The Effect of a Hyaluronic Acid Nasal Pack, and Insulin-like Growth Factor 1, on Mucosal Healing After Endoscopic Sinus Surgery, in a Sheep Model of Sinusitis

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By

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Abstract

Introduction

Chronic rhinosinusitis is a common condition that is responsible for considerable morbidity and utilisation of health care resources. Surgery remains the mainstay of treatment of sinus disease that does not respond to medical management. Despite recent advances in Endoscopic Sinus Surgery(ESS), the most common complications of surgery are adhesion formation and scarring, which result in recurrence of disease and poor patient outcomes. Hyaluronic acid based nasal packing and Insulin-like growth factor 1 (IGF-1), are recent therapeutic interventions that have shown promise in enhancing the healing process after surgery. They have not however been trailed in a model of chronic rhinosinusitis. The aim of this study was to validate an animal model of chronic sinusitis and then assess their efficacy in enhancing mucosal healing after ESS, in such a model.

Methods and Materials

Under endoscopic guidance, mucosal injuries were created in 18 sheep that were infested with the nasal parasite oestrous ovus. This infestation induces an eosinophilic chronic rhinosinusitis. In 9 sheep, one side of the nasal cavity was packing with Merogel, a hyaluronic acid based pack, and in the other 9, Merogel impregnated with

IGF-1 was used. The opposite side of the nasal cavity was left unpacked as a control. Serial biopsies were taken at days 28,56,84 and 112-post injury and assessed via light and electron microscopic techniques for mucosal reepithelialisation, epithelial height, ciliary beat frequency and the macroscopic presence of intranasal adhesions.

Results

In comparison to healthy sheep, the presence of the oestrous ovus parasite and the ensuing eosinophil driven inflammation resulted in significantly impaired mucosal healing. The use of Merogel packing had no significant effect on mucosal healing. The use of Merogel/IGF-1 packing had a significant deleterious effect on epithelial reciliation. Neither packing had any effect on adhesion formation.

Conclusions

The sheep model has been validated as a suitable one for chronic rhinosinusitis. The use of Merogel packing has no significant effect on mucosal healing after ESS. The use of Merogel/IGF-1 packing has a deleterious effect on mucosal reciliation after ESS. The sheep model will prove useful to study the effect of various interventions to improve healing after ESS.

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 of 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.

Suresh Prasanna Rajapaksa

Preface

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

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Aims

The aims of this study were as follows.

1) To develop and validate a sheep model of chronic sinusitis which is suitable for the study of endoscopic sinus surgery techniques and therapeutic interventions to enhance post-operative healing.

2) To determine whether a hyaluronic acid-based nasal packing, Merogel®, would confer any beneficial effect on nasal mucosal healing after endoscopic sinus surgery

3) To determine whether a hyaluronic acid-based nasal packing, Merogel®, impregnated with Insulin-like growth factor 1, would confer any beneficial effect on mucosal healing after endoscopic sinus surgery.

Introduction

Chronic rhinosinusitis

Chronic rhinosinusitis is one the most common disease processes seen by medical practitioners and represents a significant social and economic burden on our society. In the USA and Australia, it affects about 18% of the population at some time or another[1]. In the USA, it is believed to affect approximately 30 million patients and thus accounts for about 2% of all visits to physicians.[2]. It has been reported as the most commonly reported chronic disease process in health surveys, with at least 15% of the respondents having reported an episode of sinusitis lasting at least 3 months. It is the 5th leading reason for antibiotic prescription, and the total yearly cost in the USA was estimated at 2.4 billion dollars, not including the costs of surgery or of investigative imaging [3]. The initial management of theses patients involves the use of medical therapy such as nasal steroid sprays, oral antibiotics, nasal saline douching, nasal decongestants and analgesics[4]. However, it is estimated that up to 80% of these patients develop chronic rhinosinusitis which may requires surgical intervention[1]. Endoscopic sinus surgery (ESS) is the most common Ear Nose and Throat procedure performed in the USA, accounting for over 50% of ENT procedures performed[5-7].

Anatomy

In order to understand the pathophysiology of chronic sinusitis, the concepts that underlie ESS, and the rationale behind the attempts to enhance mucosal healing, it is important to briefly consider some fundamental aspects of paranasal sinus anatomy and physiology. The human paranasal sinuses consist of a number of hollow, bony walled "cells" which drain via their ostia to each other and ultimately to the nasal cavity via a complex series of drainage pathways. These cells lie adjacent to the orbits laterally, the skull base and olfactory fossa superiorly, the nasal cavity medially and the oral cavity inferiorly. The mucosal lining of the nasal cavity and paranasal sinuses is pseudo-stratified, columnar epithelium which is ciliated There are, on average, 5 ciliated cells present for every one mucous cell, and each ciliated cell has about 200 cilia. The goblet cells are responsible for the secretion of nasal mucous. The epithelium, as well as being the integral part of the mucociliary clearance system which will be discussed below, also acts as a physical barrier to exogenous agents. [3, 8, 9]

