salman, 1992; Gnoy et al., 1998; Ganjian et al., 1999). In more recent times, bacterial superantigens, biofilms, and fungal colonization have been included as potential aetiological factors of interest (Meltzer et al., 2004).

Work is therefore currently being undertaken to establish the validity of endoscopic sinus surgery. The first randomized controlled trial directly comparing the efficacy of FESS to that of medical therapy has recently been published (Ragab et al, 2004). Patients initially underwent six weeks of medical therapy in the form of topical decongestant, topical steroid, and alkaline douching. Those who continued to be symptomatic were randomized to receive a 12 week course of oral erythromycin as well as continuing the topical nasal steroid and alkaline douching or to undergo functional endoscopic sinus surgery. Assessments were made before starting

treatment, and then after six and twelve months. Both treatment arms significantly improved almost all the subjective and objective parameters of CRS, with no significant difference between medical and surgical treatment (except for increased total nasal volume in those undergoing surgery). The authors concluded that surgical treatment should be reserved for cases refractory to medical therapy, and that CRS should certainly be treated with maximal medical therapy in the first instance.

2.2.2 Wound Healing

At an even more basic level, research into the very processes that follow sinus surgery (conventional or endoscopic) has been limited. Whilst cutaneous wound healing and healing of fractured bones has been extensively studied, healing of wounds of the respiratory tract has been poorly investigated (Watelet et al., 2002).

Following injury, Watelet et al. (2002) delineate the healing process as moving through stages of fibroplasia, angiogenesis, and reepithelialization. The regeneration of the epithelium involves four processes: migration from adjacent epithelium, multiplication of undifferentiated cells, reorientation, and differentiation. Finally, tissue remodeling takes place. However information regarding the control of these processes is scarce, and therefore our ability to positively influence healing remains limited. So whilst there has been less emphasis on healing of the sinonasal mucosa on a microscopic and molecular level, there has been somewhat more focus on macroscopic healing and factors related to adverse outcome.

In his review of 120 patients who had undergone FESS, Kennedy (1992) found that the only factor that predicted surgical outcome was extent of disease as demonstrated on preoperative radiography. However, he notes that if intensive postoperative care had not been utilized the incidence of poor outcome generally would have been higher due to scarring and stenosis of ostia. In his initial report of the technique of functional endoscopic sinus surgery, Kennedy (1985) reported scar tissue developing between the middle turbinate and lateral nasal wall in 5 of his first 16 patients. However, in his later report the middle meatal adhesion rate had dropped to 4.0% (Kennedy, 1992). Stammberger (1986b) has categorically stated that the most frequent problems following endoscopic endonasal surgery are scars and synechiae. Rice (1989) stated that in his series of patients undergoing FESS, all unsuccessful operations seemed to occur from scarring in the middle meatus. Seven percent of his cases were affected by excessive middle meatus scarring. Likewise, Smith & Brindley (1993) stated that the majority of their surgical failures resulted from synechiae. Lund et al. (1991) noted only occasional adhesion formation within the middle meatus. Lazar et al. (1992) reported that 63 patients (9.4%) from their series of 673 patients that had undergone FESS required revision surgery, and 43% of these had significant adhesions. These were mostly located between the lateral nasal wall and the middle turbinate, obstructing the ostiomeatal complex. Chambers et al. (1997) found that scarring of the middle meatal antrostomy and scarring of the ethmoids was associated with poor outcome. In his series of 52 patients undergoing revision ESS, Ramadan (1999) found that 46% required revision surgery due to incomplete primary surgery (due to residual diseased air cells or the creation of a middle meatal antrostomy that was separate to the natural ostium leading to recirculation of mucous). However, 27% had ongoing disease due to maxillary sinus

ostium stenosis and 25% had ongoing disease due to frontal sinus ostium stenosis. In total 56% of patients were noted to have adhesions which were related to the failure of the primary procedure. In contrast to this, Richtsmeier (2001) found that adhesions were responsible for surgical failure in only 4 patients in his series of 128 patients who had failed endoscopic maxillary sinus surgery. Stankiewicz (2002) states that most cases of failure in ESS are related to lateralization of the whole or part of the middle turbinate, and hence prevention of scarring and obstruction is a priority.

The number of sinonasal procedures carried out at the one sitting may have an impact. Shone and Clegg (1987) found adhesion rates of 11% at six weeks after sinonasal surgery. All patients that developed adhesions had undergone radical trimming of the inferior turbinates and most had also undergone some other nasal procedure, and then were packed with non-absorbable packing for 24-48 hours postoperatively. The authors concluded that adhesions are more common with extensive surgery or synchronous surgery to septum and lateral wall. They also found that if patients had an abnormality at two weeks (marked mucosal oedema, crusting, or exudate), there was a 22% chance they would develop adhesions by six weeks. Postoperative nasal toilette was not performed.

Given the importance of healing following ESS, particularly with regard to adhesion formation and subsequent surgical failure, a number of intraoperative and postoperative factors have received attention and continue to be an ongoing source of study and debate.

2.2.3 Intraoperative Techniques

2.2.3.1 Surgical Technique – Preservation of Mucosa

Forsgren et al. (1995) studied the histopathologic mucosal changes following functional endoscopic sinus surgery compared to the Caldwell-Luc procedure in patients suffering chronic maxillary sinusitis. They found that inflammatory cells, oedema, and submucosal glands were reduced, but fibrosis increased, in patients who underwent the Caldwell-Luc procedure compared to those who had undergone functional endoscopic sinus surgery. However, this study did not take into account the functional outcome of a mucosal stripping procedure. Moriyama et al. (1996) examined healing of sinus mucosa following sinus surgery. They compared the healing of areas subjected to complete mucosal removal with limited mucosal removal. Endoscopic examination revealed almost normal epithelialization of areas of superficial surgery after six months, whilst bone-exposed regions had significant scar tissue and incomplete epithelial regeneration. After 18 months, it appeared that epithelial regeneration had occurred. However, electron microscopy demonstrated sparse ciliated cells in the group with total mucosal removal as compared to near normal cilia numbers in those who underwent superficial surgery. Moriyama et al. (1996) argue that a clean cut through diseased mucosa and bone is preferable to any stripping procedure given the significant granulation and scarring that can result. Furthermore, the lack of cilia found suggests that full-thickness mucosal injury has a poorer functional outcome with regard to mucociliary clearance.

These results have also been demonstrated in animal models. Full thickness mucosal stripping procedures have been found to be associated with increased fibrosis and bone neogenesis (Benninger et al., 1989; Benninger et al., 1991; Forsgren et al., 1993), and this may disrupt mucociliary clearance leading to ongoing disease. Benninger et al. (1989) found that whilst ciliary respiratory epithelium was reestablished after a mucosal stripping procedure in an animal model, mucociliary function appeared to be abolished. Benninger et al. (1991) found that the mucosa that regenerated after a full thickness injury had less regions that were ciliated, and the areas that were ciliated had decreased numbers of cilia. Furthermore, the cilia that were present exhibited increased numbers of abnormalities. There were no differences in ciliary motility compared to controls. Kennedy & Shaalan (1989) found that even though widening of the natural ostium did not change mucociliary clearance patterns, there was some disruption to mucociliary clearance. These authors state that this indicates the need for conservative surgery.

Ultra-conservative endoscopic sinus surgery procedures have been described. Setliff (1996) described the minimally invasive sinus technique (MIST) which involves removal of any structures that may “cover” sinus ostia (such as the uncinate process, medial wall of the bulla ethmoidalis, and perhaps the posterior wall of the agger nasi cell, as well as any gross polyps), but does not include enlargement of sinus ostia nor resection of any diseased mucosa. The rationale behind this is that mucosal disease, even if severe, is reversible if ventilation of the sinuses is restored. Certainly this was the finding of Stammberger (1986b), and is supported by a recent outcome study (Catalano and Roffman, 2003a). Proponents of this technique also

argue that it has a comparatively low rate of adhesion formation which reflects the minimal mucosal manipulation (Catalano and Roffman, 2003b).

However, a number of concerns regarding this technique have been documented by Chiu and Kennedy (2004). They state that correction of intrasinus hypoxia is not the sole pathogenic factor in CRS, and MIST does not address inflamed or infected bone known to be associated with CRS, with the resulting thickened and/or infected bone continuing to be a nidus of ongoing disease. Furthermore, underlying medical problems are not addressed, topical medications are unable to reach diseased intrasinus mucosa as ostia remain small, thick eosinophilic mucin is not able to be removed. Finally, they argue that the MIST technique has no role in revision sinus surgery, and that there are no long-term outcome studies of this technique. This concurs with the findings of Rajapaksa (2004), who noted that if an inflammatory state continued following ESS in his animal model, healing was adversely affected. He advocated removal of all inflammatory tissue and debris at the time of surgery, as any material left behind may perpetuate inflammation which then has implications on the healing process.

2.2.3.2 Surgical Technique – Powered Instrumentation

As noted above, Moriyama et al. (1996) found that denuded bone results in delayed healing and possibly decreased functionality. They therefore argue that a clean cut through diseased mucosa and bone is preferable to any stripping procedure in order to minimize granulation tissue and scarring. This may be achieved by removing diseased tissue with powered microdebriders and/or through-cutting forceps. Initial

reports suggested that microdebridors were associated with decreased rates of synechia formation (Krouse and Christmas, 1996; Bernstein et al., 1998). Although powered instrumentation may be associated with increased patient comfort if performing the procedure under local anaesthesia as well as perhaps decreased length of surgery, no advantage has been demonstrated when comparing powered instrumentation, through-cutting forceps, and non-through-cutting forceps with regard to adhesion formation, postoperative healing, and early postoperative outcome (Kingma et al., 1999; Vauterin et al., 2000; Selivanova et al., 2003).

Orlandi and Lanza (2004) emphasized the need for preservation of the mucosa of the leading edge of the middle turbinate using meticulous placement of instruments into the middle meatus. Such care can prevent the formation of adhesions in an important area that can otherwise be the site of surgical failure (Rice, 1989; Lazar et al., 1992). A further caution relates to the possibility of significant complications with the use of powered microdebridors (Duncavage, 2004). Graham and Nerad (2003) report on a case series of orbital complications which they related to the use of microdebridors with their associated suction and rapidly rotating sharp blades.

2.2.3.3 Surgical Technique – Middle Turbinectomy

This is a controversial component of the FESS technique of some surgeons. The argument for performing it is that synechia or other obstructions leading to surgical failure commonly involve the middle turbinate, and this can be remedied by resecting it. Proponents such as Wigand (1990) also argue that it improves the view of the posterior ethmoids, sphenoids, and the maxillary ostium, thereby improving

access and increasing the degree of safety, as well as possibly relieving nasal obstruction.

However, Kennedy (1985) and Stammberger (1986b) argue for preservation of the middle turbinate. Their reasons include the increased risks of atrophic rhinitis and hyposmia or anosmia as well as a cerebrospinal fluid leak if the resection disrupts the insertion of the middle turbinate onto the skull base. Middle turbinate resection may also be related to postoperative frontal recess scarring and obstruction (Swanson et al., 1995). Furthermore, the middle turbinate forms a surgical landmark which has particular importance in patients coming to revision surgery. Smith and Brindley (1993) argue that these risks may be minimized by performing only a partial middle turbinectomy instead of total removal. In addition, they argue that atrophic rhinitis has not been identified in any series where middle turbinectomy is routinely performed. LaMear et al. (1992) found no complications, including atrophic rhinitis, in their series of 298 patients who had undergone partial middle turbinectomy. Havas and Lowinger (2000) found a decreased rate of adhesions and decreased rate of revision surgery in patients who had undergone partial middle turbinate resection as opposed to patients who had preservation of their middle turbinate. This was particularly true in patients with more severe disease. In a review of almost 3000 patients who had undergone ESS with partial middle turbinate resection, Toffel (2003) reported excellent rates of symptom improvement/resolution and an impressively low rate of synechiae formation requiring revision surgery (2.5%).

2.2.3.4 Surgical Technique – Medialization of the Middle Turbinate

As discussed above, adhesions are to be avoided. However it may be argued that if adhesions are located where they do not interfere with mucociliary clearance, they do not require further treatment (Catalano and Roffman, 2003b). Both Bolger et al. (1999) and Friedman et al. (2000) have described how an intentionally created adhesion between the medial aspect of an unstable middle turbinate and the adjacent nasal septum can be used to prophylactically guard against potential lateralization of the middle turbinate. If lateralization were to occur, obstruction of mucociliary clearance pathways can almost certainly be expected. Alternatively, Thronton (1996) described the use of a through-and-through polyglactin suture to secure the middle turbinates to the septum. Medialization of the middle turbinate has been found to have no adverse effect on olfaction, either subjectively or objectively (Friedman et al., 1999).

2.2.3.5 Nasal Packing – Non-absorbable

One method to reduce the formation of scar tissue between two adjacent surfaces is to place a physical barrier between these surfaces. In the sinonasal setting, such a barrier may also serve to provide pressure haemostasis, and numerous substances have been described for this purpose. Illum et al. (1992) found that fingerstall packing was more comfortable and easier to remove than Merocel or gauze packs with ointment, with no difference in macroscopic healing demonstrated at three months. Garth & Brightwell (1994) prospectively examined four non-absorbable packs in patients undergoing sinonasal surgery. They found little difference between

the packs whilst they were in situ. However, on removal Telfa and paraffin-impregnated gauze were associated with less patient discomfort than bismuth iodoform paraffin paste (BIPP)-impregnated gauze or Merocel. Furthermore, Merocel was often associated with a brisk bleed on removal, although not to the extent that required repacking.

In addition to potential issues regarding patient comfort, it has been argued that non-absorbable nasal packing can be detrimental to healing of the sinonasal mucosa. Rajapaksa (2004) noted that the combination of pack expansion and tissue oedema can lead to pressure on adjacent mucosa, with possible growth of granulation tissue into the pores of the pack. Shaw et al. (2000) found that use of a non-absorbable pack such as ribbon gauze leads to a 68% loss of the ciliated surface within the nose, which was significantly more than controls. Even the use of neuropatties, which are thought to be a “kinder” non-absorbable pack and are often used for preoperative decongestion, lead to a 50% loss of the mucosa.

Non-absorbable packs are not without other risks, some of which are potentially lethal. In a review article, Fairbanks (1986) listed the possible complications of nasal packing which included cardiovascular collapse secondary to the nasal-vagal reflex, adverse reaction to topical anaesthetics or vasoconstrictors, failure to control bleeding, dislodgment of packing with possible aspiration, laceration or injury to adjacent structures, septal perforation, hypoxia leading to disorientation, myocardial infarcts, or cerebrovascular accidents, iatrogenic obstructive sleep apnoea, local infection, systemic infection, and finally toxic shock syndrome.

2.2.3.6 Nasal Packing – Absorbable

In recent times, absorbable packs which have haemostatic properties have gained increasing favour following endoscopic sinus surgery. This is particularly true in the paediatric population, where the patient would otherwise require sedation or even a second general anaesthetic in order to removal the non-absorbent pack. Fanous (1980) perhaps first described the potential benefits of absorbable packs when he reported on his experience with oxidized regenerated cellulose in nasal surgery. Other products have been developed, and include gelatin (Gelfoam, Gelfilm), gelatin plus thrombin (FloSeal), microfibrillar collagen (Avitene), and hyaluronic acid (MeroGel, Seprapack, Sepragel) (Chandra and Kern, 2004).

Miller et al. (2003) have compared the use of a non-absorbable pack (Merocel) with an absorbable pack (MeroGel) in patients who had undergone ESS. Although the percentage of adhesions requiring lysis was non-significantly higher with the absorbable pack, the same rate of adhesions (8%) was found in each group at the final eight week review. Baumann and Caversaccio (2003) also compared a non-absorbable pack (Merocel) with an absorbable pack (FloSeal), although their Merocel group was from a previous study. They detected no difference in postoperative healing or adhesion formation. However the group who were packed with an absorbable pack had increased patient comfort and satisfaction. Both Miller et al. (2003) and Baumann and Caversaccio (2003) argue against the use of non-absorbable packs due to their associated comorbidities, and endorse the utilization of absorbable packs due to equivalent rates of healing with increased patient comfort.

Hyaluronic acid is an important player in the healing process that takes place in the upper airway. It forms part of the loose connective tissue matrix that is seen approximately four days after injury. As part of this extracellular matrix, it provides a network for cell mobility and guidance and can also act as a reservoir for cytokines (Watelet et al., 2002). Sinusitis has been found to be associated with an increased amount of hyaluronic acid in the extracellular matrix of affected sinuses (Saito and Nakamura, 1977). Hyaluronic acid has been found to be bacteriostatic, but not bacteriocidal, in the in vitro setting (Pirnazar et al., 1999). The production information sheet for one of its commercially available forms, MeroGel, states that it transforms into a mucoadhesive gel by absorbing up to ten times its weight in liquids. The resultant expansion assists in haemostasis and stents the middle meatus, thus preventing adhesions. Its other purported benefits include minimization of inflammation, decreased crusting, faster healing, faster patient recovery, and dissolution after two weeks, obviating the need for nasal toilette (Medtronic Xomed, Jacksonville, FL). McIntosh et al. (2002) have found that this pack remains adherent to wounded nasal mucosa and slowly dissolves over a period of 14 days. By impregnating hyaluronic acid with therapeutic substances, it has been suggested that the delivery of the latter may be controlled and sustained (Illum, 1994; Rajapaksa, 2004).

The effect of hyaluronic acid on healing of the sinonasal mucosa has been studied in both animals and human patients. In a mouse model, Jacob et al. (2002) found that hyaluronic acid could lead to osteoneogenesis where bone was exposed in the sinuses. However, they used a relatively large amount of MeroGel which was not

moistened and therefore may persist for longer periods of time than previously noted. Maccabee et al. (2003) reported the effect of no packing, FloSeal, and Merogel on full thickness injuries created within the maxillary sinuses of healthy rabbits. They identified increased fibrosis and a lymphocytic response to both packs used, with a greater response noted with Merogel. Furthermore they found that both packs became incorporated into the regenerating mucosa. However, these were early (two-week) findings in what was a qualitative study. Interestingly, there was no osteoneogenesis and no evidence of bio-incompatibility when hyaluronic acid was used in the middle ear after full thickness mucosal injuries of the medial middle ear wall in guinea pigs (Li et al., 2001). McIntosh et al. (2002) found that Merogel improved epithelial regeneration at 12 weeks following full thickness mucosal injury in a healthy sheep model. No effect was seen on cilia regeneration. This was further assessed in a diseased sheep model by Rajapaksa (2004), who found that the use of hyaluronic acid packing had no beneficial effect on mucosal recovery (reepithelialization, reciliation, or ciliary beat frequency) or adhesion formation following endoscopic sinus surgery.

In human patients, hyaluronic acid packing used with absorbable gelatin sponge was found to be associated with less fibrous connective tissue in the middle ear compared to gelatin sponge alone (Laurent et al., 1986). When hyaluronic acid was used following nasal surgery, it was found to prevent extensive crust formation during the first week of healing, and improved subjective measures of healing (Soldati et al., 1999). Kimmelman et al. (2001) found that a gel combination of hyaluronic acid and normal saline lead to decreased adhesions, decreased middle meatal stenosis, decreased mucosal oedema, and improved mucosal regeneration

when compared to no packing. However they had a small sample size, no blinding of assessors, and the primary author had a financial interest in the development of the gel combination being assessed. Frenkiel et al. (2002) found a hyaluronic acid gel to be an effective agent for haemostasis in ESS. As detailed above, Miller et al. (2003) found no difference in healing and adhesion rates when comparing Merogel with a non-absorbable pack. Catalano and Roffman (2003) examined the use of Merogel and Gelfilm following MIST surgery. Adhesions were noted with the use of Gelfilm, although all were clinically insignificant. They also found that both packs often extrude from the nasal cavity even though they are absorbable.

Chandra and Kern (2004) state that the use of FloSeal and Avitene within the nose and sinuses is to be avoided due to their fibrogenic potential. This is supported by the work of Chandra et al. (2003), who found that the use of FloSeal lead to significantly increased granulation tissue and adhesion formation compared to Gelfoam soaked in thrombin at six to eight weeks following ESS. A similar adverse finding was also reported with Gelfilm when compared to contralateral unpacked sinuses two weeks following ESS (Tom et al., 1997).

When discussing the current status of nasal packing, it should be noted that some argue that routine nasal packing can be avoided altogether on most occasions (Nunez and Martin, 1991; Samad et al., 1992; Shaw et al., 2000). Orlandi and Lanza (2004) found that the regular use of nasal packing and/or haemostatics was not necessary to prevent postoperative bleeding complications in their series of patients. Although packing may not be required if patients do not bleed excessively following ESS, if improved rates of healing and decreased rates of adhesion

formation (with decreased rates of subsequent revision surgery) could be demonstrated with substances introduced at surgery, there may be an argument for their routine use in order to improve outcomes following surgery. However, a final consideration in this instance would be the associated cost.

2.2.4 *New Developments – Growth factors*

Growth factors are a class of chemical messengers that specifically stimulate the proliferation of cells (Greenhalgh, 1996). As the wound healing process is primarily dependent on the orderly recruitment of cells, the release of growth factors in appropriate amounts at the appropriate time is of vital importance. If this does not occur, the result is a chronic wound or excessive scarring (Brissett and Hom, 2003). Normal wound healing fluid has an abundance of growth factors (Hom, 1995). It is presumed that a spectrum of growth factors are involved in the maintenance and repair of nasal mucosa, as such processes are likely to require complex interactions between different cell types (Hansson et al., 1991). Growth factors play their part in wound healing by controlling the proliferation and migration of cells that are involved in epithelialization, angiogenesis, and the production and remodeling of collagen and the extracellular matrix (Greenhalgh, 1996; Hom et al., 2002).

As Hom (1995) points out, the manner of growth factor delivery is vital to appropriate progression of wound healing. Some growth factors require repeated or prolonged exposure to the target cell, while others need only be present at certain points in the cell cycle. Other growth factors are required in large doses during narrow windows of opportunity for activity. Finally, growth factors often act

synergistically and the absence of one of these growth factors may result in no effect (Hom, 1995).

Insulin-like growth factor 1 (IGF-1) has recently received attention owing to its potential role in healing of respiratory mucosa injuries, and as such it is of ongoing interest to our research group (McIntosh, unpublished data; Rajapaksa, 2004). IGF-1 was first identified in 1957, and its main site of production for systemic function as a hormone is the liver (Kolaczynski and Caro, 1994). However, IGF-1 can be produced as a cytokine by a variety of cells including fibroblasts, plasma cells, cartilage, and muscle. Its main target cells are fibroblasts, endothelial cells, osteocytes, and chondrocytes, resulting in increased growth, cellular differentiation, and angiogenesis (Watelet et al., 2002) – all vital processes for wound healing. IGF-1 also induces the synthesis of glycogen, protein, and glycosaminoglycans (Hom, 1995).

Of most relevance to healing following sinonasal surgery is the proposal that IGF-1 may possibly play a role in directing epithelial cell proliferation of the airway. Retsch-Bogart, et al. (1990) found that canine tracheal epithelial cells proliferated in response to IGF-1 in vitro and they therefore suggested that IGF-1 might therefore play an important role in the growth and differentiation of large airways epithelium. Hansson et al. (1991) found intense cytoplasmic IGF-1 immunoreactivity in epithelial cells that were invading denuded areas of the nasal cavity with exposed basement membrane. IGF-1 immunoreactivity was detected at an early stage of cellular differentiation but was relatively absent when cells were approaching maturity suggesting a vital role in at least the early stages of repair of the airway.

In a healthy sheep model, McIntosh et al. (unpublished) found that hyaluronic acid packing impregnated with IGF-1 had a significantly beneficial effect on tissue healing (89% reepithelialization) compared to controls (44% reepithelialization). Rajapaksa (2004) examined this same combination in a diseased sheep model and found that it had no effect on reepithelialization, and had a detrimental effect on reciliation and ciliary beat frequency. However, it should be noted that the inflammatory-provoking stimulus remained untreated throughout his experimentation. It was suggested that IGF-1 may augment any inflammation that may be present, and hence a need exists to still deal with any underlying inflammatory focus in the standard manner when utilizing novel agents.

2.2.5 Postoperative Protocols

2.2.5.1 Debridement

A number of surgeons perform postoperative debridement of the operative area, suggesting that this in turn results in improved outcomes. Stammberger (1986b) cleans the ethmoid cavity 2 to 4 days after surgery, and then every 3 to 5 days for the next 10 days. Rice and Schaefer (1988) clean the sinus cavity of their patients between the second and fourth postoperative day, and then twice a week for three weeks. Lund et al. (1991) reviewed their patients 3 to 4 days after surgery, and then weekly for endoscopic examination and suction clearance until the surgical area had healed. Kennedy (1992) recommends debridement on the first postoperative day and then on the third or fourth day following surgery, followed by weekly

debridement until the cavity has re-epithelialized. Smith and Brindley (1993) treated their patients by cleaning the cavity 2 to 4 days after surgery, and then every week for 4 to 6 weeks. Kuhn and Citardi (1997) note that in their early experience they reviewed their patients on the first or second postoperative day, then every 3 to 4 days for 2 weeks, then weekly through to the sixth week.

The suggested need for an intensive postoperative program such as those described above is said to be to debride clots, remove granulations, and lyse synechiae (Fernandes, 1999). Thaler (2002) states that the underlying rationale for an intensive postoperative debridement protocol is as follows:

- (i) Mucous may be trapped by large crusts and clot, which in the setting of chronic infection will reinfect the sinuses. Old blood itself may also act as a culture medium for bacteria.
- (ii) Crusts may act as a bridge for adhesion formation, which can lead to an obstructed cavity – particularly if formed across the middle meatus.
- (iii) Any remaining bone fragments or denuded bone can be removed as these can lead to reinfection.

Rates of adhesion formation following sinonasal surgery have been discussed above. In recent times, however, the potential benefits that extensive postoperative debridement may have on possible surgical failure due to adhesion formation has been challenged. Nilssen et al. (2002) performed a randomized control trial of postoperative debridement versus no postoperative debridement of 32 sinus cavities. They found that there were four adhesions within each group, and all of these except for one were between the anterior end of the middle turbinate and the lateral nasal

wall. There was no difference found between adhesion rates, postoperative symptom scores, or endoscopic appearance scores.

Fernandes (1999) argues that the external manipulation of debridement interferes with rational surgical judgment, likening it to removal of a scab from a cutaneous wound every few days. Indeed, even some surgeons who advocate intensive postoperative debridement state that extensive removal of all inflammatory tissue promotes future synechiae formation (Mair, 1996). The increased demand on outpatient resources to facilitate such an intensive program for each patient is also an important consideration. In his series of 45 patients (90 ethmoid cavities), Fernandes (1999) noted 10 synechiae postoperatively – only half of these, however, were in the middle meatus, giving a synechia rate of 5.6%. He suggested that this was a similar rate to patient series utilising intensive postoperative debridement, with his patients reporting similar rates of symptom improvement.

Thaler (2002) states that postoperative debridement is not possible in the paediatric population, and yet success rates are equivalent to those seen in adults who undergo the intensive protocols detailed above. Postoperative paediatric protocols where children undergo a second procedure under general anaesthesia to remove splints and perform debridement, and even a third procedure if required, have been documented (Mair, 1996; Thaler, 2002). However Tom et al. (1997) found less granulation tissue and adhesions on the side which was not packed compared to the side packed with gelfim in paediatric patients two to three weeks following surgery.

There is also debate as to whether all adhesions need lysis (Fernandes, 1999). Clearly some synechiae may be beneficial (such as those between the middle turbinate and the nasal septum described above). Therefore it may be that division of adhesions is required only if they are at risk of significantly obstructing mucociliary flow.

Some have advocated the prolonged use of smooth-surfaced non-absorbable packing in order to reduce the incidence of adhesions and reduce, if not obviate, the need for postoperative debridement (Brennan, 1996). Obviously the use of such a protocol carries the concomitant risks of nasal packing described above. With regard to absorbable packs, Chandra and Kern (2004) suggest that these packs have significant haemostatic effects, and that they may be associated with excess fibrin deposition, and thus may promote adhesion formation. It is uncertain whether additional endoscopic debridement is warranted in this setting.

2.2.5.2 Intranasal Saline Douching

In healthy volunteers, hypertonic saline was found to significantly improve mucociliary clearance as measured by saccharin clearance times compared to normal saline (Talbot et al., 1997; Homer et al., 2000). Keojampa et al. (2004) found that both buffered hypertonic saline and buffered normal saline significantly improved saccharine clearance times, but this was greater with the hypertonic solution. Rabago et al. (2002) demonstrated that the use of hypertonic saline was associated with improvements in quality-of-life and symptoms and decreased use of topical steroids in patients with chronic rhinosinusitis or recurrent acute sinusitis.

The use of hypertonic saline and normal saline nasal wash has been compared in paediatric patients with chronic rhinosinusitis (Shoseyov et al., 1998). After four weeks of usage, hypertonic saline significantly improved cough, nasal secretions/postnasal drip, and mucosal thickening on plain radiography (Waters' view). However four weeks of normal saline improved only nasal secretions/postnasal drip and had no significant effect on the other two parameters.

Intranasal hypertonic saline irrigations in the postoperative setting are said to reduce crusting and oedema, clear nasal mucous, pus, and debris, and keep the ethmoid cavity cleaner as well as possibly decrease the risk of adhesions by clearing postoperative blood clots (Talbot et al., 1997; Kuhn and Citardi, 1997). The hypertonicity is thought to be important in that it may have more potential to reduce intranasal oedema (Kuhn and Citardi, 1997), although this has not been identified objectively using acoustic rhinometry in healthy volunteers (Keojampa et al., 2004). Furthermore, the use of hypertonic saline may improve mucociliary clearance in the postoperative setting as it does in healthy volunteers (Talbot et al., 1997; Keojampa et al., 2004).

2.2.5.3 Antibiotics

Some surgeons advocate the routine use of postoperative oral antibiotics (Kennedy, 1992), whilst others do not prescribe them unless infection is noted at the time of surgery (Kuhn and Citardi, 1997). The role of antibiotics in the postoperative period is yet to be established, and studies examining their indications are few. In a prospective randomized placebo-controlled trial, Annys and Jorissen (2000) found

no benefit on symptoms, endoscopic appearance, or incidence of infections in the early postoperative period following ESS in patients who received oral cefuroxime.

A further role for macrolide antibiotics may evolve in the postoperative setting based on their anti-inflammatory effects in addition to their antibiotic effects (Wallwork and Coman, 2004; Ragab et al., 2004). It appears that long-term macrolides may improve symptoms, endoscopic appearance, and mucociliary clearance in patients with persistent symptoms following ESS (Cervin et al., 2002).

2.2.5.4 Corticosteroids

The rationale for using steroids in the perioperative period is to reduce inflammation due to disease and its treatment. This may decrease intraoperative bleeding as well as potentially avoiding adverse outcomes as a consequence of poor healing due to unchecked inflammation. Kennedy (1992) notes that the majority of his patients continued topical steroids postoperatively. Any patients that were on preoperative oral steroids for polyposis had them tapered postoperatively. Often steroids are continued in the postoperative period in patients with allergic fungal sinusitis, aspirin-sensitive asthma, or nasal polyposis (Kuhn and Citardi, 1997).

However, like postoperative antibiotics, the use of perioperative steroids has not been studied and proven indications remain ill-defined. In a randomized, double-blind, placebo-controlled trial, Dijkstra et al. (2001) found that topical fluticasone nasal spray for one year had no effect on the recurrence rate of chronic sinusitis or nasal polyposis following ESS. Ramadan (2001) studied the use of intraoperative

intravenous dexamethasone in children undergoing ESS. When they were examined under general anaesthesia 2 to 3 weeks later, those who had received steroids had decreased mucosal oedema and scarring and increased patency of sinus ostia.

2.2.6 Complications of FESS

A number of potential complications of FESS warrant discussion as part of the process of obtaining informed consent. Scarring and the need for further surgery have already been alluded to. The other risks specific to FESS and their reported incidence include:

Cerebrospinal fluid leak	0.2% to 0.4%	
Orbital injury	0.5%	
Bleeding	0.2% to 3.9%	
Anosmia/hyposmia	0% to 34%	(Wolf et al., 2002)

Risks of anaesthesia and infection also need to be discussed with any patient contemplating this procedure.

2.3 THE ASSESSMENT OF FESS

2.3.1 Subjective Outcomes Assessment

2.3.1.1 Symptom Assessment

Almost all surgeons examining outcomes of sinonasal surgery use change in symptoms following intervention as a primary measure. Symptoms of importance include nasal obstruction, nasal discharge (anterior and posterior), facial pain and headache, and hyposmia (Meltzer et al., 2004). Lund et al. (1991) used a visual analogue scale to assess common sinonasal symptoms. They found that all symptoms significantly improved following ESS, with nasal obstruction, headache, and facial pain showing most improvement. Rhinorrhoea, post-nasal discharge, and sense of smell were less significantly improved by surgery.

2.3.1.2 Quality of Life

The impact of chronic rhinosinusitis on health-related quality of life has been assessed by Glicklich and Metson (1995a). They found that chronic rhinosinusitis has a significant impact on a number of domains of general health as determined by the Short Form Medical Outcomes Questionnaire (SF-36). When compared to patients with diseases such as congestive cardiac failure, angina, chronic obstructive airways disease, and chronic back pain, patients with chronic rhinosinusitis had lower scores (worse quality of life) in the domains of bodily pain and social functioning. Notably, ESS had a positive impact on patient-perceived global health

status as measured by the SF-36 at 6 and 12 months following surgery (Winstead and Barnett, 1998).

In addition to this general measure of health-related quality of life, numerous sinonasal-specific quality of life instruments have been developed (Leopold et al., 1997; White 2004; Meltzer et al., 2004). Improvements in disease-specific quality of life have been noted following ESS when assessed with the Sino-Nasal Outcome Test-20 (Piccirillo et al., 1998; Jones et al., 1998; Gosepath et al., 2000) and the Chronic Sinusitis Survey (Gliklich and Metson, 1995b; Stewart et al., 2000).

2.3.2 Objective Outcome Assessment

2.3.2.1 Structure – Endoscopic appearance

Kennedy (1992) noted that endoscopic examination enables persistent disease to be identified, often before it becomes symptomatic. He also found that patient symptoms and endoscopic evidence of persistent disease do not correlate. Smith and Brindley (1993) found that the usual time of healing was 4 to 8 weeks, but occasionally up to 12 weeks. Fang (1994) found that 87% of the mucosa of the maxillary sinus recovered as measured by nasendoscopy 16 weeks after ESS. Abdel-Hak (1998) noted that sinonasal mucosa recovered to appear endoscopically normal three months after ESS.

Weber et al. (1996) prospectively analysed the healing process using videoendoscopic examination. For the first two weeks, examination was largely

obscured by crusts. Granulation tissue was present for the first five weeks, whereas oedema persisted for 16 weeks. However they found persistent oedema in 26% of their patients after six months. Finally, topical budesonide was felt to shorten the duration of each wound healing phase, although they did not provide any quantitative evidence for this.

However, assessment by endoscopy alone does not provide a complete picture.

Benninger et al. (1993) found that gross visible sinus inflammation may resolve before regeneration of normal mucosa on histologic and ultrastructural examination.

Moriyama et al. (1996) found that following sinonasal surgery, mucosa that is normal as assessed by endoscopy can have a poor covering of cilia. Likewise, Asai et al. (2000) found that maxillary sinuses can appear endoscopically normal, and yet have poor mucociliary clearance and extensive loss of cilia.

2.3.2.2 Structure – Light Microscopy

Forsgren et al. (1995) examined the changes on histological examination of the maxillary sinus mucosa one year after sinus surgery. They found no significant change in the extent of goblet cells, subepithelial thickening, squamous cell differentiation, polypoid formations, submucosal glands, or pathological glands following ESS. However, inflammatory cells and oedema had decreased and the extent of fibrosis had increased when compared to preoperative samples.

Interestingly, these changes were more pronounced when the Caldwell-Luc procedure was compared to the ESS procedure.

There are surprisingly few studies examining the histological changes resulting from ESS in human patients. Whilst this may be due to difficulties in obtaining biopsies following treatment, there are comparatively more studies utilizing electron microscopy where postoperative biopsy is also required (although biopsy for the latter examination may be easier). Therefore, this remains an area where further investigation is warranted, particularly in the comparison of histological outcome due to different surgical techniques.

2.3.2.3 Structure – Electron Microscopy

A number of researchers have examined the ultrastructural changes that take place following ESS utilizing electron microscopy. Moriyama et al. (1996) found that ciliary regeneration was worse with full thickness injury compared to partial thickness injury when examined 1.5 years after surgery. Guo et al. (1997) found a significant increase in the density of cilia in the region of the ostium and superolateral wall of the maxillary sinus when examined 6 to 12 months after ESS. Keleş et al. (2001) reported complete recovery of the architecture and ultrastructure of ciliated epithelium in 75% of the patients they studied (with a reduction in abnormalities noted in the remaining 25%) six months after ESS. Toskala and Rautiainen (2003) also examined patients 6 months following ESS and found that there was very definite evidence of recovery and/or reversal of ciliary abnormalities. However, ultrastructural recovery was incomplete, and therefore they recommended that final assessment of outcome following ESS take place no earlier than 12 months post-surgery.

2.3.2.4 Structure – Radiography

Plain radiographs have poor specificity and sensitivity in the investigation of chronic rhinosinusitis, and therefore this imaging modality has no role to play in the management of this disease (Jones, 1998; Meltzer et al., 2004).

Computed tomography (CT) has become the initial, and usually only, imaging modality of choice in the management of chronic rhinosinusitis. Given the degree of detail of the bony anatomy and mucosa of the sinonasal area, CT scanning has become invaluable in managing patients with chronic rhinosinusitis and also in guiding the surgeon during ESS (Meltzer et al., 2004). Owing to associated costs and risks of radiation, however, computed tomography is not routinely employed postoperatively unless the patient continues to be symptomatic, abnormalities persist on endoscopy, and/or revision surgery is being contemplated (Smith and Brindley, 1993). Anecdotally, disease on CT appeared to resolve when patients' symptoms were noted to have improved (Rice, 1989).

Magnetic resonance imaging (MRI) is particularly useful in differentiating between soft tissue and secretions (Younis et al., 2002). Given its poor resolution of bony anatomy, its increased cost, and difficulties with patients who are claustrophobic, it is not regularly used in routine cases of chronic rhinosinusitis (Seltzer et al., 2004). However, it certainly is indicated when dealing with complications of rhinosinusitis or sinonasal neoplasia (Younis et al., 2002; Lloyd et al., 2000).

In 1893, Caldwell stated that “a staging system is necessary to have meaningful results” in the assessment of sinusitis (Lund et al., 1991). With our current level of knowledge, use of CT perhaps represents the best method of staging disease and numerous staging systems have been proposed (Lund and Kennedy, 1997). Given the simplicity of the Lund Mackay staging system (Table 1), as well as its high level of inter- and intra-observer agreement, this system was recommended for use in future outcomes research (Lund and Kennedy, 1997; Seltzer et al., 2004).

Table 1 Staging of disease based on Computed Tomography – Lund-Mackay
CT score (Lund and Mackay, 1993)

STRUCTURE	Left	Right
Maxillary sinus (0,1,2)		
Anterior ethmoids (0,1,2)		
Posterior ethmoids (0,1,2)		
Frontal sinus (0,1,2)		
Sphenoid sinus (0,1,2)		
Ostiomeatal unit (0,2)		

0 = no disease

1 = partial opacification

2 = total opacification

Past studies examining the possibility of a correlation between symptoms and/or quality of life and staging scores based on CT appearance have found no correlation (Ikeda et al., 1996; Bhattacharyya et al., 1997; Stewart et al., 1999; Krouse, 1999; Arango and Kountakis, 2001). However, more recently a weak but statistically significant correlation has been found between the Lund-Mackay CT staging system and either the Chronic Sinusitis Survey or the Visual Analogue Symptom Score, but not the Sino-Nasal Outcome Test-20 (Wabnitz et al., in press).

2.3.2.5 Structure – Acoustic Rhinometry

This technique was first described by Hilberg et al. (1989) and utilizes the introduction of acoustic energy into the nasal cavity to provide information regarding cross-sectional area of the nasal cavity as a function of the distance from the entrance of the nose, as well as total nasal volume. Software analysis of the reflected sound provides three minimum values corresponding to the nasal valve, inferior turbinate, and nasopharynx. It has been found to have measures comparable to those determined by other modalities assessing nasal patency, such as endoscopy, CT scanning, MRI scanning, and rhinomanometry (Corey et al., 2002). Whilst it has been used as an objective measure in documenting treatment efficacy following septal, turbinate, and nasal valve surgery (Corey et al., 2002), its usefulness in assessing ESS is still being established. Using acoustic rhinometry, Rowe-Jones and Mackay (1997) found that nasal volume had increased in patients six weeks after they had undergone ESS, and that this correlated to improvements they found in subjective measures of olfaction. This may indicate the adverse impact that extensive mucosal thickening and/or nasal polyposis has on nasal airflow and,

hence, on olfaction. Gosepath et al. (2000) found that in patients undergoing various types of sinonasal surgery (including ESS), improvements were noted two months after surgery in both the cross-sectional area at the level of the inferior turbinate as measured by acoustic rhinometry and in sinonasal-related quality of life. They also found that greater improvements in sinonasal-related quality of life were experienced by those who had a combined (left and right) cross sectional area of ≥ 1 cm² at the level of the inferior turbinates. Therefore, acoustic rhinometry may have a role in predicting outcome in those contemplating sinonasal surgery as well a possible role in guiding surgery.