The Nasal Mucociliary System

The nasal mucociliary system plays a central role in the primary defence of the respiratory tract from inhaled material. The function of mucociliary clearance was initially described in the 1830s[10, 11]. Since then, much has been done to describe the function of this system and its associated components

Cilia are actively motile, microscopic projections from epithelial cell surface surfaces. They are composed of 9 sets of paired microtubules, laying in a vertical

plane, which lie peripherally surrounding 2 central microtubules, the 9+2 configuration. These paired microtubules are linked to each other by filaments of a substance called nexin, and are also linked radially by dynein arms with ATPase activity. These also have dilated heads that can link with structures on the central microtubules. Ciliary movement occurs in two phases, the effective (power) strike, and the recovery (preparatory) stroke. Movement occurs due to movement of the 9 paired microtubules against each other, powered by interactions between ATP and the ATPase in the dynein arms, resulting in movement of the erect cilia in an arc which is vertical relative to the cell surface during the active phase, and then a sideways movement of bent cilia near the surface of the cell during the recovery phase. The movement of many cilia in a coordinated fashion is described as metachronous movement[11-16].

Integral to mucociliary function is mucous. It is composed of glycoproteins, water and ions, and is secreted by serous glands, mucous glands, goblet cells and also produced by transudation from blood vessels[11]. The nasal mucous exists in a double layer. This consists of a sol, or aqueous layer, which lies closest to the epithelial surface, and a gel layer lying superficially. During the active phase, the tips of the cilia project into the gel layer, but move only in the sol layer during the recovery phase. In this way, the active phase produces movement of the gel layer.[11, 16, 17]. Further discussion of the intricacies of the nasal mucous, and its interaction with the cilia lies beyond the scope of this work.

In the upper respiratory tract, cilia are universally found on the epithelium except for the nasal vestibule, the squamous lined areas of the larynx, pharynx and nasopharynx and the olfactory epithelium. In the lower respiratory tract, ciliated epithelium extends to the respiratory bronchioles to the level of the non-alveolar walls[15]. The function of the mucociliary system is the removal of trapped inhaled particles from the respiratory tract. Particles larger than 12.5 microns in size become trapped in the mucous, and are eventually transported towards the nasopharynx where they can then be expelled or digested.[11, 15].

The natural drainage pathways of paranasal sinuses

The concept of the mucociliary drainage pathways is central in the understanding of chronic rhinosinusitis and ESS. In a series of classic studies in canine models, Hilding demonstrated the course of the natural mucociliary pathways in the paranasal sinus complex and showed that the majority of mucociliary clearance was directed posteriorly towards the nasopharynx[18]. He also characterised the drainage of the frontal sinus complex in a canine model[19]. In human subjects, King examined mucociliary clearance pathways and demonstrated that clearance continued to occur via the natural ostia of the sinuses, even when an inferior meatal antrostomy had been performed[20]. Friedmann further confirmed this preferential drainage through the natural ostia using a canine model, and nuclear medicine scanning to track mucociliary clearance, which demonstrated drainage via the natural pathways and the middle meatus, even when drainage was gravitationally more advantageous via the surgically created inferior meatal antrostomy[21]. Messerklinger showed that in the

human maxillary sinus, the natural pathway of mucociliary flow originates in the floor of the sinus, flows in a star shape and then ascends in a spiral fashion towards the middle meatus (the natural ostium of the maxillary sinus)[22]. In the human frontal sinus, there is a lateral pathway from the lateral aspect of the floor of the sinus to the frontal ostium and then down on the lateral wall of the frontal recess, and a medial pathway which ascends from the frontal sinus ostium along the medial wall of the frontal sinus to the level of the intersinus septum[23]. A thorough understanding of these natural pathways of mucociliary drainage, and the observation that this system would work in preference to artificially created pathways form the basis of the theories underlying functional endoscopic sinus surgery (FESS)

Pathogenesis of sinusitis – disruption of mucociliary clearance

The basic underlying process in the pathogenesis of chronic rhinosinusitis is disruption to the natural function of mucociliary clearance from the paranasal sinus complex. Stammberger, in one of his landmark papers on the anatomic and physiological considerations relevant to ESS, described an ongoing cycle where disorders in mucociliary clearance, and changes in the mucosa and mucous composition result in blockage of the natural ostia and drainage pathways, which in turn leads to further metaplasia of the mucosa, further loss of cilia and then further derangement of the mucociliary system. The result of this is mechanical blockage of the middle meatus which then allows for the sinus to become permanently infected, and allows spread of this infection throughout the paranasal sinus complex[24]. Numerous studies have been performed which support this theory. As far back as the