2.3.2.6 Function – Ciliary Beat Frequency

This is the most frequently measured parameter to establish function of a single cilium (Jorissen, 1998). A number of ciliated cells can be sampled, the CBF measured, and then the mean calculated. Numerous methods to accomplish this have been described which probably accounts for the relatively wide range of normal values that have been reported – from 7.9 to 15.3 Hz (Luk & Dulfano, 1983; Phillips et al., 1990; de Jongh and Rutland, 1995; Jorissen et al., 2000; Chilvers and O’Callaghan, 2000). However the most widely utilized method uses a photosensitive cell that converts reflections or interruptions of light from beating cilia into an electric current that is then converted by fast Fourier transformation to a spectrum of frequencies (Rutland and Cole, 1980; Lale et al., 1998). The frequency with the highest peak is deemed the CBF. Other methods described use oscilloscopes or ultra-fast video image analysis (Lale, et al., 1998; Min et al., 2001). The only truly in vivo means to measure CBF utilizes spectral analysis of the

scattered light from laser introduced into the nasal cavity. However this has only been used in anaesthetized animals (Wong et al., 1988).

A number of authors have determined the CBF in patients with chronic rhinosinusitis. Wilson et al. (1986) found the CBF to be 14.3 Hz in controls whereas it was 12.1 Hz in patients with chronic rhinosinusitis. Nuutinen et al. (1993) found that 23% of their patients with chronic rhinosinusitis had no detectable ciliary movement. The CBF in the remaining 77% was 16.6 Hz, with a CBF of 15.9 Hz noted in their controls. Similarly, Joki et al. (1998) found that 18% of their patients had a CBF of 0 Hz, with a CBF of 9.2 HZ in the remaining 82%.

Lund et al. (1991) found that the CBF improved from 12.3 Hz to 14.1 Hz following ESS, which was a statistically significant improvement. However, Hafner et al. (1997) found no improvement in CBF following ESS in patients suffering from chronic rhinosinusitis. Abdel-Hak (1998) reported that the CBF steadily improved in chronic rhinosinusitis patients who underwent ESS to reach normal levels at six months after surgery.

2.3.2.7 Function – Mucociliary Transport

This is felt to be the easiest parameter to measure when assessing the complex function of nasal clearance (Passàli et al., 1984). Various substances can be introduced into the nasal cavity, and the time taken for that substance to move over a specified distance, or to appear in the nasopharynx, or to be tasted, is measured (Ferguson and Mabry, 1997; Lale et al., 1998). This can then facilitate comparisons

under different conditions to determine any effect those conditions may have on mucociliary transport. Tracers that have been used include soluble and insoluble dyes, soluble sweeteners, and radioactive particles (Duchateau et al., 1985; Ferguson and Mabry, 1997; Lale et al., 1998; Shaw, 2001). It is likely that insoluble substances are carried on the gel layer without any interaction with the sol layer (Passàli et al., 1984), whereas soluble substances are carried in the sol layer (Talbot et al., 1997; Lale et al., 1998). Passàli et al. (1984) found that insoluble dyes such as charcoal had relatively shorter transit times and less variability when compared to saccharin for the assessment of mucociliary clearance. As such, they felt that more accurate results could be obtained with the former substance. Furthermore, they noted comparable results with the use of radioactive substances, but felt that the use of charcoal was still desirable given its low cost, safety, and simplicity. However, saccharin transit time is probably the most commonly used method as it is easy, inexpensive, safe, and still is a valid measure of mucociliary clearance (Ferguson and Mabry, 1997). The normal range of saccharin clearance time (with saccharin placed on anterior end of inferior turbinate) is 7 to 15 minutes, with a clearance time of greater than 20 minutes indicative of pathological mucociliary clearance (Lale et al., 1998; Jorissen et al., 2000). The mean mucociliary transport rate, as determined by utilizing radiolabelled substances, is 5 to 6 mm/minute (Lale et al., 1998). Transport rates less than 3 mm/minute are considered abnormal (Jorissen, 1998). Duchateau et al. (1985) found that mucociliary transport (as measured by clearance of saccharin and indigo carmine) correlated to the ciliary beat frequency in a logarithmic fashion. This has not been replicated by other researchers, although some correlation between the two measures of mucociliary function is thought likely (Jorissen, 1998).

Asai et al. (2000) observed that as the severity of mucosal disease increased, the saccharin clearance time of the maxillary sinuses increased. Behrhohm and Sydow (1991) found that mucociliary clearance improved following ESS as measured by movement of radioactive technetium particles. Hafner et al. (1997) found that mucociliary clearance as measured by the saccharin clearance technique improved seven months after ESS, although they found no change in CBF postoperatively. Elwany et al. (1998) also found that saccharin clearance time had improved three months following ESS in patients suffering from chronic rhinosinusitis. İnanlı et al (2000) found that whilst mucociliary clearance had improved at 12 weeks following ESS, it was still significantly worse than the clearance times of healthy controls, suggesting that mucociliary function may take longer than 12 weeks to completely recover following surgery. Min et al. (1995) noted an abnormal mean saccharin transit time of 27 minutes in patients with chronic rhinosinusitis. They determined that mucociliary clearance rates improved after surgery, and remained that way for at least the first twelve months following ESS.

2.3.2.8 Function – Olfaction

The functioning of olfaction is essentially tested with either smell identification or threshold testing. In the former, a panel of odours is presented individually by opening microencapsulated material (“scratch and sniff”) with each odour having a number of responses from which the patient chooses (Ferguson and Mabry, 1997). The 40-item University of Pennsylvania Smell Identification Test is the most commonly used example of such a test. This was used by Lund et al. (1991) to test

olfaction following ESS. They found no objective improvement, even though their cohort of patients reported a significant subjective improvement. However, Abdel-Hak et al. (1998) reported improvements in subjective and objective measures of olfaction in their patients undergoing ESS, although they used the Erlangener Smell Identification Test.

The second method of assessing olfaction is through the use of odour detection threshold testing where variable concentrations of a substance is presented (most commonly phenyl ethyl alcohol, which has a rose-like smell) until the odour is just perceived (Ferguson and Mabry, 1997). This method was used by Rowe-Jones and Mackay (1997) who found thresholds were significantly improved at six weeks following ESS with adjuvant medical treatment, as were subjective measures of olfaction. Min et al. (1995) assessed olfactory thresholds pre- and post-ESS utilizing butanol, which has a characteristic alcohol odour. They found that thresholds were substantially improved (significantly reduced) 12 months following surgery, with the greatest improvements in those with more severe disease.

However, Ferguson and Mabry (1997) caution that there are reports of patients who have “smell blindness” to a limited number of odours, which may include the test odour. They therefore advocate including odour identification tests in addition to threshold testing if the latter is of particular interest to researchers.

2.3.2.9 Function – Anterior Rhinomanometry

This technique measures transnasal pressure and nasal airflow simultaneously to provide a measure of nasal resistance, and may be combined with acoustic rhinometry to provide complementary objective measures of nasal patency (Ferguson and Mabry, 1997). There have been contradictory results when changes in nasal airway resistance have been evaluated following ESS. Lund et al. (1991) found no improvement in nasal airway resistance after FESS despite subjective improvements in nasal obstruction. They note that as the nasal valve region that provides greatest resistance to airflow, and this was not surgically altered, nasal resistance may not be affected by ESS. Keleş et al. (1998) assessed subjective changes in nasal obstruction in patients undergoing ESS as well as any change in total nasal resistance as measured by rhinomanometry. Both measures were found to improve, and this was thought to be due to decreased mucosal oedema following surgery rather than change in sinonasal anatomy. Giger et al. (2003) also found that ESS improved nasal airway resistance both subjectively (symptoms and quality of life) and objectively (rhinomanometry) at four months and two years following surgery. Others have noted no correlation between subjective and objective measures of nasal airflow (Jones et al., 1989).

2.3.2.10 Function – Properties of Mucous

Clearly, mucous forms an important component of mucociliary transport and its rheological properties must be carefully matched to its biological function (Lale et al., 1998). In chronic rhinosinusitis, absorption of sodium and water occurs with the

stasis of secretions, which in turn leads to mucous with increased viscosity and elasticity (Quraishi et al., 1998). This has been associated with prolonged saccharin clearance times in patients with chronic rhinosinusitis (Lale et al., 1998).

The properties of mucous that can be assessed include its viscoelasticity, its spinnability, and its adhesiveness (Quraishi et al., 1998). Perhaps the simplest means by which to globally assess the nature of mucous is to place individual samples of mucous on mucous-depleted frog palate and calculate nasal mucous transport rates (Puchelle et al., 1982). Using this method, Lioté et al. (1989) found that the faster the in vitro nasal mucous transport rate, the shorter the in vivo nasal mucociliary transport time (as measured with the saccharin clearance test). There was no correlation between the in vitro measure and CBF. However, it should be noted that the study of rheological properties of mucous is not currently used as an outcome measure following surgery as reliable testing and clinical applicability has not been established (Lale et al., 1998).

In addition to the assessment of its rheological properties, numerous substances and cells have been isolated from nasal mucous and quantified under various conditions (Kramer et al., 2000; Bolard et al., 2001; Besançon-Watelet et al., 2002). However, this also remains largely an experimental avenue of enquiry rather than a parameter that currently has a role as a surgical outcome measure.

2.4 ANIMAL MODELS

The “gold standard” with regard to assessment of different interventions on a disease process is the double-blinded randomized placebo-controlled trial. Clearly this presents difficulties in the assessment of surgical interventions. At the very least, the surgeon is not blinded as to which procedure is performed on the patient, and this can also be obvious to blinded assessors postoperatively due to changes in anatomy. Furthermore, there are ethical considerations in performing sham surgery (Meltzer et al., 2004), which would form the placebo arm of a surgical trial.

Therefore, animal models may be used in order to identify potential benefits of different surgical interventions before they are examined in the diseased human setting.

A number of different animal models have been used to examine the response of the sinuses to various treatments. Typically the smaller animal models have been used to investigate the medical treatment of rhinosinusitis. Clearly, these animals are unsuitable for investigating endoscopic sinus surgery given the size of standard endoscopic instrumentation.

2.4.1 Mouse Model

Mice have the advantages of being relatively pathogen free, having minimal antigenic priming, and demonstrating minimal sibling genetic variability.

Furthermore, genetic knockout and transgenic mice have revolutionized animal research to the extent that Jacob et al. (2001) argue that use of the murine model is

required in any further research. Both an acute rhinosinusitis model (Bomer et al., 1998) and a chronic rhinosinusitis model have been described (Jacob et al., 2001). However, in the chronic rhinosinusitis model, drilling through the bony nasal dorsum was required to enter the sinonasal cavity in order to introduce the bacterial suspension (Jacob et al., 2001).

2.4.2 Rabbit Model

The rabbit model has been the most commonly used animal for examining sinonasal response in health and disease. However, studies utilizing the rabbit model have required a transcutaneous external approach to the small sinuses of the rabbit (Benninger et al., 1989; Kennedy and Shaalan, 1989; Benninger et al., 1991; Benninger et al., 1993; Czaja and McCaffrey, 1998), which clearly is not analogous to the human approach. Furthermore there are difficulties with ensuring that the rabbits are *Pasturella*-free (Friedman and Toriumi, 1989), with colonization of the sinuses occurring early (Glass and Beasley, 1989). Infections with *Pasturella* may confound findings when examining the paranasal sinuses in this setting (Benninger et al., 1989; Kennedy & Shalaan, 1989; Friedman & Toriumi, 1989).

2.4.3 Pig Model

With regard to sinonasal research, the pig has only been used in examining the effect of endoscopic sinus surgery on the growing sinuses (Mair et al., 1995; Carpenter et al., 1997). Shaw et al. (2001) examined a sagittally cut pig's head and

suggested that the sinus alignment and turbinate structure made it unsuitable for repeated endoscopic sinus surgery.

2.4.4 *Dog Model*

There appears to have been a vogue in the 1970's for using the dog in studying diseases of the frontal sinuses and their treatment (Schenck et al., 1974; Neel et al., 1976; Schenck & Rauchbach, 1976; Abramson et al., 1978). It is unclear why this animal model has fallen out of favour, but a significant difficulty is that it is not widely available for research (Shaw et al., 2001).

2.4.5 *Sheep Model*

Although it is somewhat larger in volume, the sheep nasal cavity is very similar in appearance to the human nasal cavity and the paranasal sinuses lie in the same orientation. The bone of the ethmoturbinal complex is slightly thicker in the sheep, but not to the extent that this causes any problem with dissection (Gardiner et al., 1996). In fact the larger nasal cavity facilitates endoscopic manipulation, and this animal model has been found to be suitable for training in endoscopic sinonasal surgery (Gardiner et al., 1996). Furthermore, histological study of the sheep's nasal mucosa has shown it to be identical to that of humans (Illum, 1996).

For these reasons, the sheep is an excellent model for studying various endoscopic manipulations that may impact on healing. However, in order to access the paranasal sinuses, the large middle (ventral) turbinate needs to be removed. This

manoeuvre has been found to have no significant effect on the cilia covering of the nasal cavity, nor was there any adverse effect on mucociliary transport (Shaw et al., 2001).

2.4.6 *A Diseased Animal Model*

As stated previously, the treatment for chronic rhinosinusitis that is refractory to medical therapy is functional endoscopic sinus surgery. Surgery is performed in the human patient in the setting of inflammation, and often this is an eosinophil driven response. The assessment of different therapies would therefore be best carried out in a diseased animal model that is closely analogous to the human situation.

In order to mimic this disease process, most animal models rely on occlusion of the sinus ostium by artificial means in order to create a bacterial infection of the closed maxillary sinus (Marks, 1998; Jacob et al., 2001). However, Marks (1998) points out that there are two problems with this nonpathophysiologic situation. First, infection is limited to the occluded sinus, and whilst this does occur in humans it usually leads to a mucocoele or a mucopyocoele. Secondly, there is a significant, almost necrotizing infection that resembles an abscess more than it does a sinus infection. The result may be an animal model that more closely resembles sinonasal diseases other than chronic rhinosinusitis.

Oestrosis is a very common condition affecting sheep and other animals (such as goats and camels) where larvae of the *Oestrus ovis* nasal bot fly become resident in the nasal cavity of sheep. Human infestation has been reported (Badia & Lund,

1994; Masoodi & Hosseini, 2003). The inflammatory response that occurs in sheep was thought to be due to mechanical and traumatic damage caused to the nasal mucosa by the oral hooks and cuticular spines of the larvae. However, recent work provides evidence for a hypersensitivity response, with increased numbers of mast cells and eosinophils in the mucosa (Dorchies et al., 1998). The major immunogens have been found to be salivary gland polypeptides, and to a lesser extent polypeptides from the larval cuticle (Innocenti et al., 1995; Tabouret et al., 2001). The resulting mucosal swelling occludes the orifices of the sinuses leading to accumulation of exudates and purulent secretions (Jubb et al., 1993). As Rajapaksa (2004) points out, other animal models of CRS require inoculation by bacteria as well as occlusion of ostia, whereas this model requires no such manipulation and therefore closely parallels the disease process seen in humans. This animal model of CRS provides an excellent means by which benefits and detriments of potential treatments may be studied, and has been previously validated for this very use (Shaw, 2001; McIntosh et al., 2002; Rajapaksa, 2004).

CHAPTER 3

**THE EFFECT OF HYALURONIC ACID
PACKING, WITH AND WITHOUT INSULIN-
LIKE GROWTH FACTOR 1, ON
EPITHELIAL REGENERATION AFTER
FULL THICKNESS NASAL MUCOSA
INJURY IN AN ANIMAL MODEL OF
CHRONIC RHINOSINUSITIS**

3.1 INTRODUCTION

The primary treatment for chronic rhinosinusitis is medical therapy, with endoscopic sinus surgery (ESS) reserved for cases that do not respond to intensive medical treatment (Ragab et al., 2004). Some patients are recalcitrant to surgical treatment also, and both medical and surgical reasons have been identified as causes of failure of ESS (Ramadan, 1999; Richtsmeier, 2001).

Most investigators would agree that timely and uncomplicated healing following ESS is an important factor, if not the most important factor, affecting the outcome of surgery. Adhesion formation is thought to be the biggest problem following ESS, which can lead to recurrence of disease if it occurs. Much has been written about the importance of surgical technique, intraoperative packing, and postoperative care on the success of ESS and the impact of these factors on adhesion formation (Kennedy, 1985; Stammberger, 1986b; Rice, 1989; Richtsmeier, 2001; Thaler, 2002). At times these claims have been made with little scientific evidence, representing the opinions of respected leading surgeons in the field. However, although slow in coming, there has been an increase in credible scientific examination of healing following ESS in recent times.

Non-absorbable packing has long been used in sinonasal surgery, with the primary aim of obtaining haemostasis. However, these packs can have detrimental effects on healing following surgery (Shaw et al., 2000), and can be associated with significant patient discomfort as well as complications (Fairbanks, 1986; Garth & Brightwell, 1994; Orlandi and Lanza, 2004). Hence, absorbable packs have received increasing

attention with no clear evidence of any significant advantage with one pack over another (Miller et al., 2003; Catalano and Roffman, 2003).

In the last few years, there has also been an exponential increase in the information we have regarding the wound healing process and the role of cytokines and growth factors in this process. The possibility of exploiting this process by utilizing growth factors during and following surgery in order to achieve improved surgical outcomes is an attractive prospect. Although the complex mystery of wound healing is still yet to be completely unraveled, there is some evidence that insulin-like growth factor 1 (IGF-1) may have a positive impact on the healing of the airways. Retsch-Bogart et al. (1990) have shown that canine tracheal epithelial cells proliferate in response to IGF-1 in vitro. They suggested that IGF-1 might therefore play an important role in the growth and differentiation of large airways epithelium. Furthermore, Shoji et al. (1990) demonstrated that insulin and IGF-1 act as chemotactic agents for bronchial epithelial cells in vitro.

We sought to assess reepithelialization following full-thickness injury to the sinonasal mucosa in a chronic rhinosinusitis animal model, and to determine if the use of an absorbable hyaluronic acid pack with or without impregnation with insulin-like growth factor 1 has any impact on this outcome.

3.2 METHODS

Approval was granted for this study by the Animal Ethics Committees of the University of Adelaide, Adelaide, South Australia, and the Queen Elizabeth Hospital, Woodville, South Australia.

Eighteen sheep that had not been treated for the nasal bot *Oestrus ovi* were utilised for this study. Infestation by the nasal bot was confirmed during the study period through endonasal examination of each sheep. Sheep were deprived of food and water for 24 hours prior to general anaesthesia (GA) in order to limit regurgitation. GA was induced by an injection of sodium thiopentone (19mg/kg body weight) into the jugular vein, the skin over which had previously been well shorn, cleaned, and sterilised. Following endotracheal intubation, anaesthesia was maintained by inhalation of a gas mixture of 1.5 – 2% halothane with oxygen.

There were two separate procedures performed on each animal under the conditions of GA, and these were separated by a four week interval. The first was that of endoscopic ventral turbinectomy to allow access to the nasal cavity. This has previously been shown to have no adverse effect on mucociliary clearance or on the ciliary covering of the nasal cavity (Shaw et al. 2001). Neuropatties soaked in 2mL of 10% cocaine were inserted onto the ventral turbinate and then removed some minutes later prior to the resection of the ventral turbinate from its lateral attachment using turbinectomy scissors. Haemostasis of the posterior nasal artery (analogous to the sphenopalatine artery in the human) was achieved using suction diathermy. This was then allowed to heal over the four week period. The second

procedure to be performed under general anaesthesia was stripping of full thickness lateral nasal wall mucosa. This was carried out in four sites in each sheep – above and below the stump of the ventral turbinate on each side. This was performed endoscopically using a sharpened Freer dissector to make the incisions and lift the mucosa. A sample of this tissue was sent for light microscopy as baseline measure.

Each defect measured 4 x 1 cm, and extended 4cm anteriorly from the level of the ethmoid terminal posteriorly. This was accurately measured and recorded for guiding subsequent biopsies. The two mucosal defects of one side of each sheep was then covered using hyaluronic acid packing (MeroGel[®] – Medtronic Xomed Surgical Products, Jacksonville, FL). The side to be packed was randomly assigned in each sheep. The first nine sheep received hyaluronic acid packing (HA) alone. The other nine sheep received hyaluronic acid packing impregnated with insulin-like growth factor-1 (HA+IGF1) at a concentration of 10 μ g/cm² (Fidia Advanced Biopolymers, Abano Terme, Italy). Ovine IGF-1 structurally differs from human IGF-1 by only one amino acid residue, and are functionally identical (Francis et al., 1989). Each sheep was then treated with 8mL of Closal[®] (37.5g/L closantel, 19g/L albendazole – Coopers Animal Health, Baulkham Hills, NSW) per orally at day 2 postoperatively to eradicate the *O. ovi* infestation.