1930s, Hilding demonstrated that interference with natural mucociliary clearance resulted in chronic rhinosinusitis in a canine model[19, 25]. Since then, much work has been performed to highlight the importance of a normally functioning mucociliary clearance system to the health of the paranasal sinuses. In a rabbit model, Czaja et al. showed that obstruction of the mucociliary drainage pathways of an infected maxillary sinus resulted in chronic infection in that sinus, with the resultant histopathological changes in the ciliated epithelium described previously, with then further deleterious effects on mucociliary clearance. These changes did not occur in experimental subjects whose sinuses were inoculated with bacteria but whose natural drainage pathways were left unobstructed, nor in subjects whose sinuses were obstructed but not infected, suggesting the important role both factors play in the development of chronic rhinosinusitis[26]. A number of other authors have also induced chronic rhinosinusitis in murine models by occluding the natural mucociliary drainage pathways of the paranasal sinuses with exogenous materials. Histopathological analysis of mucosal samples showed similar changes to those described previously [27, 28]. In a study on patients who had undergone ESS, Waguespack performed endoscopic assessment of mucociliary transport pathways using powdered graphite as a visible tracer. Mucociliary stasis in the ethmoid cavity was the most common finding associated with recurring sinusitis after ESS [29]. In a series of 100 patients, Schaefer et al commented that the formation of post operative adhesions which lead to obstruction of the mucociliary drainage pathways of the ethmoid and maxillary sinuses lead to sinusitis[30].

Further evidence of the importance of the mucociliary clearance system is evidenced by studies performed in patients with congenital derangements in ciliary structure and function. There are a number of conditions collectively know as primary ciliary dyskinesia, who have ultra structural abnormalities in their cilia resulting in decreased ciliary action or in the case of Kartagener's syndrome, immotile cilia. Patients with these conditions have poor clearance of mucous and debris from their lungs and resultant chronic chest disease[31-33] [34]. With regards to sinus disease, other work has noted increased sinus infections[34-39], and documented alterations in mucociliary function[35, 40, 41].

Animal Models of Chronic sinusitis – histopathological changes

When the histopathological changes that accompany chronic sinusitis are studied, one can understand the mechanisms of disruption to mucociliary clearance. Analysis and documentation of these changes also allows the assessment and validation of newer models of sinusitis that are more relevant modern ESS techniques. Numerous studies have been performed in animal models, which document the effect of chronic rhinosinusitis on the mucosal lining of the paranasal sinuses and the mucociliary transport system. In a rabbit model of bacterial sinusitis, after an initial increase in CBF, there was an almost complete loss (86%) of viable ciliated epithelium was seen after 5 days of infection, and the authors also suggested that this was due to ischaemic

and other changes secondary to the infection rather that due to bacterial toxins[42] Again in a rabbit model of chronic sinusitis, Czaja et al showed a significant increase in ciliary ultra-structural abnormalities as demonstrated by electron microscopy, and that once over 10% of the cilia were abnormal, there was a significant effect on ciliary beat frequency[43]. This model also demonstrated that chronic sinusitis resulted in loss of ciliated epithelium, cell necrosis, basement membrane thickening, and disorganised patterns of mucociliary clearance and resulting pooling and stagnation of mucous [26]. In further rabbit models, Benninger et al demonstrated that the presence of chronic sinusitis resulted in a loss of epithelium[44], Marks showed epithelial necrosis, loss of cilia and sloughing associated with an intense polymorphonuclear infiltration[27], as did Schlosser et al[45].

Human studies on Chronic Sinusitis - histopathological changes

In addition to the work performed in animal models, the numerous studies have been conducted in human subjects to demonstrate the detrimental effect of rhinosinusitis on both cilia and mucociliary clearance in humans. In a series of over 2500 patients undergoing ESS for chronic sinusitis, mucosal samples showed features such as increases in glandular structure, thicker mucous, thickened basement membranes, squamous metaplasia, an increase in ultra structural ciliary abnormalities and an overall decrease in cilia[46]. In studies on nasal brushings from patients with rhinosinusitis, Maurizi et al. demonstrated numerous non specific ciliary abnormalities as assessed by electron microscopy, and also, slower mucociliary transport times measured by the saccharin test technique [47]. In further work on

patients with chronic sinusitis, slower mucociliary transport was noted compared to controls, but there was no difference in ciliary beat frequency between the controls subjects and those with sinusitis. These findings suggested that the sinus infection may have affected the nasal mucous, resulting in overall decreased transport times[48]. In another study on human patients, no significant differences in ciliary beat frequency were seen between control subjects and patients with chronic sinusitis [49]. Toskala also demonstrated a significant loss of ciliated cells and replacement with non-ciliated columnar epithelium, and an increase in compound and shortened cilia, in patients with chronic rhinosinusitis[50]. Similar ultra structural ciliary abnormalities were seen in electron microscopy studies on samples from patients with chronic sinusitis and recurrent infections in a study by Fontoillet et al [51], and also by Carsons et al who specifically noted the presence of focal areas of micro tubular adhesions as well as a generalised loss of ciliated epithelium in mucosal samples from children with viral upper respiratory tract infections[52]. Mygind et al. demonstrated ciliary ultra structural abnormalities as well as delays in mucociliary transport time in patients with viral upper respiratory tract infections[37], and Albegger et al. described abnormal "hair like" filaments in the ciliated epithelium patients with chronic rhinosinusitis.[53].