Subsequent biopsies were then performed every four weeks for a period of sixteen weeks to assess the progression of healing. These were performed under conditions of mild to moderate sedation, and animals were sedated using an intramuscular injection of 4mg of xylazine. Co-Phenylcaine Forte[®] spray (50mg/mL lignocaine hydrochloride, 5mg/mL phenylephrine hydrochloride – ENT Technologies Pty Ltd,

West Perth, WA) was also used in order to anaesthetize the area of biopsy. An incision was made and a flap raised using a sharpened Freer elevator. Biopsies were taken from this flap using a punch biopsy forceps at each four week interval. Following the final biopsy, euthanasia was performed by intravenous injection of sodium pentobarbitone (>100mg/kg).

Specimens for light microscopy were fixed in formalin for four hours, and then placed in 70% ethanol until processing took place soon after. Specimens were processed, embedded in paraffin blocks, sectioned at 4µm thickness, and mounted on glass slides. They were then stained using Haematoxylin and Eosin.

Each specimen was examined under lighter microscopy utilising image capture software (Image Master Pro Software, Media Cybernetics, Inc., Silver Spring, MD). Measurements and calculations were made as to what percentage of total linear surface had an epithelial covering of any thickness, as opposed to areas that had exposed lamina propria. Results were pooled according to whether specimens were controls (no packing), had HA packing, or had HA+IGF1 packing.

Differences in reepithelialization rates between these groups was examined. Student's *t*-test was used for statistical evaluation with $p < 0.05$ accepted as statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 9.0 software system (SPSS, Inc., Chicago, IL).

3.3 RESULTS

The percentage of epithelialization for each site of injury is documented in Table 2.

A number of biopsies had associated cartilage or bone artifact, and these are

indicated by an asterisk (*) on Table 2. The degree of epithelialization is

summarized at different time points by pack used in Table 3. Baseline

epithelialization was a mean of 90.3% (SD 25.3%, SEM 4.2%, R 0-100%).

Analysis of the difference in epithelialization between the packs at each time point

is summarized in Table 4. The only statistically significant finding was between the

control group and the HA+IGF1 group at 8 weeks after injury (Student's paired *t*-

test, $p < 0.05$).

No adhesions were noted as a result of surgery at any time point.

Table 2 Percentage of epithelialization for each site of injury

Sheep	Site	Pack Used	% epithelialization				
			Baseline	Week 04	Week 08	Week 12	Week16
1	left	HA+IGF1	100.0	6.8	70.9	69.6	82.5
1	right	None	100.0	38.3	9.0	68.9	80.1
2	left	None	100.0	22.2	42.3	0.0	72.3
2	right	HA+IGF1	100.0	4.5	88.9	89.8	52.8
3	left	HA+IGF1	100.0	0.0	39.6	67.7	81.3
3	right	None	92.1	0.9	0.0	0.0	67.5
4	left	HA	100.0	23.3	64.8	91.6	0.0
4	right	None	100.0	37.3	7.7	0.0	100.0
5	left	HA	100.0	46.8	74.9	0.0*	0.0
5	right	None	39.8	32.5	91.9	74.4	82.0
6	left	HA	100.0	0.0	41.7	17.2	25.1
6	right	None	100.0	0.0	64.9	53.4	88.7
7	left	HA+IGF1	100.0	54.2	26.8	45.5	93.2
7	right	None	100.0	64.7	0.0	96.9	-
8	left	HA+IGF1	100.0	28.8	88.1	94.2	-
8	right	None	100.0	46.5	26.8	81.2	85.9
9	left	None	93.0	47.3	-*	90.3	69.3
9	right	HA	89.2	33.8	0.0	85.9	93.5
10	left	None	100.0	0.0	58.6	68.3	0.0
10	right	HA+IGF1	100.0	0.0	78.1	0.0	100.0
11	left	HA	100.0	88.1	92.1	60.4	85.9
11	right	None	100.0	100.0	81.8	54.2	93.5
12	left	None	70.4	11.6	63.6	87.5	0.0
12	right	HA	100.0	15.3	14.4	79.8	60.0
13	left	None	100.0	73.0	64.6	59.7	-*
13	right	HA	100.0	0.0	65.8	23.4	91.2
14	left	None	100.0	0.0	60.1	79.6	100.0
14	right	HA	0.0	8.3	0.0*	91.5	75.0
15	left	HA	67.1	35.9	67.9	5.5*	69.5
15	right	None	100.0	25.4	55.8	63.5	89.3
16	left	None	100.0	64.5	0.0*	46.4	97.4
16	right	HA+IGF1	100.0	46.8	17.1	82.2	58.1
17	left	None	100.0	24.5	0.0*	37.7	0.0
17	right	HA+IGF1	0.0*	25.6	78.9	36.8	100.0
18	left	None	100.0	0.0	68.7	81.1	76.3
18	right	HA+IGF1	100.0	17.2	42.6	63.2*	0.0

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

* indicates specimen had cartilage or bone artifact

Table 3 Percentage of epithelialization by time and pack used

Time	Control (no pack)	HA pack	HA+IGF1 pack
Baseline	94.2%	84.0%	88.9%
4 weeks after injury	32.7%	27.9%	20.4%
8 weeks after injury	40.9%	46.8%	60.0%
12 weeks after injury	57.9%	50.6%	61.0%
16 weeks after injury	68.9%	55.6%	71.0%

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

Table 4 Two-tailed *p*-values for Student’s paired *t*-test analysing differences between packs at different time points

Time	Control and HA pack	Control and HA+IGF1 pack	HA pack and HA+IGF1 pack
Baseline	0.599	0.846	0.226
4 weeks after injury	0.726	0.057	0.510
8 weeks after injury	0.417	0.019 [#]	0.396
12 weeks after injury	0.952	0.592	0.619
16 weeks after injury	0.320	0.383	0.348

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

[#] *p* < 0.05

3.4 DISCUSSION

Chronic rhinosinusitis has been defined as a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses of at least 12 consecutive weeks' duration (Benninger et al., 2003). The most common finding on histological examination of the sinonasal mucosa of patients with this disease is an inflammatory cellular infiltrate, desquamation of epithelial cells, as well as thickening of the basement membrane and oedema within the submucosa (Goldwyn et al., 1995; Harlin et al., 1988). The potential role of the eosinophil in this disease process has been well described, and it is through the production and release of inflammatory mediators such as major basic protein and eosinophil cationic protein by the eosinophil that the mucosa is damaged (Harlin et al., 1988; Baroody et al., 1995; Szucs et al., 2002; Wei et al., 2003). There is some evidence that the extent of eosinophilia correlates with the severity of disease as determined by histological examination (Szucs et al., 2002) or radiological staging (Newman et al., 1994; Hoover et al., 1997; Bhattacharyya et al., 2001). Our animal model is based on an *O. ovi* infestation of sheep, which leads to an eosinophilic-driven chronic rhinosinusitis. Of note, baseline epithelialization was found to be 90.3% suggesting that the *O. ovi* infestation did indeed lead to mucosal injury. Denuded epithelium was noted in all regions of the specimens, and not just at the edges where an epithelial injury may have been sustained by taking the biopsy. This has also been found by Rajapaksa (2004) when he examined this animal model. There were no significant differences between this baseline measure when comparing the three groups. Eosinophils were noted in the submucosa of specimens on light microscopy, but they were not quantified.

Following treatment with Closal[®], *O. ovi* nasal bots were no longer detected. It was felt that this treatment was important in order to mirror the reduction of infective load through the use of perioperative antibiotics in the human situation. In a similar study, Rajapaksa (2004) found that an ongoing infestation with *O. ovi* lead to a significantly worse rate of healing following injury when compared to disease-free sheep.

All three of our groups had progressive improvement in their re-epithelialization rates. Eight weeks after injury, animals in which the HA+IGF1 pack was used had a re-epithelialization rate of 60.0%, whereas the control group had a re-epithelialization rate of 40.9% at this time point. This was the only statistically significant difference found between any of the groups at any time point. Although there was a difference of 13.2% between the HA group and the HA+IGF1 group at eight weeks, this did not achieve statistical significance. It is interesting to note that from week four to week eight, the re-epithelialization rate of the control group improved by 8.2%, the rate improved by 18.9% in the HA group, and by 39.6% in the HA+IGF1 group. However, following this large increase, the increases in subsequent measures in the HA+IGF1 group were not as dramatic and indeed no different to the measures in the other two groups. These results suggest that the presence of insulin-like growth factor 1 at the time of injury increases the rate of mucosal healing as observed at eight weeks, but no improvement following this time point compared to hyaluronic acid alone or no packing. Thus, there is no evidence of any significant benefit with these packs in the medium to long term.

Although there is an abundance of growth factors in a healing wound, the role each growth factor plays in wound healing is incompletely understood. Insulin-like growth factor-1 (IGF-1) is a potent chemotactic agent for vascular endothelial cells, and is therefore thought to play a role in vascularization of a healing wound (Grant et al., 1987). With regard to healing following sinonasal surgery, IGF-1 is thought to play an active role in directing epithelial cell proliferation following injury to the airway. Retsch-Bogart et al. (1990) found that canine tracheal epithelial cells proliferated in response to IGF-1 in vitro and they therefore suggested that IGF-1 might therefore play an important role in the growth and proliferation of cells of the large airways epithelium. Similarly, Shoji et al. (1990) found that IGF-1 is a chemoattractant for bronchial epithelial cells in vitro. McIntosh et al. (unpublished) found that topical IGF-1 had a beneficial effect on respiratory epithelial wound healing in vitro. Hansson et al. (1991) found intense cytoplasmic IGF-1 immunoreactivity in epithelial cells that were invading denuded areas of the nasal cavity with exposed basement membrane. They also found that these cells appeared to have immunoreactivity for more than one peptide but this was not further characterized. The authors concluded that a cocktail of growth factors is likely to be required to facilitate repair and normalization of the nasal mucosa after an insult. However, IGF-1 immunoreactivity was detected at an early stage of cellular differentiation but was relatively absent when cells were approaching maturity. This may account for the improved reepithelialization rates seen in our HA+IGF-1 group at the eight week time-point. It seems unlikely that a slow-release formulation of IGF-1 will have any effect on our findings given the relatively early expression of IGF-1 immunoreactivity. It may be that the balance of IGF's, IGF receptors, and IGF-binding proteins is an important part of the healing process, as has been found

in cutaneous and bone healing (Jyung et al., 1994; Tsuboi et al., 1995; Toung et al., 1999). Alternatively, the role of synergistic growth factors may be important (Lynch et al., 1987). The introduction of other yet-to-be characterized growth factors prior to the eight-week time point may lead to reepithelialization rates that are both improved and sustained.

There appears to be no advantage in using a HA pack alone in the setting of injured sinonasal mucosa. Maccabee et al. (2003) examined healing in rabbits which had mucosal stripping procedures and compared results between controls, a HA pack, and a thrombin/gelatin haemostatic sealant at two weeks following injury. All three groups had loss of cilia and lamina propria fibrosis, but this appeared to be greatest in the hyaluronic acid group and least in the control group. This was a descriptive study with no statistical analysis. McIntosh et al. (2002) found that a HA pack improved reepithelialization at 84 days following injury, but there was no difference compared to controls at 112 days following injury. They found no improvement in reciliation when using a HA pack compared to controls in their healthy sheep model. Rajapaksa (2004), who used a diseased animal model identical to ours, found no difference in reepithelialization when using a HA pack with and without IGF-1 compared to controls. However, the oestrosis in his animals remained untreated for the duration of his study and hence his specimens were taken in the setting of ongoing inflammation. In a small study, Miller et al. (2003) found no difference in the adhesion rate when using a HA pack compared to using a non-absorbable pack following ESS in human patients.

The findings of studies in animal models by Benninger et al. (1989), Benninger et al. (1991), and Forsgren et al. (1993) suggest that complete regeneration of the sinonasal mucosa takes at least six to nine months. Moriyama et al. (1996) demonstrated poor healing in patients with chronic rhinosinusitis who had areas of the sinonasal complex that had been stripped of mucosa down to bone. They found that epithelial regeneration still had not occurred at six months after injury as assessed by endoscopic examination. After 18 months, they found that the bone-exposed areas were finally covered with mucosa. Four months after full thickness injury, our animal subjects had reepithelialization rates of somewhere between 55.6% and 71.0%. This is similar to the findings of Rajapaksa (2004), who found reepithelialization rates of around 65% four months after injury. Clearly, an examination of findings after at least six to nine months is required in order to fully characterize healing end-points. In fact, there is some evidence to suggest that final assessment should not take place before twelve months, although this is based on electron microscopic examination (Toskala and Rautiainen, 2003).

There were a number of specimens that had artifact from either cartilage or bone that were somehow included in the specimen (as indicated in Table 1), even though care was taken to raise the mucosal biopsy in the subperiosteal plane. Forsgren et al. (1993) found that there can be new bone formation when a full thickness injury is created in the sinonasal mucosa. Furthermore, the presence of hyaluronic acid adjacent to denuded mucosal surfaces has been shown to lead to neosteogenesis (Jacob et al., 2002). Finally, IGF-1 has also been implicated in the stimulation of new bone formation in animal models (Hock et al., 1988). These factors may well

account for the artifact seen as this problem was not seen in baseline or week four specimens, whereas it was a feature in later specimens of all three groups.

3.5 CONCLUSION

Following full thickness nasal mucosa injury in a chronic sinusitis animal model, a HA pack impregnated with IGF-1 lead to a significant improvement in epithelial healing compared to controls at eight weeks following injury. There was also a trend towards improved healing compared to a HA pack alone, but this was not statistically significant. However, there was no difference between the three groups in the longer term, suggesting that further exposure of the damaged mucosa to other growth factors at that time point may be required for sustained increased rates of re-epithelialization.

CHAPTER 4

**THE EFFECT OF HYALURONIC ACID
PACKING, WITH AND WITHOUT INSULIN-
LIKE GROWTH FACTOR 1, ON CILIAL
REGENERATION AFTER FULL
THICKNESS NASAL MUCOSA INJURY IN
AN ANIMAL MODEL OF CHRONIC
RHINOSINUSITIS**

4.1 INTRODUCTION

The complex process of mucosal healing involves not only re-epithelialization, as examined in section Chapter 3, but also regeneration of the cilia which are so integral to the healthy functioning of the sinonasal mucosa. Therefore, in order to fully assess the structural components of the sinonasal mucosa, examination by both light microscopy and electron microscopy is necessary (Kupferberg et al., 1998). Indeed, Benninger et al. (1993) found that gross visible sinus inflammation may resolve before normal ultrastructure is re-established. Moriyama et al. (1996) have also demonstrated that re-epithelialization does not necessarily indicate a return to normality for the mucosa of the sinonasal complex. Following sinonasal surgery, they found that endoscopically normal mucosa can continue to have a poor covering of cilia. They concluded that ciliary regeneration constitutes the most important requirement for successful surgery.

A number of authors have examined the electron microscopic changes in patients undergoing endoscopic sinus surgery for chronic rhinosinusitis. Inanli et al. (2000) found that histological and morphological recovery as assessed by electron microscopy was incomplete 12 weeks after ESS, although improvements were noted. Keleş et al. (2001) found that the normal architecture and ultrastructure of the ciliated epithelium was restored at six months following endoscopic sinus surgery. Toskala and Rautiainen (2003) found that numbers of non-ciliated cells and microvilli had increased and the degree of metaplasia and disorientation and the number of compound cilia had decreased following surgery. However they also found many persistent pathological findings at six months after endoscopic sinus

surgery, suggesting that some changes persist longer than six months or may even be irreversible.

Clearly, then, an examination of reciliation by electron microscopy is an important component of assessing the healing process following endoscopic sinus surgery.

This is of particular importance when considering different treatment options, given that improved re-epithelialization does not necessarily imply improved reciliation or functionality typical of normal mucosa. We therefore sought to assess whether our interventions lead to improved reciliation as compared to controls.

4.2 METHODS

Approval was granted for this study by the Animal Ethics Committees of the University of Adelaide, Adelaide, South Australia, and the Queen Elizabeth Hospital, Woodville, South Australia. The same eighteen sheep that had not been treated for the nasal bot *Oestrus ovi* were also utilised for this study.

As described in previous chapter (3.2 – Methods), all sheep underwent endoscopic ventral turbinectomy followed by the creation of four regions of full thickness mucosal injury per sheep four weeks later. Both of these procedures were under conditions of GA.

A sample of tissue from the injury site was sent for baseline scanning electron microscopy examination. Mucosal injury sites were then left unpacked or packed with HA with and without IGF-1 as described previously. Subsequent biopsies were taken at weeks 8 and 16 following full thickness injury creation. Sites of biopsy were carefully ordered and recorded during the sixteen week period in an attempt to sample untouched areas. Following the final biopsy, euthanasia was performed by intravenous injection of sodium pentobarbitone (>100mg/kg).

Specimens were placed in phosphate buffered saline and then washed for 20 minutes using an ultrasonic cleaner in an attempt to remove mucous, debris, and fungus. They were then placed in fixative (4% paraformaldehyde/1.25% glutaraldehyde in phosphate buffered saline + 4% sucrose, pH 7.2) until processed. This involved washing with phosphate buffered saline + 4% sucrose, exposure to 1% osmium tetroxide in phosphate buffered saline, and dehydration in progressively increasing concentrations of ethanol. Finally, the specimens were placed in acetone for critical point drying, and then mounted and coated with carbon and gold.

Each specimen was examined by scanning electron microscopy (Philips XL30 Field Emission Scanning Electron Microscope) at 500x magnification, avoiding the margins of the specimens where they may have been affected by artefact. Every specimen was graded according to the grading system in Table 5. If clarification was required, specimens were also examined at higher magnifications (2000x and 5000x). Results at the different time points (baseline, 8 weeks, 16 weeks) were analysed to ascertain if packing with HA with or without IGF-1 had any impact on reciliation as compared to no packing (controls). Pearson's χ^2 test and Fisher's exact

test were used for statistical evaluation with $p < 0.05$ accepted as statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 9.0 software system (SPSS, Inc., Chicago, IL).

Table 5 Grade of reciliation based on scanning electron microscopy appearance

Grade	Appearance on Electron Microscopy
I	normal cilia with normal orientation
II	ciliated epithelium but disorientated
III	stumps of cilia, regenerating cilia
IV	no identifiable cilia
V	unusable (crust or clot covering epithelium)

4.3 RESULTS

The grading of reciliation for each site of injury is documented in Table 6. The grading of specimens at baseline, 8 weeks after injury, and 16 weeks after injury is indicated by Figure 1, Figure 2, and Figure 3 respectively.

As all grade V specimens were covered by crust, clot, or mucous, further analysis of ciliary regeneration was not possible, and therefore no meaningful data is obtainable from these samples. They were thus deleted from further statistical analysis. The occurrence of these at different time points is summarised in Table 7.