Principles of Endoscopic Sinus Surgery

As stated previously, the mainstay of treatment of chronic sinusitis is medical management. However, for those patients whose symptoms are refractory to medical treatment, ESS becomes the next treatment option. ESS was initially practiced in the mid 1980s based on the theories of Messerklinger. His theories were based on the animal and human studies previously discussed, which described the complex system of pathways by which the paranasal sinuses drained in to the nasal cavity, and the importance of these pathways in maintaining the health of the sinuses. He described the relationship between obstruction of these pathways, mucous retention, inadequate ventilation and then subsequent infection. Very importantly, he realised the importance of the observations that mucociliary clearance was directed towards the natural sinus ostium, even in the presence of artificially created ostia. His technique was then focussed on the removal of diseased tissue, restoration of the natural drainage pathways and preservation of as much of the healthy sinus mucosa and architecture. Due this aim of preserving the physiological functioning of the sinuses, Kennedy coined the term Functional Endoscopic Sinus Surgery (FESS)[6]. This technique was also facilitated by the development and advancements in endoscopic technology, which afforded improved access with minimal morbidity and a magnified view of the paranasal sinuses, surgical instruments, both non powered and powered which allowed manipulation of the sinus tissues, and finally, advanced radiology techniques such as computerised tomography which afforded the surgeons improved diagnostic abilities and also and anatomic road map for their surgery.[6, 7, 24, 30, 54].

ESS was then pioneered and popularised in the USA by Kennedy[7] and in Europe by Stammberger[24].

Efficacy Of ESS

However, at present there is a paucity of good long-term outcome studies which help predict which groups of patients may most benefit from ESS[3].

Kennedy reported the results of 120 patients who had undergone ESS and were followed up for an average of 18 months post operatively. Of these patients, 71% were having revision surgery, so they were classified as having disease that was resistant to both medical and surgical treatment. A major improvement in symptoms was noted by 85% of patients, while a mild improvement was noted in a further 12.5%. When these subjective results were compared with objective endoscopic assessment of the healed sinuses, it was noted that only 45% of the patients had normal, disease free sinuses on examination. The Kennedy system of grading sinus disease, as assessed by computerised tomograph, was a reliable predictor of a continuing disease process when assessed objectively by the surgeon, but did not correlate with subjective improvements reported by the patient[55]. Schaefer et al reported on a series of 200 consecutive patients undergoing ESS who were followed up for an average of 5 month. Of these, 83% reported their symptoms improved significantly and were also noted to have a patent ostiomeatal complex on endoscopy, while a further 10 % were improved but did have 1 episode of sinusitis during the follow up period. Of this group, 80% had a normal healthy ostiomeatal complex at postoperative nasoendoscopy.[30]. Toffel reported that in a series of 785 patients

followed up at 12 months post ESS, 90.75% had complete resolution of the symptom of nasal obstruction, 83.3% reported complete resolution of post nasal drip, and 84.4% had a complete resolution of midfacial pain. Of the remaining patients, almost all reported some improvement, with less than 1% of patients reporting that any of these 3 symptoms had not changed after ESS. At the 3 year follow up mark, the number of patients reporting complete resolution of these three symptoms had reduced to 88.3%, 81.5% and 80% respectively, but the number reporting no change was still less than 1% in each symptom category[56]. Levine presented a series of 250 patients, all of whom had failed medical management, and 13 of whom had previous surgery. Of the patients with nasal polyposis, 89.7% were judged by both the surgeon and the patient as having been successfully treated, after a follow up period of 18 months. Of those with chronic sinusitis, the success rate was 80.2%[57]. In a series of 145 patients followed up for 24 months, 91.8% of patients who responded to a postoperative questionnaire (61 patients) indicated that their symptoms were improved or indeed, completely resolved. At this same time period, 94.08% of the middle meatuses were still patent[58]. Using the Chronic Sinusitis Survey (CSS), and the SF-36 Health survey, both previously validated health assessment questionnaires, Metson and Glicklich assessed the change in symptomatology in 108 patient with chronic rhinosinusitis who were treated with ESS. With regards to the CSS scores, 82% of patients had a clinically and statistically significant improvement in their symptoms after ESS. Using the SF-36, which is a better indicator of overall quality of life and global function, 75% of the general health categories showed a significant improvement 12 months after ESS[59]. In the field of frontal sinus surgery, which has

historically been a clinically difficult problem, the same authors reported a 37% improvement in symptom scores 12 months after ESS, and also noted significant reduction in medication use after surgery. They noted that these results were not as promising as those seen when ESS was performed for non-frontal disease, but was nevertheless clinically significant[60].