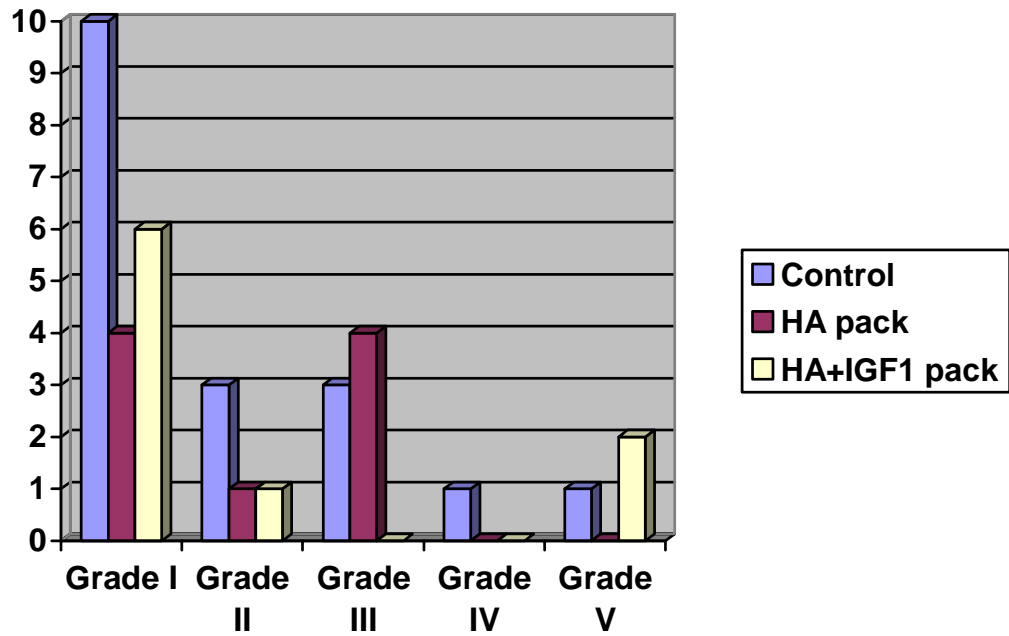
Table 6 Grade of reciliation for each site of injury

Sheep	Site	Pack Used	Grade of reciliation		
			Baseline	Week 08	Week16
1	left	HA+IGF1	I	V	V
1	right	none	II	II	I
2	left	none	II	III	V
2	right	HA+IGF1	V	III	I
3	left	HA+IGF1	V	IV	II
3	right	none	I	IV	III
4	left	HA	I	III	IV
4	right	none	II	I	IV
5	left	HA	I	V	IV
5	right	none	I	V	IV
6	left	HA	II	V	IV
6	right	none	V	V	IV
7	left	HA+IGF1	I	V	I
7	right	none	I	V	I
8	left	HA+IGF1	I	II	V
8	right	none	I	III	I
9	left	none	III	II	IV
9	right	HA	III	V	III
10	left	none	IV	V	III
10	right	HA+IGF1	I	II	I
11	left	HA	I	III	I
11	right	none	I	V	V
12	left	none	III	I	III
12	right	HA	III	II	IV
13	left	none	III	I	IV
13	right	HA	III	V	IV
14	left	none	I	III	I
14	right	HA	I	I	I
15	left	HA	III	V	II
15	right	none	I	III	I
16	left	none	I	V	III
16	right	HA+IGF1	I	V	I
17	left	none	I	III	I
17	right	HA+IGF1	I	II	II
18	left	none	I	III	III
18	right	HA+IGF1	II	I	I

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

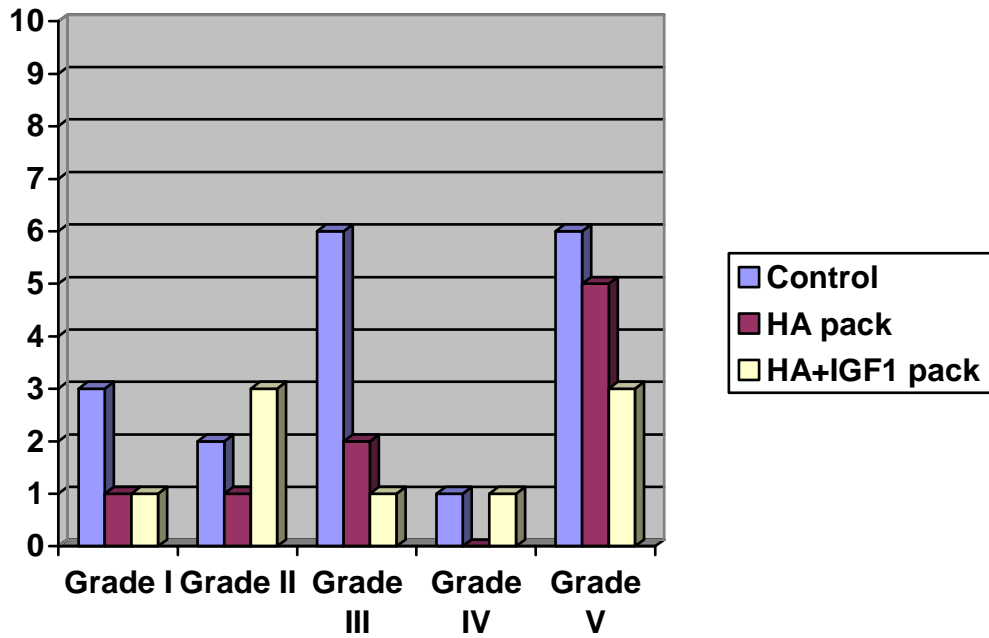
Figure 1 Electron Microscopy Grade by pack used at baseline



HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

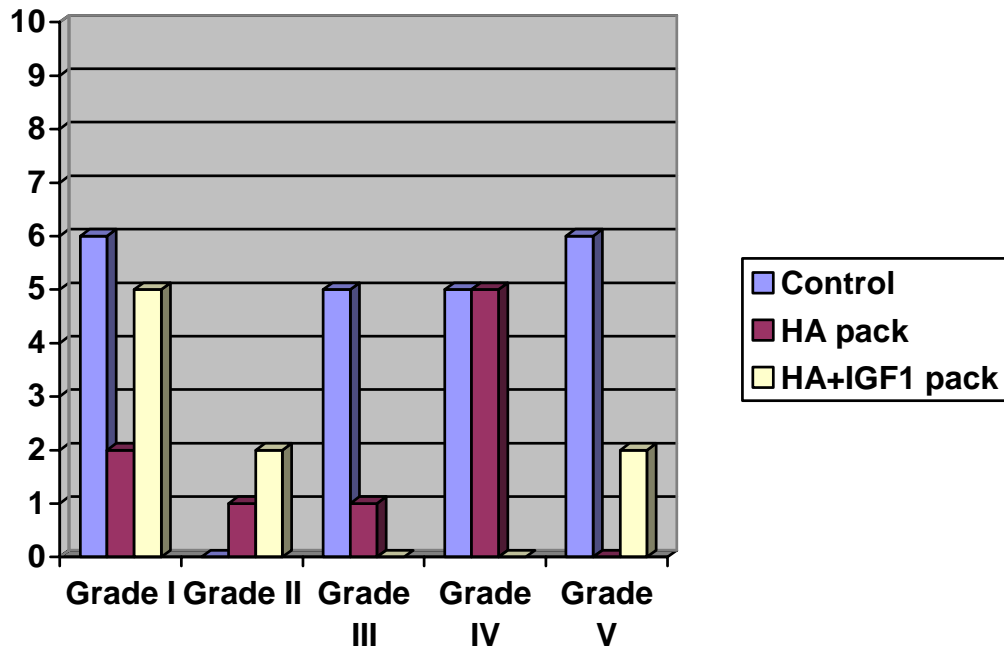
Figure 2 Electron Microscopy Grade by pack used at 8 weeks after injury



HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

Figure 3 Electron Microscopy Grade by pack used at 16 weeks after injury



HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

Table 7 Summary of Grade V specimens by pack used at different time points

Time	Control (no pack)	HA pack	HA+IGF1 pack	Total
Baseline	1	0	2	3
8 weeks after injury	6	5	3	14
16 weeks after injury	2	0	2	4

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

The conditions for the Pearson's χ^2 test were not met, and therefore data was grouped accordingly to enable analysis with Fisher's exact test. In order to form the 2x2 contingency tables for this statistical analysis, the absolute number of specimens graded I or II were combined (Grade A), as were those graded III or IV (Grade B). The *p* values for Fisher's exact test comparing different packs at the three time points are detailed in Table 8.

Table 8 *p*-values for Fisher's exact test comparing the grade of reciliation (Grade A versus Grade B)^ as determined by scanning electron microscopy at each time point

Time	Control versus HA pack	Control versus HA+IGF1 pack	HA pack versus HA+IGF1 pack
Baseline	0.382	0.283	0.088
8 weeks after injury	1.000	0.620	1.000
16 weeks after injury	1.000	0.007*	0.011*

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

^Grade I and II have been combined (Grade A), Grade III and IV have been combined (Grade B).

4.4 DISCUSSION

Electron microscopy has become an important modality for assessing the respiratory mucosa. Some researchers have analysed ciliary ultrastructure using transmission electron microscopy (Herzon, 1981; Benninger et al., 1991; Benninger et al., 1993; de Iongh and Rutland, 1995; Rayner et al., 1995; Keleş et al., 2001), whilst others have used scanning electron microscopy (Toskala et al., 1995; Joki et al., 1998; Shaw, 2001; McIntosh et al., 2002; Rajapaksa, 2004), and still others have used both modalities (Toskala et al., 1994; İnanlı et al., 2000; Toskala and Rautiainen, 2003). Transmission electron microscopy has primarily been used to assess the ultrastructure of individual cilia. Whilst this is an important avenue of investigation, abnormalities of individual cilia does not necessarily translate into poor functionality (Herzon, 1981; Maurizi et al., 1984; Benninger et al., 1993; Jorissen, 1998). Abnormalities affecting between 10% (Czaja and McCaffrey, 1998) and 15% (Jorissen, 1998) of the total number of cilia can still be associated with a normal ciliary beat frequency. Our primary goal was to assess the extent of regeneration and/or orientation of cilia in a three-dimensional manner, and as such, scanning electron microscopy was thought to be the best method to assess this (Toskala et al., 1994; Toskala et al., 1995; Joki et al., 1998).

One method to assess the degree of reciliation based on scanning electron microscopy images utilises computerised image capture and imaging analysis programs. As cilia are electron dense and therefore bright compared to the background tissue, reciliation has been calculated based on an adjustable grey scale that is matched to the degree of reciliation seen on the image providing a percentage

of surface area (Shaw, 2001; McIntosh et al., 2002; Rajapaksa, 2004). However this “shade” can be influenced by various parameters of the imaging protocol – for instance, the angle of the electron beam relative to the specimen, or the presence of crust and clot which can also appear electron dense – and this may confound results, particularly when the observer is not blinded. Although this technique is said to have little inter- and intra-observer variability (Shaw, 2001), it may be that observers are following the same inaccurate path to arrive at the same incorrect result. Of note, Shaw (2001) has reported a poor correlation between such an assessment of reciliation based on scanning electron microscopy images with that based on light microscopy, with a mean difference of 30.6% between the two assessment modalities. Rajapaksa (2004), who also used this method, reported that his biopsies had an epithelial covering of 65% and yet had a cilia covering of 87%. Rather than using a grey scale, Guo et al. (1997) circled areas that were ciliated on their images with software calculation of the total percentage of ciliated area for each specimen. This certainly appears to be a valid method, but remains labour-intensive.

In order to assess reciliation, we utilised a grading system based on the image obtained at 500x magnification (and higher degrees of magnification when required). The endpoint of mature cilia that were orientated was assigned to grade I. Cilia that appeared mature but were disorientated were designated grade II. As an isolated finding, disorientation of cilia is of doubtful significance and may even be due to processing of the specimen (Jorissen, 1998). However it has been noted in patients with rhinitis and sinusitis (Herzon, 1981; Rayner et al., 1995; Toskala and Rautiainen, 2003) as well as in patients suffering from immotile cilia syndrome

(Eliasson et al., 1977). When de Iongh and Rutland (1989) examined the degree of orientation at three different levels along the length of cilia, they found little variation. As such, measurements made anywhere along the cilium are valid, and by inference so too is assessment by scanning electron microscopy (Toskala et al., 1994; Toskala et al., 1995). Decreasing degrees of disorientation is thought to be a sign of recovery of mucosa (Rayner et al., 1995; Toskala and Rautiainen, 2003). Benninger et al. (1993) have previously noted short bleb-like epithelial surface structures in their electron microscopic examination of regenerating mucosa. Toskala et al. (1995) have also found microvilli and short cilia to be present in patients with chronic or recurrent sinus infections, and suggested that these were signs of ciliogenesis. McIntosh et al. (2002) noted that short or stubby cilia represented immature forms of cilia. Therefore, cilia that were in the process of maturing were deemed to be grade III, whereas the absence of cilia was assigned to grade IV. As compound cilia appear not to significantly contribute to a decline in functioning (Toskala et al., 1995; Torkkeli et al., 1997; Czaja and McCaffrey, 1998), this was not included in our grading system. An example of each grade is shown in figure 4 (Grade I), figure 5 (Grade II), figure 6 (Grade III), and figure 7 (Grade IV).

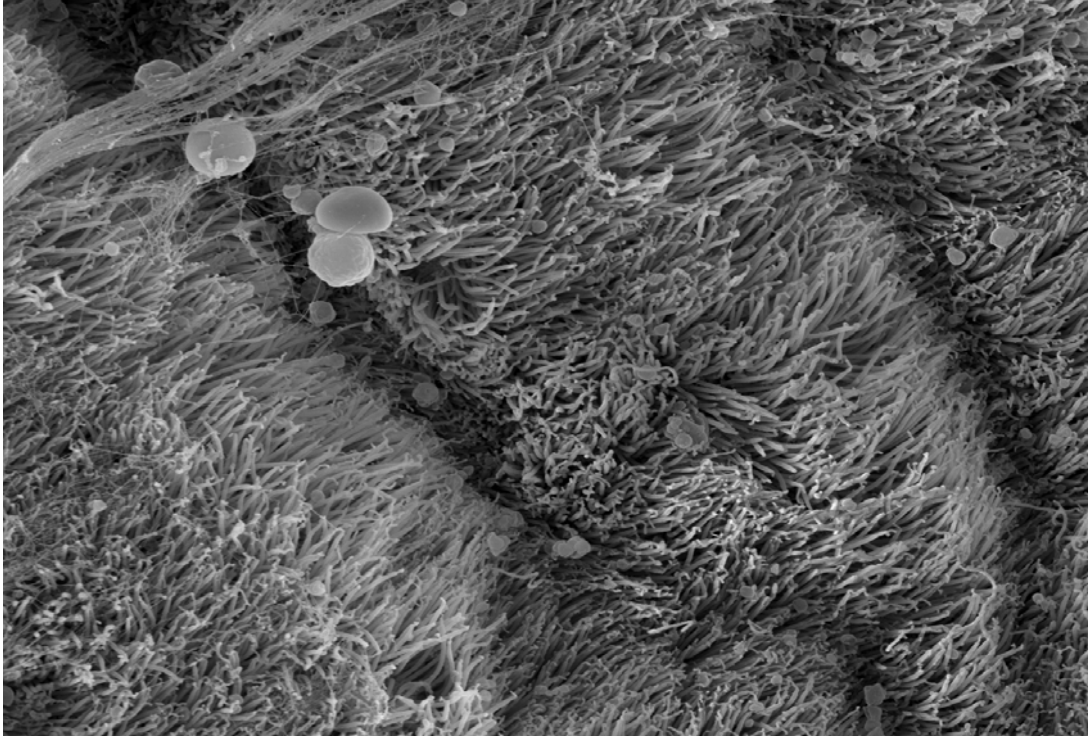


Figure 4 Grade of reciliation based on scanning electron microscopy appearance (2000x magnification) – Grade I: normal cilia with normal orientation

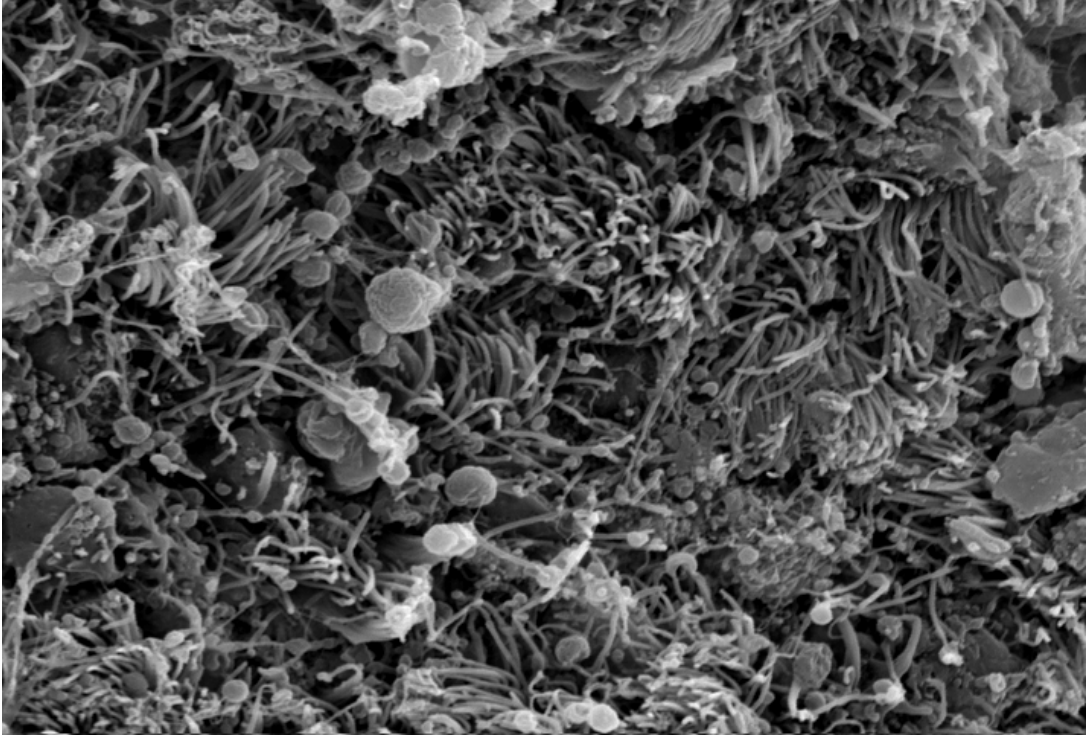
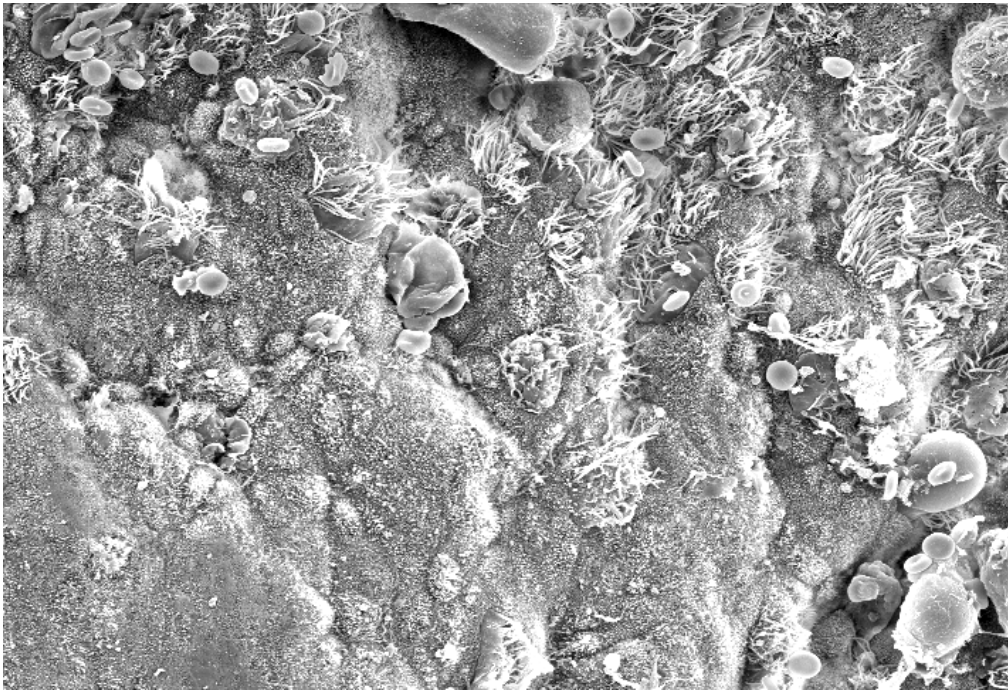


Figure 5 Grade of reciliation based on scanning electron microscopy appearance (2000x magnification) – Grade II: ciliated epithelium but disorientated

(a)



(b)

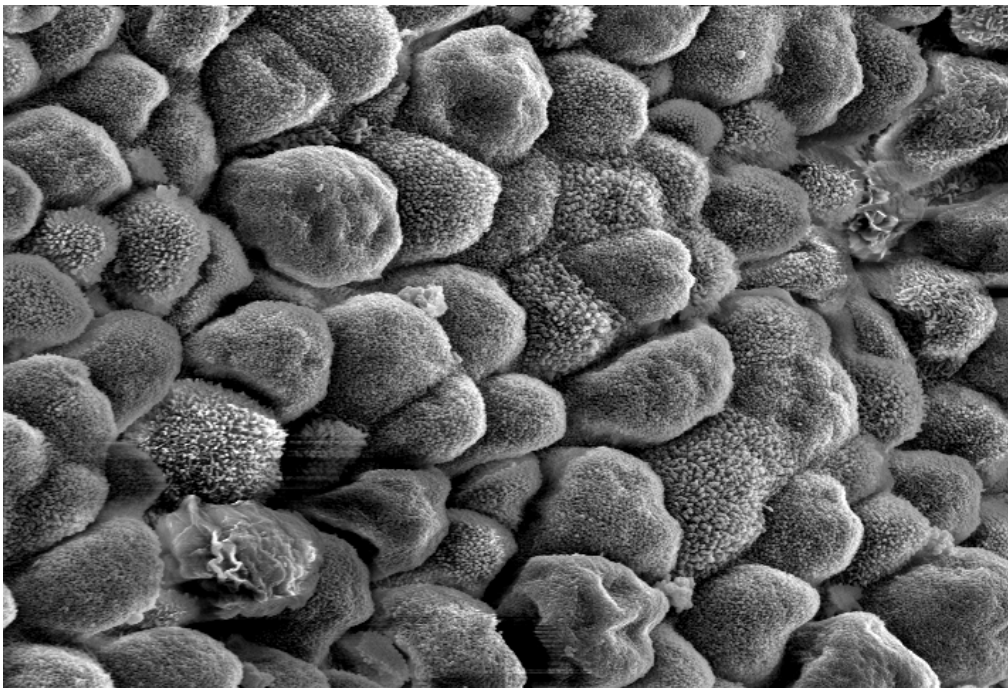


Figure 6 Grade of reciliation based on scanning electron microscopy appearance – Grade III: stumps of cilia, regenerating cilia

a) example at 2000x magnification

b) example at 5000x magnification

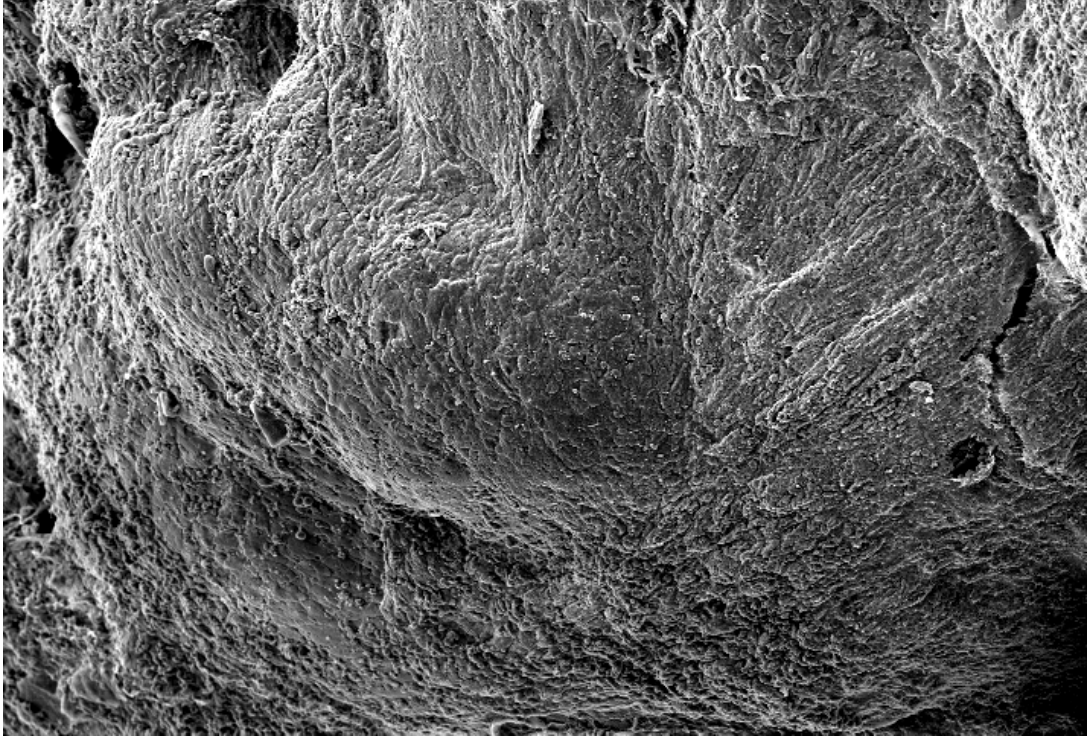


Figure 7 Grade of reciliation based on scanning electron microscopy appearance (500x magnification) – Grade IV: no identifiable cilia

Disappointingly, a substantial number of specimens (19.4%) were rendered unusable by crust or clot (Grade V) and these were deleted from further analysis. The technique of washing specimens using an ultrasonic cleaner has previously been used with satisfactory results (McIntosh, 2002; Rajapaksa, 2004). It is noted, however, that Toskala et al. (1995) and Shaw (2001) had similar difficulties with 15.1% and 44.4% of their samples respectively similarly affected. An example from our series is provided in figure 8.

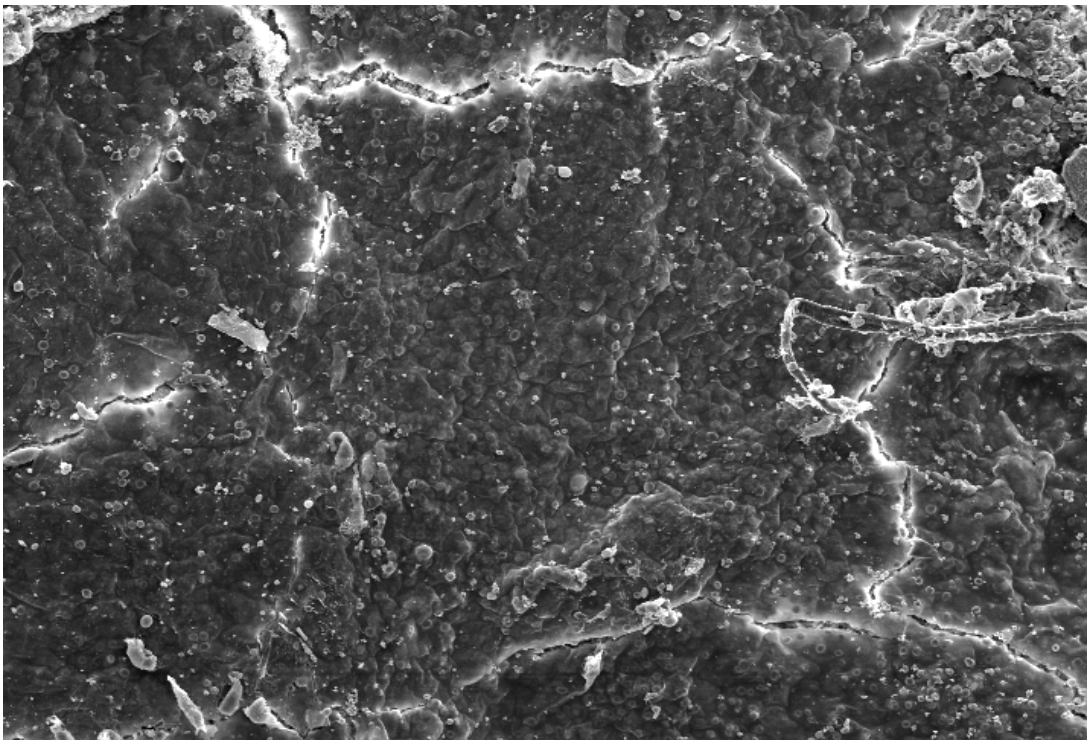


Figure 8 Grade of reciliation based on scanning electron microscopy appearance (500x magnification) – Grade V: unusable

Given the number of specimens excluded, the conditions for Pearson's chi-square analysis were not satisfied (as the expected count was less than 5 in > 20% of cells in tables drawn). The statistical solution to this dilemma is to combine categories until a 2x2 table may be drawn, and then proceed with Fisher's exact test analysis (Norušis, 1995). Obviously this results in difficulties drawing conclusions if the distinction between categories is lost. It was noted that the ciliary beat frequency [see Section 6.3 – Results] was similar in grades I and II (CBF of 8.2 Hz and 7.9 Hz respectively), and hence these were combined. Furthermore the CBF of grades III and IV were also similar (6.9 Hz and 6.6 Hz respectively) suggesting these could be combined. (Note that there is statistical support for this combination based on CBF [see Section 6.3 – Results]).

At baseline, 13 of 33 usable specimens (39.4%) had evidence of an abnormality as defined by grades II, III, or IV. Benninger et al. (1993) have previously found that chronic inflammation of the sinuses can result in loss of ciliated epithelium, and this obviously has implications for the functioning of that sinus.

There was no difference in reciliation at baseline or at eight weeks between the three groups. However, there was a significantly better grade of reciliation in the HA+IGF1 group compared to either the control group or the HA alone group at sixteen weeks. Rajapaksa (2004) previously reported that the use of a HA pack with or without IGF-1 lead to worse reciliation at 16 weeks after full thickness injury in a diseased animal model. However, there was an inflammatory focus present in his sheep throughout his study which may have impacted his results. McIntosh et al. (2002) found no difference in reciliation when comparing presence and absence of

HA packing in a healthy sheep model. The reciliation grade of our HA alone group was also not significantly better than that of the control group, suggesting that the presence of insulin-like growth factor 1 at the early stages of healing may result in improved reciliation in the later stages of healing. Hansson et al. (1991) have demonstrated that regenerating nasal mucosa cells have IGF-1 immunoreactivity at early stages of differentiation, which disappears in later stages of maturation. Therefore, rather than IGF-1 having a direct influence on the regeneration of cilia, it is more likely that the foundation of improved reepithelialization seen at eight weeks with IGF-1 translates into improved reciliation at sixteen weeks. This is supported by the work of Shaw (2001) who found that partial thickness injuries to respiratory mucosa had far superior epithelial regeneration compared to full thickness injuries, and as a consequence also had significantly improved ciliary regeneration. A healthy foundation established early may lead to improved regeneration of structures built on this platform.

It is important to consistently sample the same area, as there is some variability in the degree of ciliation of different regions within the same sinus (Guo et al., 1997). We were obviously confined to a small area where we had previously created a full thickness injury, and hence the potential impact of variability based on anatomical site was negligible.

4.5 CONCLUSION

There was no difference in reciliation at baseline or at eight weeks between the three groups. However, there was a significantly better grade of reciliation in the HA+IGF1 group compared to either the control group or the HA alone group at sixteen weeks. This suggests that the introduction of insulin-like growth factor 1 at the early stages of healing may promote reciliation in later stages. However, a more likely explanation may be that the improved reepithelialization seen at eight weeks translates into improved reciliation at sixteen weeks. The utilization of IGF-1 in this setting deserves further investigation.

CHAPTER 5

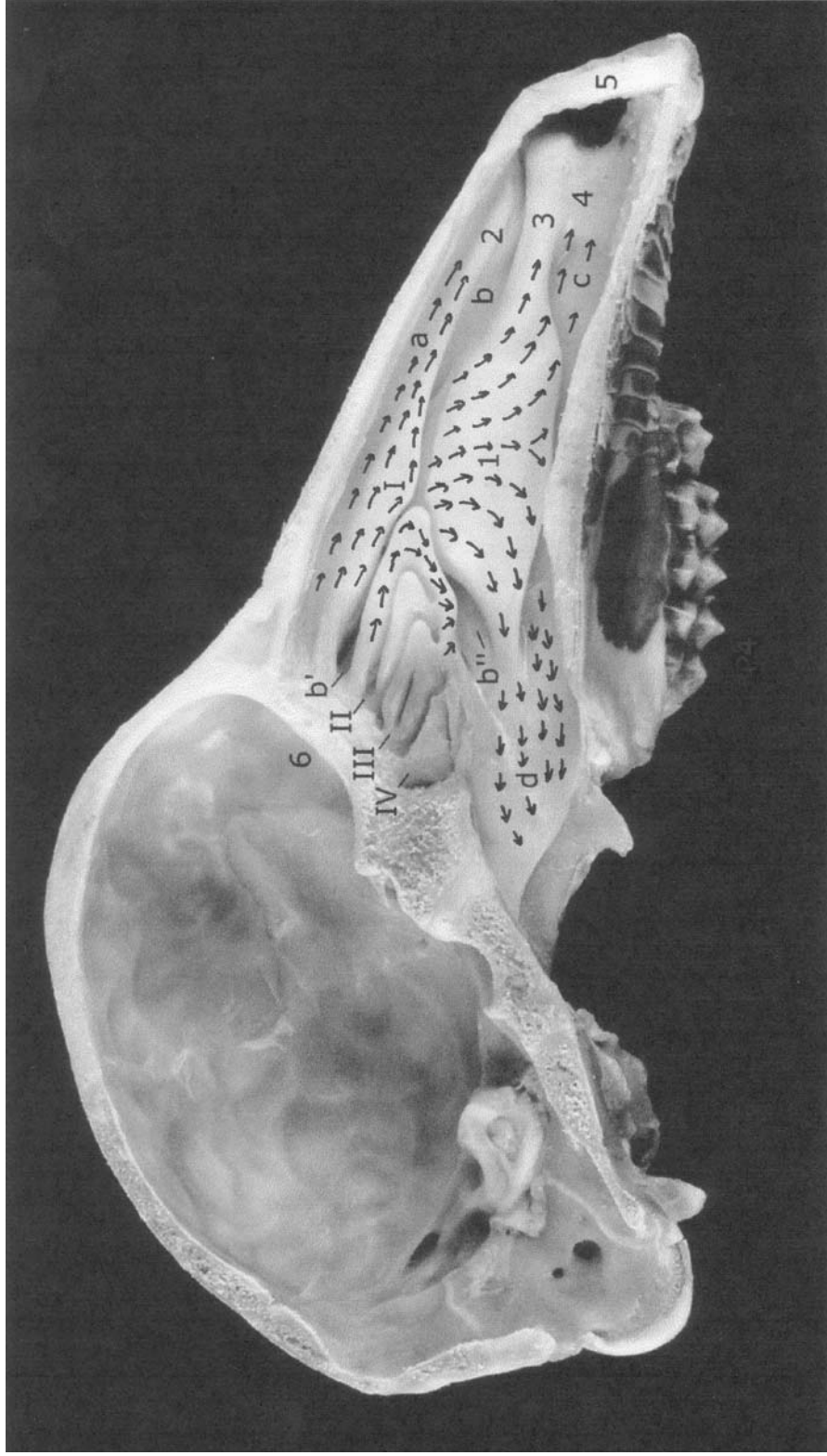
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5.1 INTRODUCTION

Mucociliary clearance (MCC) is one of the most important functions of the sinonasal complex. Clearly, the assessment of any new intervention involving this region of the body requires not only an assessment of the structural recovery following the intervention, but also an assessment of the impact of the intervention on functionality. Although electron microscopy provides information regarding ciliary ultrastructural alterations, this information is only of value when coupled with an assessment of MCC (Maurizi et al., 1984; de Jongh and Rutland, 1995).

There are a number of means by which MCC may be assessed. In man, the method of placing saccharin within the anterior nasal cavity and then observing the time it takes for the saccharin to be tasted provides an objective measure of overall MCC (Talbot et al., 1997; Lale et al., 1998; Keojampa et al., 2004). However, it relies on the subject reporting when they first experience the sweet taste of saccharin, and obviously is not possible to utilize in an animal model. Passali et al. (1984) have reported that an insoluble substance such as charcoal powder is more accurate and consistent is the assessment of MCC based on the time taken for it to appear in the nasopharynx. This technique has been used previously in the sheep model (Shaw, 2001). In man, the direction of mucous flow is towards the nasopharynx without any clearance towards the anterior nares. However, within the sheep nasal cavity, Lucas and Douglas (1934) found that cilia beat towards one of two different directions depending on their location (Figure 9). A diagonal line extending anteriorly from the superoposterior angle toward the inferior margin may be drawn – anterior to this, cilia beat anteriorly; posterior to this line, cilia beat posteriorly.

Figure 9 Direction of beating of cilia of the lateral nasal wall of the sheep (as determined by Lucas and Douglas, 1934)



I Dorsal turbinate; II/III/IV Ethmoturbinates; 1 Ventral turbinate; 2 Plica recta; 3 Plica alaris; 4 Plica basalis; 5 Septum nasi (part of); 6 Base of skull; a Dorsal nasal meatus; b/b'' Middle nasal meatus; c Ventral nasal meatus; d Nasopharynx
(Adapted from Menke, 2003)

These same authors also found that regions of greatest velocity were superior, whereas weak ciliary movement was detected inferiorly (particularly on the nasal floor). Given the variability on the direction and speed of cilia within the sheep nasal cavity, there is some doubt as to the validity of utilising an insoluble substance as a marker for MCC in the sheep model. The same may be said for any other insoluble dyes used.

The use of radioactive particles and radio-opaque particles within the nasal cavity have also been described (Lale et al., 1998). However, these methods present difficulties with cost, safety, and the need for specialized equipment, training and/or personnel (Passali et al., 1984), as well as possibly being confounded by the multidirectional nature of sheep nasal mucosa.

An examination of the ciliary beat frequency (CBF) was first performed in 1844 using a stroboscope (Lale et al., 1998). The CBF represents an important component of the MCC (Joki et al., 1998). Ciliated cells are relatively easy to harvest and their beating frequency is relatively easy to determine. Lund et al. (1991) used CBF to measure mucociliary function in patients in preference to the saccharin clearance test as they felt that CBF is a more accurate measure and allowed small changes in function to be determined. Therefore, this was the method chosen to assess the impact of our interventions on the function of the sinonasal complex.

5.2 METHODS

Approval was granted for this study by the Animal Ethics Committees of the University of Adelaide, Adelaide, South Australia, and the Queen Elizabeth Hospital, Woodville, South Australia. Once again, the same eighteen sheep (untreated for the nasal bot *Oestrus ovi*) were utilised for this study. Infestation by the nasal bot was confirmed during the study period through endonasal examination of each sheep.

All sheep had undergone endoscopic ventral turbinectomy under GA four weeks prior to the first sampling. Full thickness lateral nasal wall mucosal stripping was then performed under GA as before. Brushings were performed for collection of ciliated cells for baseline analysis of ciliary beat frequency at a site distant from biopsy. Collection was performed using a Cytobrush[®] Plus Cell Collector (Medscand Medical, Sweden) without local anaesthetic. The four sites of full thickness injury were accurately measured and recorded for guiding subsequent ciliary sampling. Mucosal injury sites were then left unpacked or packed with HA with and without IGF-1 as described previously. Subsequent brushings were then performed every four weeks for a period of sixteen weeks. These were performed under mild to moderate sedation, and animals were sedated using an intramuscular injection of 4mg of xylazine. Brushings were performed prior to administration of any local anaesthetic/decongestant spray in order to reduce confounding factors. Sites of brushings were carefully ordered and recorded during the sixteen week period in an attempt to sample untouched areas. Following the final biopsy,

euthanasia was performed by intravenous injection of sodium pentobarbitone (>100mg/kg).

Cells from brushings were suspended in 1mL of Dulbecco's culture medium (Modification of Earle's Medium without L-Glutamine with 4.5g/L Dextrose. ICN Biomedicals Inc, Aurora, OH) and agitated to release cells into the culture medium. This was kept at 36.5°C until analysis of the CBF was performed.

One droplet from each specimen was placed on a microscope slide warmed to 36.5°C, and phase contrast microscopy was used as previously described (Rutland and Cole, 1980). Briefly, moving cilia were identified and positioned in the centre of the viewing area of the microscope. The disruption of light shone perpendicular to the slide was detected by a photodiode. Analysis of the resulting voltage change and fast Fourier transformation to a frequency spectrum was performed using an MP100 data acquisition system and AcqKnowledge software, version 3.7.0 (Biopac Systems Inc, Santa Barbara, CA). Ten cells per specimen were individually analysed and the final CBF of the specimen was deemed to be the average of these cells.

Results were pooled according to whether specimens were from controls (no packing), were from sheep packed with HA, or were from sheep packed with HA+IGF1. Differences between the CBF of these groups were examined at each time point. Student's *t* test was used for statistical evaluation with $p < 0.05$ accepted as statistically significant. Statistical analysis was performed using the Statistical

Package for the Social Sciences version 9.0 software system (SPSS, Inc., Chicago, IL).

5.3 RESULTS

The CBF for each site of injury is documented in Table 9. There are a number of missing data points which was related to the learning curve and methodological issues (see Discussion). The baseline CBF was 8.9 Hz. The CBF of each study group at different time points is summarised in Table 10.

Analysis of the difference in ciliary beat frequency by Student's paired *t*-test at each time point is summarized in Table 11. The only statistically significant finding was between the HA group and the HA + IGF-1 group at 8 weeks after injury ($p < 0.05$).