One of the few studies to examine long term outcomes of ESS reported on a series of 72 patients with an average follow-up period of 7.8 years. These were in fact from the same cohort of patients that Kennedy reported on in 1992 [55]. Ninety eight point four percent of these patients reported via a questionnaire that they still had an improvement in their symptoms in comparison to prior to surgery. In this long term follow up, it was noted that the presence of ongoing disease in the sinuses, as assessed by endoscopy at 18 months post operatively, correlated with the need for revision surgery, while symptom scores at that time did not. These results emphasis the importance of complete healing of the mucosa, even in the absence of clinical symptoms, and add weight to the usefulness of attempts to improve the healing process after ESS[61, 62].

Benefits of Sinus Surgery – Histopathological Studies

As well as the subjective improvements in symptomatology reported by patients, it is also important to document objective changes in the health of the sinuses in order to demonstrate the efficacy of treatments. There have been numerous animal studies to demonstrate the benefit of sinus surgery in the treatment of sinusitis. In a rabbit

model of chronic sinusitis, Czaja and McCaffrey demonstrated that performing middle meatal antrostomies could reverse the ciliary and other epithelial histopathological abnormalities, and return the ciliary beat frequency (CBF) to a normal rate. Six weeks after surgery was performed, the rabbits with chronic sinusitis that initially had a CBF that was statistically less than the controls, had returned to a CBF that was no different to the control subjects. Analysis of pre and postoperative mucosal biopsies revealed a return to a healthy state of ciliated epithelium, and also a "reorientation of previously disorganised cilia". The authors also assessed the patterns of mucociliary flow and noted improvements in these at 6 weeks postoperatively[63]. In a rabbit model, Kennedy et al re-examined some of Hilding's work and noted that widening of the middle meatus resulted in epithelial regeneration but did not result in reorientation of mucociliary clearance[64]. Unfortunately, until recently there has not been a suitable animal model for ESS so the specific effects of ESS on sinusitis have not been studied in an animal model[65].

Clinical studies on human patients have also demonstrated the beneficial effects of ESS on both the histopathological changes due to sinusitis and also on improving mucociliary clearance. Hafner et al. measured both CBF and also mucociliary transport time before and after ESS in patients with chronic rhinosinusitis, and showed a significant improvement in mucociliary transport time after surgery [66]. Lund et al measured CBF in patients before and after ESS and again showed a significant improvement after surgery. They also demonstrated an improvement in subjective symptom scores but no improvement in rhino manometric assessment of

nasal airflow[67]. In a series of 70 patients undergoing ESS for chronic sinusitis, preoperative CBF values were shown to be significantly reduced compared to normal subjects, but 6 months after surgery, significantly improved and had reached normal values[68]. Ikeda used Technetium nuclear medicine scanning to show a significant improvement in mucociliary transport rate after ESS, and the use of light and electron microscopy revealed in increase in ciliated epithelium, and an improvement in ciliary organisation[69]. In series of human patients undergoing ESS, Moriyama showed that after a time period of 6 months, there was an almost complete return to normal respiratory epithelium if the surgical injury had only been removal of the superficial layers of tissue. Inspection by SEM at a time of 18 months post operatively revealed normal reciliation of the epithelium. In contrast, when the surgical injury was a complete removal of mucosa to expose bone, normal epithelium was not seen at 6 months, and at 18 months, although the epithelium appeared macroscopically normal, it proved to be non ciliated when examined by SEM[70]. Again, in a series of human patients with chronic sinusitis, mucosal samples were taken preoperatively and 6 months postoperatively, examined with TEM (Transmission electron microscopy) and compared. In the preoperative samples, the most notable finding was the paucity of cilia on the epithelial cells. In contrast, in the postoperative samples, the degree of ciliation had returned to a normal healthy state in 75% of the patients, and the remaining 25%, although there was no improvement, there was no worsening in the degree of ciliation[71]. Asai et al examined mucociliary clearance as assessed by the saccharin transport test, and correlated this with the endoscopic appearance of the maxillary sinus mucosa. They noted a post ESS improvement in the saccharin

transport time in the subjects whose sinus mucosa appeared most healthy. SEM analysis was also performed on mucosal samples from those patients with the most prolonged saccharin transport time and revealed an extensive loss of cilia[72]. Kaluskar et al., likewise measured saccharin transport time in patients pre and post ESS and demonstrated an improvement after ESS[73].

The Importance of Mucosal Healing

Now that we have considered the pathological processes behind chronic rhinosinusitis and the principles and efficacy of ESS in the management of sinus disease, we can turn our attention to the vital area of mucosal healing after surgery. In a canine model, Hilding noted that when mucosal injuries were made, they were replaced by scar tissue, and sometimes, even bone, which impeded mucociliary clearance, even though they did possess some ciliated epithelium[19, 74]. In a rabbit model, Benninger et al noted an incomplete regeneration of ciliated epithelium after complete removal of the maxillary sinus mucosa via a Caldwell-Luc incision, with the proportion of the cells in the operated sinus demonstrating ciliation of only 54% of those on the control side [75]. Shaw et al created endoscopic full and partial thickness injuries in a sheep model. At day 84 post-operatively, only 80.7% of the partially injured surface had reepithelialized, and only 64.9% of the fully injured surface had reepithelialized. In terms of reciliation at day 84 post-operatively, 68.4% reciliation had occurred in the surfaces that had partial thickness injuries and only 33% of the fully injured surface showed reciliation. Interestingly, there were no significant differences in mucociliary transport time between the two injury groups at day 84 and the baseline values[76].

Shone et al examined the healing of nasal mucosa after ESS in human patients and noted that it took up to three months for mucociliary clearance to improve, suggesting a slow return to normal function[77].

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Adhesions and Their Relevance to Sinus Surgery

The most common post operative complication encountered in the field of nasal and sinus surgery is the occurrence of post operative adhesions, which are a result of impaired healing, have serious clinical consequences, especially with regards to recurrence of disease and symptoms[24, 30, 58, 78, 79]. Brennan et al. carried out a retrospective review of 479 cases, where an intranasal procedure was carried out on the nasal septum, inferior turbinate, or both, and noted adhesion formation in 30.6% to 36.2% of cases [80]. Tofffel reported that 3%(41 patients) of a series of 1363 patients developed adhesions between the middle turbinate and lateral nasal wall, and of these, 15 required revision surgery. This was despite the use of special stenting to prevent these adhesions forming [56]. Schaefer et al reported the formation of adhesions between the lateral nasal wall and the middle turbinate in 6% of their patients, and these adhesions as one of the "most frequent and frustrating complications" of ESS, which leads to obstruction to the natural mucociliary drainage pathway from the ethmoid and maxillary sinuses, and would eventually lead to sinusitis[30]. Levine reported an incidence rate of stenotic lesions of the maxillary ostium of 6.8% in a series of 250 patients, and 60% of these patients required revision

surgery[57]. In a series of 145 patients undergoing ESS, adhesions were seen in 11.7%[58]. Stammberger stated that if apposing areas of mucosa are pressed together and adherent, as occurs with adhesions, then mucociliary transport of mucous from those areas is interfered with and the resultant pooled mucous becomes an ideal area for the growth of bacteria or viruses[24]. Given that the presence of inflammation delays the return of normal epithelial coverage after surgery, the presence of active infection and inflammation in the nasal mucosa may predispose patients to the formation of post operative adhesions due to damage to the normal mucociliary clearance system which may aid removal of post operative debris including blood clots. The multiple cytokines and other bioactive agents[81] present due to the chronic inflammatory state may also predispose to tissue changes characteristic to inflammation such as fibrosis, and scarring, rather than regeneration of injured tissue to its normal state.

Nasal Packing

The use of postoperative nasal packing is one technique by which surgeons have attempted to reduce the incidence of adhesions. Since the advent of nasal surgery, the use of nasal packing has been almost universal[82]. Packing has been used pre operatively for the delivery of anaesthetic and vasoconstrictor agents, in order to improve analgesia and minimise intraoperative haemorrhage[83, 84]. Post operatively the nasal cavity has been packed for the purposes of haemostasis, provision of shape and support after surgery, and for the prevention of adhesion formation [84, 85]. In

those studies, little or no benefit was seen in terms of adhesion prevention, but deleterious effects of packing were noted. In regards to postoperative packing, patients report the pain and discomfort upon removal of their packs post operatively as being one of the most unpleasant aspects of their surgery[82]. Infection, specifically toxic shock syndrome[86, 87], has also been described. In animal models, occlusion of the maxillary sinus ostium with non absorbable packing has been performed to induce a state of sinusitis in the animal [28], and in theory, this same process could occur during the postoperative period if packing is used. In a study comparing the use of 4 types of non-absorbable packing, BIPP gauze, Jellonet, Merocel and Telfa, the authors assessed patient comfort, haemostasis and ease of removal. While in situ, there were no significant differences between the materials, but there was significantly less bleeding and discomfort on removal associated with the Telfa and Jellonet as compared to the BIPP and Merocel. This was attributed to the non-adherent surface characteristics of these materials, which allowed easy passage from the nasal cavity[82]. Shaw et el demonstrated that the use of preoperative packing for the delivery of local anaesthetic and a vasoconstrictor, consisting of either ribbon gauze or neuropatties, resulted in a statistically significant reduction in intact ciliated nasal epithelium of over 50% as compared to the unpacked side. They made the recommendation that the use of packing should be minimised, and if possible, performed endoscopically to minimise trauma to the epithelium.[88]. Another study looked at the use of Silastic splints to provide stability to the healing septum after septoplasty, and also to prevent adhesions within the nasal cavity. This randomised study showed no difference either in adhesion formation or septal stability between

the splinted and unsplinted group, but patients reported increased pain and discomfort with the removal of the splints[85].