Table 9 Ciliary beat frequency for each site of injury

Sheep	Site	Pack Used	CBF (in Hertz)				
			Baseline	Week 04	Week 08	Week 12	Week16
1	left	HA+IGF1		11.5	5.3	10.8	9.6
1	right	none		13.5	5.5	5.0	
2	left	none	11.1	7.3	3.1	9.9	5.9
2	right	HA+IGF1		7.2	5.2	8.8	6.7
3	left	HA+IGF1		8.2	5.1	5.4	4.3
3	right	none	7.7			6.2	4.1
4	left	HA		9.2	10.3		5.4
4	right	none		8.4	10.3		4.5
5	left	HA			10.2	4.3	
5	right	none			7.8	5.1	
6	left	HA	8.6		10.9		9.9
6	right	none	8.3		13.0	5.0	5.5
7	left	HA+IGF1		7.3	4.8		4.4
7	right	none		8.3		10.3	
8	left	HA+IGF1		6.4	9.8		
8	right	none		10.2	11.2		
9	left	none	5.9		8.1	6.2	
9	right	HA			8.1	4.4	7.8
10	left	none			14.2		
10	right	HA+IGF1			10.8		
11	left	HA			6.6		
11	right	none			6.9		
12	left	none			8.8		
12	right	HA			8.1		
13	left	none			14.2		7.8
13	right	HA		6.7	10.8		7.9
14	left	none		7.7	6.6		
14	right	HA			6.9		7.3
15	left	HA			8.8	7.8	4.4
15	right	none		10.0	8.1		
16	left	none		11.5	3.5		
16	right	HA+IGF1		12.7	6.6		
17	left	none		5.5		4.1	
17	right	HA+IGF1	11.5	13.2			
18	left	none		12.7	7.1		
18	right	HA+IGF1		11.6	7.6	3.3	4.4

HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

Table 10 Ciliary beat frequency (\pm standard deviation) in Hertz by time and pack used

Time	Control (no pack)	HA pack	HA + IGF-1 pack
Baseline	8.9 (\pm 2.1)		
4 weeks after injury	9.5 (\pm 2.5)	8.0 (\pm 1.8)	9.8 (\pm 2.8)
8 weeks after injury	8.1 (\pm 3.6)	9.8 (\pm 1.6)	6.1 (\pm 1.3)
12 weeks after injury	6.8 (\pm 2.3)	5.5 (\pm 2.0)	7.1 (\pm 3.4)
16 weeks after injury	5.6 (\pm 1.4)	7.1 (\pm 2.0)	5.9 (\pm 2.3)

HA – hyaluronic acid

HA + IGF-1 – hyaluronic acid impregnated with insulin-like growth factor 1

Table 11 Two-tailed p -values for Student's paired t -test analysing differences in ciliary beat frequency between packs at different time points

Time	Control and HA pack	Control and HA + IGF-1 pack	HA pack and HA + IGF-1 pack
4 weeks after injury	0.412	0.884	0.364
8 weeks after injury	0.248	0.137	0.002*
12 weeks after injury	0.540	0.786	0.402
16 weeks after injury	0.155	0.815	0.301

HA – hyaluronic acid

HA + IGF-1 – hyaluronic acid impregnated with insulin-like growth factor 1

* $p < 0.05$

5.4 DISCUSSION

Mucociliary clearance is an important defence function of the respiratory tract (Passali et al., 1984; Rayner et al., 1995; Joki et al., 1998). There are a number of factors that can influence the MCC, including

- (i) ciliary factors – the number of vibrations per unit time, the amplitude of vibration, the length of cilia, the ratio of ciliated to nonciliated area, and the susceptibility of cilia to intrinsic and extrinsic agents which modify the rate and degree of movement; and
- (ii) mucous factors – thickness of the mucous layer, its original viscosity when secreted, and its viscosity after changes due to the passage of air and the addition of serous fluids (Lucas and Douglas, 1934).

Friedman and Toriumi (1989) found that a full thickness mucosa injury can continue to adversely affect mucociliary clearance some 12 to 24 weeks after it was created. Benninger et al. (1989) found that although ciliated respiratory mucosa can regenerate after a significant surgical insult, the mucociliary clearance can remain poor. Abdel-Hak et al. (1998) found that following endoscopic sinus surgery, the CBF did not reach normal values until six months postoperatively, despite endoscopic examination revealing normal nasal mucosa at around three months. This highlights the need to assess the function of mucosa that has been injured rather than merely accepting that normal endoscopic appearance or histological repair of mucosa is coupled with a return to function.

The ciliary beat frequency is an important component of the mucociliary clearance (Joki et al., 1998) and we chose this method to objectively assess the function of the sinonasal mucosa in our study as other methods are less reliable in the sheep model. Duchateau et al. (1985) found that there is a linear correlation between the logarithm of mucociliary transport and the CBF. It should be noted, however, that this finding has not been replicated by any study since although it seems likely that a correlation does exist (Jorissen, 1998).

Disappointingly, a number of samples could not be used due to inadequate sampling of ciliated cells. In the early experimental period, this was due to the learning curve of correct technique in sampling. A number of brushes were trialled, and it was found that the largest brush was the only one suitable for adequate sampling. However as it had to be passed through the narrow nares of the sheep, there may have been loss of cells upon exiting the narrow nasal cavity. Another difficulty that arose from the passage of the brush through this narrow portal is the contamination by bacteria that was found in a number of samples. These bacteria flourished in the culture medium, and although respiratory epithelial cells were visible, they were paralysed by the presence of these bacteria. This can be avoided in future by using culture media with added antibiotics. Finally, although every attempt was made to carefully brush areas distant from previous biopsies and distant from future biopsies, the size of the brush made this difficult. Movement from the sedated, non-paralysed sheep compounded this problem.

Despite these methodological problems, a number of samples were obtained for analysis. Given the small number of valid baseline samples, these were combined

from all groups to provide a meaningful CBF (of 8.9 Hz). The CBF found by Rajapaksa (2004) in an identical diseased animal model was slightly lower at 7.1 Hz. Scarcity of cilia was obviously an issue at week four, being relatively early in the healing process. However, as noted in Figures 1 to 3 (Section 4.3 – Results), ciliated epithelium was noted in at least some of the animals within each group.

There was a non-significant trend for decreasing CBF over time in the control group. Although there were some oscillations in the other two groups, they also appeared to have a trend for a decreasing CBF over time. It could be argued that with the passage of time the CBF should improve as the mucosa recovers, and not deteriorate as our data suggests. There are a number of factors that may have impacted our findings. Given the number of samples from which we were not able to derive a CBF, the mean CBFs within each group at each time point was widely distributed as reflected in relatively large standard deviations. Therefore the trends observed may not be a true reflection of the CBF in our experimental setting.

Secondly, in our samples where bacteria had flourished it was noted that all ciliated cells were stationary. It may be that in some of our samples where bacterial growth was in its early stages, there may have been a cilioinhibitory effect as opposed to the ciliostatic effect seen with advanced bacterial involvement. The net result of this would be to lower the CBF observed. Finally, ongoing inflammation or infection may have affected our findings. At no point after treatment with Closal[®] were any nasal bots seen which would have indicated that our original inflammatory stimulus had returned. It has already been noted that full thickness mucosal injury is associated with poor mucociliary clearance that can continue for many months (Friedman and Toriumi, 1989; Benninger et al., 1989; Abdel-Hak et al., 1998). The

resulting stasis of secretions may be a medium for secondary infection which can lead to further adverse effects on mucociliary clearance, continuing the cycle that may be associated with a decreased CBF. Yet it should be noted that we did not note any gross features of inflammation and/or infection in our sheep following mucosal injury.

From our results it appears that our introduction of HA or HA+IGF1 had no influence on the CBF compared to our control group at any time point following full thickness injury. It was noted that the CBF with the HA+IGF1 group was statistically significantly worse than the HA group at week 8. The reason for this is unclear. However, it is difficult to comment further given that so many data points were lost due to methodological difficulties, with wide distributions of the remaining data. Interestingly, Rajapaksa (2004) reported similar findings. He found that the CBF was worse with HA+IGF1 compared to controls, and a non-significant trend of worse CBF in the HA+IGF1 group compared to the HA alone group, at sixteen weeks post-injury. It should be noted that in his study, there was ongoing inflammation throughout the study period and hence the presence of IGF-1 in an inflammatory setting may augment the injury sustained by the mucosa. Rajapaksa (2004) argues that this emphasises the need for removal of all diseased tissue in ESS as this may otherwise form an inflammatory focus. This occurred in our study by the treatment of *Oestrus ovi* following full thickness injury.

Benninger et al. (1991) found that the motility of cilia that regenerated following full thickness injury was no different from the motility of cilia of control animals, even though there were decreased numbers of cilia and increased abnormalities of

cilia in the former group. It may be that the CBF of any of the cilia that are present following surgery, whether few or many, is not substantially altered by the extent of injury. Nevertheless, MCC warrants further investigation because although the CBF may essentially be normal, decreased numbers of cilia can have a negative impact on the overall function (Toskala et al, 1995b). Therefore although there were essentially no differences in CBF between our three groups at each time point (apart from the spurious finding between HA and HA+IGF1 groups at eight weeks), MCC may still be affected by the gross number of cilia and deserves further assessment. However, MCC in the sheep model is difficult for reasons elucidated above.

Disease states involving the sinuses have been found to lead to a decreased CBF (Ohashi and Nakai, 1983a; Scadding et al, 1995). Lund et al. (1991) and Abdel-Hak et al. (1998) have found that ESS improves the CBF in patients with CRS. However, this was not replicated by Hafner et al. (1997) who found that whilst overall MCC improved following ESS, this was not due to any improvement in CBF. It is hoped that ongoing investigation into the various components of MCC will elucidate how best to optimise functioning of this defence mechanism. From our findings it appears that HA packing, with or without IGF-1, has no role to play in improving the CBF of patients having undergone ESS.

5.5 CONCLUSION

The ciliary beat frequency at eight weeks was significantly worse with the HA+IGF1 group compared to the HA alone group. There was no significant difference compared to controls, and there were no other significant differences found between any comparisons at any other time points. In all likelihood, this isolated statistically significant finding is likely to represent a spurious finding of no clinical significance. Given the methodological problems encountered, the investigation of CBF following endoscopic sinus surgery in our sheep model represents a pilot study and provides constructive guidelines for future studies.

CHAPTER 6

**AN INVESTIGATION INTO THE
CORRELATION OF MEASURES OF
CILIARY ULTRASTRUCTURE AND
CILIARY BEAT FREQUENCY IN AN
ANIMAL MODEL OF CHRONIC
RHINOSINUSITIS**

6.1 INTRODUCTION

The rate of mucociliary transport depends on a number of interdependent factors, including ciliary beat frequency (CBF), density of ciliary population, length of cilia, ciliary ultrastructure, periciliary fluid, and mucous quality (Lale et al., 1998; Joki et al., 1998).

Joki et al. (1998) and Jorissen (1998) have previously demonstrated that a correlation exists between ciliary activity and ciliary ultrastructure in patients with sinus disease. This is agreement with Maurizi et al. (1984) who have stated that “ciliary motility is directly related to the normality of the structures that make up the cilium.” Czaja and McCaffrey (1998) found that as the number of ultrastructural abnormalities of cilia increased, the CBF decreased in their rabbit model of chronic sinusitis. These changes (ultrastructural and functional) reversed to normality six weeks after middle meatal antrostomy.

Whilst we did not specifically examine the individual structures that make up the cilium, it is possible that ciliary motility may also be related to the overall degree of maturation of cilia as they regenerate following injury. Our aim was to assess if improved reciliation was associated with increased CBF.

6.2 METHODS

Results were taken from the previous two chapters. Our assessment of reciliation was based on a grading scale of Grade I – normal orientated cilia, Grade II – disorientated cilia, Grade III – stumps of regenerating cilia, Grade IV – no identifiable cilia (Table 5, Section 4.2 – Methods). The potential for a correlation between the grade of reciliation based on scanning electron microscopy grade (Section 4.3 – Results) and CBF (Section 5.3 – Results) was examined. As we were interested in the degree of reciliation and its possible correlation to ciliary function, rather than the temporal recovery after injury, the timing of biopsy specimens was noted but not assessed any further. Specimens that were assessed as Grade V according to the scanning electron microscopy reciliation grading system (that is, degree of ciliation not able to be assessed due to overlying clot or mucous) were not included in the statistical analysis for reasons discussed previously.

Spearman's *rho* coefficient was used to assess overall correlation. Student's *t*-test was used to examine differences between the CBF of individual grades of reciliation. Once again, $p < 0.05$ was deemed to be statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 9.0 software system (SPSS, Inc., Chicago, IL).

6.3 RESULTS

The grade of reciliation and CBFs (where available) are listed in Table 12. The mean CBF for each grade of reciliation is detailed in Table 13. As their CBFs were noted to be similar, Grades I and II were combined to form Grade A, and Grades III and IV were also combined to form Grade B. The combined CBFs were calculated. There was a trend for decreasing CBF with increasing grade as shown on Figure 10.

Spearman's rho correlation coefficient was -0.211 , with a p value of 0.203 , indicating no statistically significant correlation between the CBF and the reciliation grade.

Table 12 Grade of reciliation and ciliary beat frequency (in Hertz) by sheep and site/pack used

Sheep	Site	Pack Used	Baseline		Week 08		Week 16	
			Grade	CBF	Grade	CBF	Grade	CBF
1	left	HA+IGF1	I		V	5.3	V	9.6
1	right	none	II		II	5.5	I	
2	left	none	II	11.1	III	3.1	V	5.9
2	right	HA+IGF1	V		III	5.2	I	6.7
3	left	HA+IGF1	V		IV	5.1	II	4.3
3	right	none	I	7.7	IV		III	4.1
4	left	HA	I		III	10.3	IV	5.4
4	right	none	II		I	10.3	IV	4.5
5	left	HA	I		V	10.2	IV	
5	right	none	I		V	7.8	IV	
6	left	HA	II	8.6	V	10.9	IV	9.9
6	right	none	V	8.3	V	13.0	IV	5.5
7	left	HA+IGF1	I		V	4.8	I	4.4
7	right	none	I		V		I	
8	left	HA+IGF1	I		II	9.8	V	
8	right	none	I		III	11.2	I	
9	left	none	III	5.9	II	8.1	IV	
9	right	HA	III		V	8.1	III	7.8
10	left	none	IV		V	14.2	III	
10	right	HA+IGF1	I		II	10.8	I	
11	left	HA	I		III	6.6	I	
11	right	none	I		V	6.9	V	
12	left	none	III		I	8.8	III	
12	right	HA	III		II	8.1	IV	
13	left	none	III		I	14.2	IV	7.8
13	right	HA	III		V	10.8	IV	7.9
14	left	none	I		III	6.6	I	
14	right	HA	I		I	6.9	I	7.3
15	left	HA	III		V	8.8	II	4.4
15	right	none	I		III	8.1	I	
16	left	none	I		V	3.5	III	
16	right	HA+IGF1	I		V	6.6	I	
17	left	none	I		III		I	
17	right	HA+IGF1	I	11.5	II		II	
18	left	none	I		III	7.1	III	
18	right	HA+IGF1	II		I	7.6	I	4.4

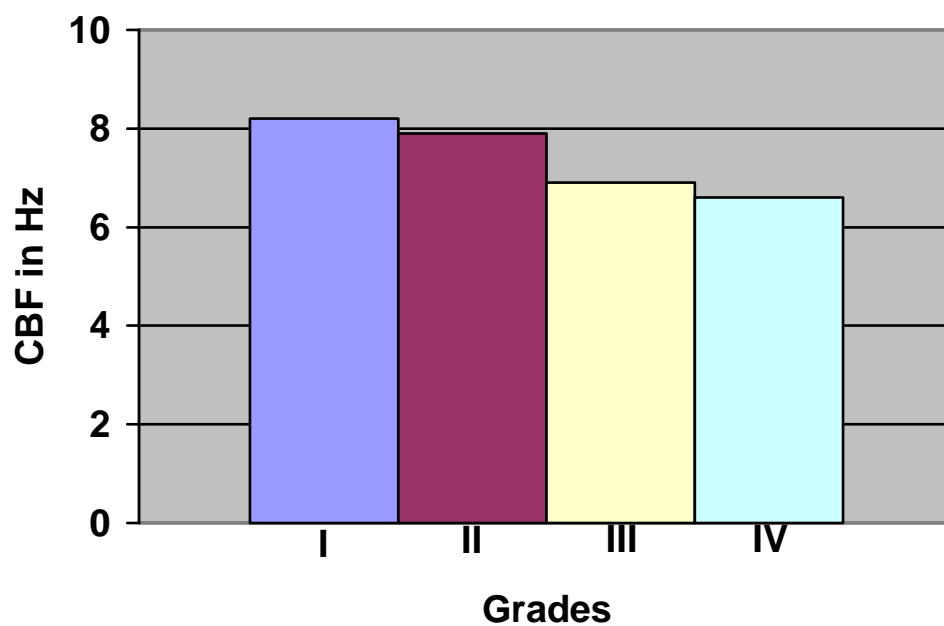
HA – hyaluronic acid

HA+IGF1 – hyaluronic acid impregnated with insulin-like growth factor 1

Table 13 Mean CBF \pm standard deviation (in Hertz) by grade of reciliation

Grade	Number of specimens	CBF
I	11	8.2 \pm 2.9
II	9	7.9 \pm 2.6
III	11	6.9 \pm 2.4
IV	7	6.6 \pm 2.0
V	16	8.4 \pm 2.9
I and II (Grade A)	20	8.0 \pm 2.7
III and IV (Grade B)	18	6.8 \pm 2.2

Figure 10 Ciliary Beat Frequency (in Hertz) for Grades I, II, III, and IV



The probability of a statistically significant difference between the individual grades is detailed in Table 14. Of note, the difference in the CBF of grades II and IV reached statistical significance ($p = 0.030$). There were no significant differences between any other combination of individual grades. The p -value for the difference between Grade A and Grade B is 0.034, indicating that although there is no difference between the values within each pooled grade, there is a statistically significant difference between the two pooled groups.

Table 14 Two-tailed p -values for Student's paired t -test analysing differences in CBF between different reciliation grades

Grade	II	III	IV
I	0.497	0.268	0.092
II		0.559	0.030*
III			0.700

* $p < 0.05$

6.4 DISCUSSION

No correlation was found between our measure of ultrastructural regeneration based on scanning electron microscopy and function of cilia based on CBF. However, a clear trend was noted, with CBF increasing as the grade of reciliation improved. Given the number of grade V specimens that were not able to be included in analysis, coupled with the number of specimens from which we were unable to derive a CBF, it may be that a correlation does exist but was hidden by an inadequate sample size due to methodological problems (type 2 error).

The possibility of a correlation between ultrastructural abnormalities of cilia and ciliary activity in patients suffering from inflammation of the sinuses has been examined previously. Ohashi and Nakai (1983b) found that the more severe the ultrastructural changes were in chronic sinusitis, the more the CBF was reduced. Jorissen (1998) found that there was a correlation (in the form of a logistic sigmoid curve) between CBF and ultrastructural abnormalities of cilia in patients with upper airway infections or chronic rhinosinusitis. He found that a proportion of ultrastructural abnormalities less than 5% does not interfere with ciliary function and is therefore essentially normal. Above 15%, the CBF decreases rapidly although normal values do remain possible. Joki et al (1998) found that in patients with sinusitis, decreased numbers of ciliated cells, disorientated cilia, significant epithelial metaplasia, and absence of secretions were all factors that were associated with decreased ciliary activity. Furthermore they found that there was a correlation between increased numbers of cilia, orientation of cilia, and CBF. These findings have also been replicated in a rabbit model of sinusitis (Czaja and McCaffrey,

1998). In keeping with these findings, in our animal model of CRS, the CBF was noted to be best with normal epithelium and worst in the setting of absent cilia. Notably, the difference between the CBF of grade A and grade B was statistically significant which supports our amalgamation of grade I with grade II and grade III with grade IV when assessing the degree of reciliation following injury (Section 4.3 – Results).

In our animal model the CBF of specimens where the epithelium was normal was 8.2 Hz whereas it was 7.9 Hz in biopsies where there was evidence of disorientation. This was not statistically significantly different. When disorientation of cilia has been specifically examined in patients with sinusitis, decreased mucociliary functioning has been noted. This has been based on mucociliary transport rate or saccharin clearance time rather than CBF (Toskala et al., 1995b; Rayner et al, 1995). This suggests that even in cases where active cilia are noted, the overall function can be poor due to disorientation of those cilia (Joki et al., 1998).

It is thought that ciliary disorientation may cause stagnation as the opposing strokes of multiple cilia negate one another, decreasing both CBF and mucociliary transport efficiency (Czaja and McCaffrey, 1998). It may be that our processing of specimens for scanning electron microscopy altered the surface of some specimens that had normal orientation rendering them disorientated, as some have previously suggested (Jorissen, 1998). Whilst others have used gentler methods of washing samples than sonication (Joki et al., 1998), we felt that this method was required in order to “uncover” specimens for analysis. Assuming that the CBF of disorientated cilia is lower than the CBF of orientated cilia, the net effect of this processing fault would

be to increase the number of samples graded as II and as well as increasing the mean CBF of that group. This would also abolish any statistically significant difference in the CBF between grades I and II that may exist.

It would seem counterintuitive to measure the CBF of specimens that were found to be grade IV (no identifiable cilia), or even those labeled grade III where presumably stumps of cilia would have no effective beating to be measured. Clearly the CBF of these specimens is derived from the occasional mature cilium within the injured region, or more likely from mature cilia in the mucosa adjacent to the denuded/regenerating area. The poor MCC that would result from such mucosa (Toskala et al., 1995b) leads to stasis and thickening of secretions, resulting in changes in local pH, changes in mucosal gas metabolism, increased inflammation, and tissue oedema (Kennedy et al., 1995). This would be reflected in a decrease in function of any mature cilia that are present in this environment, and hence lead to a decreased CBF. As such, it is not surprising to note an almost identical CBF when comparing grades III and IV, and there may be an argument for combining these grades particularly given the difference found between grade A (grades I + II) and grade B (grade III + IV). However, given the clear upward trend in CBF noted with improvements in the reciliation grade, the statistically significant difference between grade II and IV, and the trend toward statistical significance between grades I and IV, it would be worthwhile to further investigate the correlation of function (as measured by CBF) with ciliary recovery using our reciliation grading system. An alternative is to use transmission electron microscopy to calculate absolute numbers of ultrastructural abnormalities and correlate this with the CBF as performed by Jorissen (1998) in human patients. However, it would be interesting to

also examine our grading system in human patients given our observations in an animal model of chronic rhinosinusitis.