A recently developed non-dissolvable packing material, polyvinylchloride (Merocel®), has also been shown to have no beneficial effect of the healing of nasal epithelium after injury. This study was performed in the sheep model[65], and involved histological analysis of the regenerating mucosa and electron microscopic analysis of reciliation patterns and ciliary maturity (Unpublished data). Interestingly, this study showed no significant differences in terms of reciliation or reepithelialisation between the two groups, contrasting with Shaw et al's[88] work showing significant damage with the use of packing. However, the analysis for Shaw et al's study was done after only a few days post removal of pack, while McIntosh's work considered the long term healing results. Nevertheless, if in the early post operative period after the packs are removed there is damage to the cilia and epithelium and thus a disruption in mucociliary clearance, it would seem logical that this may impact negatively on the healing of the sinuses after surgery. As can be seen from these studies, the packing materials associated with nasal surgery have tended to have been non absorbable in nature and required removal. In order to avoid the various problems associated with these packs, attempts have been made to develop absorbable nasal packs.[89]

Due to the occurrence of postoperative adhesions and scarring, and the poor results obtained by some patients from their ESS, attempts are constantly being made to develop interventions or techniques that will improve healing of nasal mucosa after

surgery to prevent these problems. The aim of this thesis was to examine to effect of two therapeutic postoperative interventions on the healing process of nasal mucosa and the formation of adhesions after ESS.

Hyaluronic Acid

Basic Science

The first of these interventions was the use of a newly developed absorbable hyaluronic acid based nasal packing, Merogel® (Xomed, Jacksonville Fl). In order to understand the choice of this packing material for use after ESS, one must understand the important biological properties of hyaluronic acid. Hyaluronic acid is a linear polysaccharide that is made up of units of glucoronic acid and N-acetyl glucosamine. It is a naturally occurring substance that is present in all soft tissues in humans and other higher organisms[90]. It is found in particularly high concentrations in the extracellular matrix of foetal wounds, and believed to play in important role in foetal wound healing[91]. The main reason for the interest in hyaluronic acid is the nature of foetal wound healing. Unlike in adults, foetal wound healing is characterised by rapid progress, the lack of scar formation and fibrosis and the absence of an inflammatory process[92] [93]. This results in essentially "perfect healing", that is, regeneration rather than repair, with none of the derangement of structure and compromise of function seen in adult wound healing.[94]. Studies in foetal wound healing have demonstrated a paucity of the collagens usually associated with fibrosis, and an abundance of hyaluronic acid in a well organised extracellular matrix. The collagens present are well organised unlike the patterns seen in scars[95]. In regards to skin

wounds, the foetal fibroblast appears to be the cell responsible for coordinating the healing process[96]. The hyaluronic acid also appears to remain present in the wound extracellular matrix for far longer than in adults, most likely due to the presence of HASA (hyaluronic acid stimulating agent)[94]. As yet, the exact mechanisms of foetal wound healing, and the role of hyaluronic acid in the process is not understood. However, it has been speculated that an extracellular matrix rich in hyaluronic acid provides an environment that facilitates cell migration and proliferation, and also the orderly deposition of appropriate amounts of collagen[93, 97, 98]. Hyaluronic acid may also interact with inflammatory mediators[99], and has been shown to inhibit platelet function including the platelet driven activation of inflammatory pathways[100].

Hyaluronic acid nasal packs (Merogel®)

There are numerous formulations of hyaluronic acid available, including solutions of differing concentrations and a hyaluronic acid-carboxymethylcellulose film (Seprafilm ®). A newly developed formulation of hyaluronic acid is Merogel®, composed of an esterified hyaluronic acid compound. This process induces functionalisation of carboxyl groups in the HA, which allows attachment of bioactive compounds. This functional esterified hyaluronic acid has been termed HYAFF. HYAFF can then be manufactured into fibres or membranes, which in turn can be made into different products. Freeze-drying then allows the manufacture of sponge like materials like Merogel [90]. It is fully biodegradable, resorbable material that can be easily manipulated while dry. Human trials of using this material as middle ear packing after ear surgery have been performed, and have demonstrated decreased

unresorbed packing material, improved hearing results and an absence on new bone formation when compared to the traditional absorbable gelatine sponge used in ear surgery[101]. Due to its bioactive profile with potential pro-healing effects, and the fact that it is dissolvable, Merogel® is a potentially useful nasal packing material.