There were a number of factors that were not examined in our study, such as absolute numbers of cilia and quality of mucous, that may have had an impact on the CBF. Furthermore we ignored the use of HA packing with and without IGF-1 which was crucial to our earlier experimentation. It is unlikely that the use of these substances had any effect on the arm of enquiry within this chapter as baseline samples were taken before the introduction of packing, and HA packing is known to dissolve after approximately two weeks (McIntosh et al., 2002) and thus would be unlikely to be physically present at eight weeks to directly effect the CBF. The introduction of exogenous HA into cultured ovine epithelial cells which were depleted of endogenous HA did not have any effect on CBF during the first week of culture (Lieb et al., 2000). It also seems unlikely that IGF-1 would have been physically present at eight weeks following injury. Any residual IGF-1 would have been digested by proteases or by intracellular lysosomes well before eight weeks (Ganong, 1997). The complex of IGF-1 and its binding protein has a half-life in serum of 12 to 15 hours (Kolaczynski and Caro, 1994). The absence of any significant effect on the CBF by HA packing, with or without IGF-1 (Section 5.3 – Results), also supports our supposition that these introduced packs can be ignored in this study.

6.5 CONCLUSION

Although a clear trend was noted with a statistically significant difference between grades II and IV, there was no correlation found between our measure of ultrastructural regeneration of cilia and their functioning based on CBF. A statistically significant difference was found between the combined CBFs of grades I and II (grade A) and grades III and IV (grade B), which provides support to our combination of these grades when assessing ultrastructural recovery. It would be of interest to examine the possible correlation between CBF and ciliation grade in human patients suffering with chronic rhinosinusitis.

CHAPTER 7

THE EFFECT OF NORMAL SALINE AND HYPERTONIC SALINE ON CILIARY BEAT FREQUENCY IN HEALTHY HUMAN VOLUNTEERS

7.1 INTRODUCTION

Endoscopic sinus surgery has been widely accepted as the treatment of choice for chronic sinusitis resistant to medical therapy. However, debate continues regarding optimal postoperative care. Treatment regimes may include a combination of decongestants, antihistamines, antibiotics, intranasal or oral steroids, and intranasal saline. Postoperative care may also involve debridement of the nasal mucosa. Protocols proven to improve outcomes are yet to be established.

Our interest in CBF in the postoperative healing phase resulted in our examination of various therapies which have been used in an attempt to improve mucociliary function in the postoperative setting. Whilst we had established a methodology for assessment of postoperative outcomes in a diseased sheep model, we were also interested in the effect of different concentrations of saline on ciliary functioning. In some centres, intranasal saline sprays, nebulisers and douches have been used in the management of chronic rhinosinusitis and for postoperative care. Potential benefits include clearance of nasal mucous, pus, debris and crusts as well as possibly decreasing the risk of adhesions by clearance of postoperative blood clots (Talbot et al., 1997). However, there is some speculation regarding the concentration of saline that may be beneficial. Talbot et al. (1997) found that 3% saline increased the mucociliary clearance (MCC) as measured by saccharin clearance time compared to isotonic saline, whereas Homer et al. (2000) found that saccharin clearance time was only increased with 5% saline and that there was no difference between 3% and 0.9% saline. Others have found that laboratory testing of saline, be it isotonic or hypertonic, has a negative impact on ciliary beat frequency (CBF) (Boek et al.,

1999; Min Y-G et al., 2001) which is thought to be a key component of MCC. As a number of studies contradict each other, our aim was to investigate this further by examining the intranasal effect that normal saline and hypertonic saline have on CBF rather than in the laboratory setting (which may not be as relevant to clinical practice).

7.2 METHODS

This study was approved by the ethics committee of the Queen Elizabeth Hospital, South Australia. Eight healthy volunteers were included in this study. None of the volunteers had rhinitis or a history of an upper respiratory tract infection in the preceding six weeks. Furthermore, none had used a topical nasal medication in the preceding three weeks, and none were smokers.

Sodium chloride nasal sprays were made to concentrations of 0.9% and 3.0% and buffered to pH 7.6. No preservatives were added. Both concentrations of saline were administered to each subject, and the bottles were randomly labelled right or left nostril as predetermined by computer allocation. Volunteers and investigators were blinded to the concentrations administered on the respective sides of the nasal cavity.

Nasal mucosa cells were collected from the anterosuperior aspect of the inferior turbinate immediately prior to administering the saline spray for determination of

baseline CBF. Blinded concentrations of saline were administered from a spray-pump bottle. Each subject was instructed to place the tip of the spray bottle slightly lateral to the midline and approximately 10° above the horizontal plane. Four sprays were administered per nostril. Further cells were then collected from immediately adjacent regions of the inferior turbinate at 5 minutes and 60 minutes following administration of saline. Collection was performed using a 3.0mm Bronchial Cytology Brush (TeleMed Systems Inc, Hudson, MA) without local anaesthetic. The brush was then inserted into 1mL of Dulbecco's culture medium (ICN Biomedicals Inc, Aurora, OH) and agitated to release cells into the culture medium. This was kept at 36.5°C until analysis of the CBF was performed.

One droplet from each specimen was placed on a microscope slide warmed to 36.5°C, and phase contrast microscopy was used as previously described (Rutland and Cole, 1980). Analysis of the voltage change and fast Fourier transformation to a frequency spectrum was performed using an MP100 data acquisition system and AcqKnowledge software, version 3.7.0 (Biopac Systems Inc, Santa Barbara, CA). Ten cells per specimen were individually analysed and the final CBF of the specimen was deemed to be the average of these cells.

Differences between the effects of the two concentrations on CBF were examined. Student's *t* test was used for statistical evaluation with $p < 0.05$ accepted as statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 9.0 software system (SPSS, Inc., Chicago, IL).

7.3 RESULTS

Of the eight volunteers, six were male and two were female. The age range was 23 to 41 years.

CBF measurements are presented in Table 15 and summarised in Table 16. The mean baseline CBF overall was 9.6 Hz. There was no statistically significant difference in the baseline CBF between the two groups prior to administration of the saline ($p = 0.132$).

Isotonic saline reduced the baseline CBF measured at 5 minutes and 60 minutes, but this was not statistically significant ($p = 0.102$ and $p = 0.167$ respectively). Use of 3.0% saline increased the CBF at 5 minutes before returning to near-baseline levels – however these measures were also not statistically different from baseline ($p = 0.127$ and $p = 0.658$ respectively).

When comparing the CBF between the concentrations, there is a statistically significant difference at 5 minutes ($p = 0.039$) which is not present at 60 minutes ($p = 0.734$), as shown on Table 16.

Table 15 Ciliary beat frequency measurements (in Hertz) in healthy volunteers after administration of 0.9% saline and 3.0% saline

Subject	CBF					
	0.9% saline			3.0% saline		
	0 min	5 min	60 min	0 min	5 min	60 min
1	10.5	11.9	12.2	11.9	12.1	7.0
2	8.4	7.2	6.7	7.8	9.2	7.3
3	8.9	8.0	9.2	8.6	9.3	9.9
4	10.0	10.3	-	7.6	10.5	11.7
5	13.1	11.3	8.1	8.3	-	11.8
6	10.7	8.1	7.5	10.5	10.3	8.6
7	9.0	8.1	9.3	7.2	9.5	8.5
8	10.7	9.9	8.7	10.4	9.5	11.3

Table 16 Ciliary Beat Frequency by concentration of saline and time after saline administration (mean \pm standard error of the mean)

	Baseline CBF	5 minute	60 minutes
0.9% NaCl	10.2 \pm 0.5 Hz	9.1 \pm 0.6 Hz	8.8 \pm 0.7 Hz
3.0% NaCl	9.0 \pm 0.6 Hz	10.1 \pm 0.4 Hz	9.2 \pm 0.7 Hz
<i>p</i> value	0.132	0.039	0.734

7.4 DISCUSSION

There has been an increase in research into mucociliary physiology of the sinonasal system in recent times. It is accepted that mucociliary clearance plays an important role in the functioning of the respiratory tract. Disease states such as allergic rhinitis and chronic sinusitis lead to a significant decline in this functioning by adversely affecting the mucous layer or slowing the ciliary beat frequency, or both (Holmstrom et al., 1992; Scadding et al., 1995; Quraishi et al., 1998). Precise mechanisms remain unknown, but the potential of manipulating these key components remains a tantalising prospect for treating physicians. Some researchers have found that endoscopic sinus surgery improves the ciliary beat frequency in patients with chronic rhinosinusitis (Lund et al., 1991), whilst others have found that the CBF does not improve although the overall MCC does (Hafner et al., 1997). Certainly, non-surgical treatment options avenues are being explored (Scadding et al., 1995; Talbot et al., 1997; İnanlı et al., 2002).

Our ex vivo findings suggest that 0.9% saline and 3% saline do not alter the CBF significantly from baseline. However, normal saline did tend to decrease the CBF at 5 minutes whereas 3% saline did tend to increase the CBF at 5 minutes – to the extent that there was a significant difference between the two. This had resolved by 60 minutes after administration. Therefore, 3% saline appears to have a ciliostimulatory effect over and above normal saline.

It seems unlikely that the increased CBF seen with 3% saline is due to a direct effect on cilia. A number of in vitro studies have examined the effect of continued

exposure of nasal cilia to various concentrations of saline. Hypotonic saline (0.06% and 0.12% saline) does not have any significant effect on CBF even after exposure for 60 minutes (Min Y-G et al., 2001). Luk & Dulfano (1983) found that CBF did not change significantly in the presence of saline with concentration between 0.5% and 1.2%. However, the CBF declined below this range with a marked reduction below 0.05% concentration. When the in vitro effect of 0.45% saline has been examined in an animal model, the CBF was found to decrease by 14-50% (van de Donk et al., 1980). In vitro studies examining the effect of isotonic saline have found that CBF is either decreased (Boek et al., 1999; van de Donk et al., 1980) or remains unchanged (Min Y-G et al., 2001). Hypertonic saline leads to ciliostasis which is reversible at lower concentrations (3% or 7% saline) and irreversible at higher concentrations (14.4% saline) (Min Y-G et al., 2001; Boek et al., 1999). Therefore there is no evidence to suggest any concentration of saline (be it hypotonic, isotonic, or hypertonic) has a ciliostimulatory effect in vitro – and it is therefore unlikely that saline has a direct effect on the cilia to account for our findings, whether the saline reaches the cilia in its original concentration or is diluted as it passes through the mucous. In fact, extracellular sodium has been found to specifically and competitively inhibit an ATP-gated channel that is responsible for calcium influx which in turn stimulates ciliary motility (Ma et al, 1999). Increased sodium within airway surface fluid inhibits this channel to result in decreased CBF.

It would seem likely then, that our observations are due to changes in the properties of the mucous layer which then has an indirect impact on the CBF. Initially, the nasal spray dissolves into the mucous layer increasing the proportion of fluid within

this layer. The increased osmolarity of the 3% saline solution over the 0.9% solution attracts additional fluid to the mucous layer from adjacent compartments.

Exactly where this occurs within the mucous layer, and how it leads to increased CBF, is speculative. If it was to affect the gel layer, this may result in decreased viscosity. This would then lead to increased MCC as there is a decreased resistance to shear forces (Quraishi et al., 1998). Increase of mucous viscosity has been shown to lead to a decreased CBF (Luk and Dulfano, 1983; Sleight, 1990), and therefore the reverse may also be true to an extent. Clearly the function of this system can be compromised, as the mucous layer requires sufficient viscosity to support its load of airborne particles (Quraishi et al., 1998; Sleight, 1990; Proctor, 1977).

If the incorporation of additional fluid primarily affects the sol layer, it may result in a deeper compartment. The ciliary tips need not be in contact with the gel layer for it to be cleared by ciliary motion. It has been suggested that provided the ciliary tips are no further than 2.5 μ m away from the gel layer, there will be sufficient ciliary shear for propulsion of the mucous (Winet, 1987). The frequency of beating becomes faster as the cilia are now free of their interaction with the viscous gel layer. Once again, the function of this system can be compromised by “too much of a good thing” leading to a sol layer that is too deep, which then results in mucociliary uncoupling (Proctor, 1977; Quraishi et al., 1998; Sleight, 1990). Previous findings suggest that this uncoupling does not occur with 3% saline (Talbot et al., 1997).

Whether it is the gel layer or the sol layer that is affected, the result is that cilia are able to beat at a rate which is presumably closer to their genetically intrinsic rate. Given the dynamics of fluid homeostasis, it would seem likely that both the gel layer and the sol layer are ultimately affected. With ongoing MCC and further secretion from the mucosa, clearance of this effect was demonstrated at 60 minutes.

The fact that patients with chronic sinusitis appear to have a decreased nasal ciliary beat frequency that persists even after the sinusitis is successfully treated (Hafner et al., 1997) suggests that hypertonic nasal saline may be therapeutically beneficial in these patients. The effect we observed was transient, however, and it may be that frequent intranasal exposure to hypertonic saline is required for any benefit to be realised.

Boek et al. (2002) suggested that their *in vivo* measurements of the effect of different substances on mucociliary transport were qualitatively similar to *in vitro* experiments, and this provides a strong indication that CBF is a major determinant of MCT. However our results with 3% saline do not correlate with those of *in vitro* studies. We would argue that the continual bathing of cilia to a particular concentration of a substance (be it saline or another compound) *in vitro* bears little resemblance to the intranasal setting where the time of exposure to mucosa is far shorter.

The baseline CBF of 9.6 Hz measured in our study was in the range of values such as 7.9 – 13.2 Hz that have been previously reported (Luk & Dulfano, 1983; Jorissen et al., 2000; Chilvers and O'Callaghan, 2000; Phillips et al., 1990). This variation

may be due to different methods of analysis (Chilvers and O'Callaghan, 2000), the different media used for cell culture, or simply variation within normal populations. The temperature during analysis was kept as close as possible to 36.5°C to minimise any influence by changes in temperature, although this is slightly warmer than the ambient temperature of the region sampled (Phillips et al., 1990). There was approximately a 10% difference in the baseline CBF between turbinates randomised to the different concentrations, but this did not reach statistical significance. It was assumed that randomisation would decrease the chance of anatomical variants or the nasal cycle influencing results, and there were certainly no significant abnormalities (such as gross septal deflections) noted in our subjects. Use of concentrations of saline greater than 3% were not considered in our in vivo study. There is an intense burning sensation associated with increased concentrations of saline (even with buffering) which precludes their widespread clinical use.

Our findings from the previous chapter (Section 6.3 – Results) suggest that there may be a correlation between increased CBF and improved reciliation. To date, there has been no investigation into the degree of reciliation and the associated effect of intranasal saline (of any concentration) on CBF in the postoperative setting. There is no reason why hypertonic saline in this setting should not improve the CBF as extrapolated from our results. Certainly no adverse effect has been noted with 3% saline and as such its use is to be encouraged in the postoperative patient. However its therapeutic efficacy in this particular setting requires further scientific investigation, including an assessment of cilial regeneration, to provide validity to the commonly held belief that postoperative intranasal saline is beneficial.

7.5 CONCLUSION

In our small ex vivo study, we have found that 3% saline stimulates CBF over and above 0.9% saline as measured at 5 minutes after administration. This effect is transient and has resolved by 60 minutes. There is no evidence to support saline directly affecting cilia leading to an increased CBF. Hypertonic saline may decrease the viscosity of the gel layer, or deepen the sol layer, or both, to the extent that ciliary beat frequency is temporarily increased. As various disease states lead to a decreased CBF, the frequent use of hypertonic saline may therapeutically counter this effect. Further investigation is required into the effect of intranasal saline on regenerating cilia in the postoperative phase. Finally, the in vitro effect of substances on cilia needs to be applied with caution to the in vivo situation.

CHAPTER 8

SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

Chronic rhinosinusitis is a common and costly condition, and a number of patients with this condition will require surgery. However, results of treatment can be compromised due to suboptimal postoperative healing. In recent times, a number of avenues have been explored in an effort to further understand the postoperative healing process and create an environment whereby optimal outcomes are achieved.

A full thickness injury of the sinonasal mucosa was created in 18 diseased sheep, representing an animal model of chronic rhinosinusitis. A control group was compared to a group that had a hyaluronic acid pack (HA) inserted at the time of injury, and a group that had an insulin-like growth factor 1 impregnated hyaluronic acid pack (HA+IGF1) inserted at the time of injury. Results were collected for 16 weeks – light microscopy to assess reepithelialization, scanning electron microscopy to assess reciliation, and ciliary beat frequency examination to assess functionality. Furthermore, the results from electron microscopy and ciliary beat frequency were examined to determine if there was a correlation between reciliation and function.

There was a progressive improvement in the reepithelialization in all groups. There was a significant improvement in epithelial healing with the HA+IGF1 pack compared to controls at eight weeks following injury. There was also a trend towards improved healing with the HA+IGF1 pack compared to the HA group, but this was not statistically significant. However, there was no difference between the three groups in the longer term, suggesting no significant medium- to long-term improvement in rates of healing. Further exposure of the damaged mucosa to other

growth factors at the eight week time-point may be required for sustained increased rates of re-epithelialization.

There was no difference in reciliation at baseline or at eight weeks between the three groups. However, there was a significantly better grade of reciliation in the HA+IGF1 group compared to either the control group or the HA alone group at sixteen weeks. This suggests that the introduction of insulin-like growth factor 1 at the early stages of healing may promote reciliation in later stages. This is likely to be a consequence of the improved reepithelialization seen at eight weeks, which then translates into improved reciliation at sixteen weeks.

The ciliary beat frequency at eight weeks was significantly worse with the HA+IGF1 group compared to the HA alone group. There was no significant difference compared to controls, and there were no other significant differences found between any comparisons at any other time points. The explanation for the finding at eight weeks is unclear and although it is statistically significant, it may represent a spurious finding. Its clinical significance is likely to be negligible. There was an overall negative trend in the ciliary beat frequency within each of the three groups. Whilst this may be a function of unrecognized ongoing inflammation/infection, it is probably due to methodological problems leading to deletion of a significant number of specimens. Wide measures of distribution were noted to support this conjecture. Alternatively, bacterial growth may have adversely affected cilia whilst in culture media awaiting assessment.

There was a clear trend between improved ciliary beat frequency and improved reciliation. When analysed with tests of correlation, no statistically significant correlation was determined. However, support for a correlation was found with a statistically significantly better CBF in grade A (combined grades I and II) over grade B (combined grades III and IV). Furthermore, a significantly better CBF was observed with grade II when compared to grade IV, and a trend towards significance was observed with grade I compared to grade IV. Our findings may have been negatively impacted by the difficulties experienced with measuring CBF leading to a type II error, and a true correlation may indeed exist between CBF and the grade of reciliation. This is certainly worthy of further examination.

Given our interest in improving postoperative outcomes coupled with our interest in assessing ciliary recovery and function, it was felt that an examination into the use of intranasal saline was warranted due to conflicting reports in the scientific literature. We found that in normal human subjects, 3% saline stimulated CBF over and above 0.9% saline as measured at 5 minutes after administration. This effect was transient and had resolved by 60 minutes. There is no evidence to support saline directly affecting cilia leading to an increased CBF. Hypertonic saline may decrease the viscosity of the gel layer of the mucous, or deepen the sol layer of the mucous, or both, to the extent that ciliary beat frequency is temporarily increased.

There are a number of further avenues that require exploration as determined by this body of work. Firstly, further investigation needs to be carried out into the molecular processes that order wound healing in the sinonasal complex. If the improved early healing seen with IGF-1 can be consolidated by the introduction of

other signal molecules vital to the healing process, at the appropriate times, then improved mucosal healing may be realised. This has implications not only for the sinonasal region, but also for the treatment of injuries sustained by the lower airways. Secondly, ciliary beat frequency examination should be repeated in the same experimental setting with improved care taken with methodology – for instance, utilising culture media with added antibiotics. Thirdly, exploration of a correlation between ciliary beat frequency and our grading system of reciliation is warranted, particularly in human chronic sinusitis patients. Finally, the effect of hypertonic saline (and perhaps other concentrations of saline) on ciliary beat frequency and mucociliary function should be investigated in the human postoperative setting, particularly highlighting any impact that the degree of ciliary regeneration may have.

Such information can only add to our understanding of the interdependent nature of the structure and function of the ciliated respiratory mucosa, and the adverse effects that may be seen with pathological conditions. It is hoped that this understanding may then be transformed into further advances in treatment for patients who are suffering from diseases of the respiratory tract.

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