Hyaluronic acid and adhesions

A clinically significant aspect of tissue healing in which hyaluronic acid plays a part is that of adhesion formation. The relevance of adhesions after nasal surgery has been previously discussed but adhesions have been most commonly described in the general surgical and gynaecologic surgical literature. An adhesion has been described as being the result of 2 injured surfaces healing in contact with each other[102]. The rate of adhesion formation in abdominal and pelvic surgery has been quoted as being between 67% and 97%, and adhesions remain the most common cause of small bowel obstruction in the western world, as well as being responsible for infertility and pelvic pain. There is also the associated morbidity and mortality of the surgical procedures sometime required to manage these symptoms. As will be discussed later, the use of hyaluronic acid based material has been shown to decrease the incidence of adhesions formation in these circumstances[103, 104] [105, 106].

IGF-1 – Basic science

The other intervention that was examined was the incorporation of Insulin like growth factor 1(IGF-1) into the hyaluronic acid based nasal packing. IGF-1, also known as somatomedin C, is a polypeptide growth factor with known cell proliferation and differentiation, metabolic and anti apoptotic properties[107]. It is one of a group of growth factors whose activity has also been implicated in wound healing[108]. In the setting of wound healing, IGF-1 is initially released from alpha granules in platelets. It is then believed to help modulate activities of other bioactive substances[109]. In latter stages of wound healing, IGF-1 is believed to play a role in macrophage function[109], and alveolar macrophages have been shown to express IGF-1, which can then act to encourage fibroblast proliferation[110]. In the next stage, IGF-1 has been shown to be a powerful chemo attractant for fibroblasts but is the also secreted by fibroblasts to act in an autocrine fashion to stimulate further fibroblasts proliferation and then collagen synthesis[109, 111, 112]. IGF-1 has also been shown to be involved with angiogenesis, an essential element of wound healing[112]. Work on a bovine respiratory epithelial culture model, IGF-1 was shown to be a powerful chemo attractant for respiratory epithelial cells, and about 1000 fold more effective than insulin for this purpose[113]. IGF-1 has also been demonstrated to play a role in the epithelialization of wounds. An in-vitro study on intestinal wound healing revealed that IGF-1 had a significant beneficial effect on intestinal epithelial cell migration during wound healing, and demonstrated the presence of IGF-1 receptor mRNA in intestinal epithelium[114]. Ando et al examined the effect of IGF-1 on

human keratinocyte migration and showed a significant increase in speed of migration with topical application of IGF-1 in a cell culture model[115].

In vitro work performed by our group on a series of growth factors including IGF-1, IGF-2, and Epidermal growth factor (EGF) suggested IGF-1 would be the growth factor most likely to show benefit on nasal epithelium (Unpublished data). Human IGF-1 has been shown to be structurally almost identical to that of sheep IGF-1, varying by only one amino acid[116]. Work by our group using immunohistochemical techniques has also demonstrated the presence of IGF-1 receptors in sheep nasal mucosa (unpublished data). The carrier vehicle for the IGF-1 was Merogel®, a hyaluronic acid based packing material whose biological effects have been previously characterised in the sheep model[117]. Incorporation of bioactive factors into hyaluronic acid based materials has been previously described as a successful method of delivery of these substances to the nasal mucosa[118]. The IGF-1 was incorporated into the packing material at the time of manufacture and its bioavailability from the pack was demonstrated by radioimmunoassay techniques (unpublished data).

Animal Models For Sinus Surgery

In order to study the effects of these therapeutic interventions, a suitable animal model was needed. For many years, animal models have been used to study the effect of surgical intervention on the nose and paranasal sinuses. In a series of classic studies in a canine model, Hilding demonstrated that removal of maxillary sinus mucosa led to the formation of scar tissue which impeded mucociliary clearance[19],

and that could also lead to new bone formation within the scar tissue[74]. In 1989, Kennedy reviewed this work in a rabbit model with similar findings of scar tissue formation if there was complete removal of mucosa[64], and these observations regarding scar formation and bony regrowth have also been noted by other authors using animal models[119] [120]. In a rabbit model, Benninger concluded that the changes in sinus mucosa after surgery were detrimental to normal sinus function due to a less than optimal number of seromucinous glands being present in the regenerating mucosa, leading to increased mucous viscosity, and there was also an absence of motile cilia. Electron microscopy also revealed decreased numbers of cilia on the epithelium, but an increase in the number of structurally abnormal cilia[75, 121]. In the same rabbit model with induced sinusitis, Benninger et al. demonstrated no difference in either patency rates, or gross and microscopic grading of mucosal inflammation, in subjects who underwent either a middle meatal antrostomy or an inferior meatal antrostomy[44]. Friedman and Toriumi also studied the effect of inferior meatal windows on mucociliary clearance using a rabbit model[21]. In a canine model, Kass evaluated the use of KTP and argon lasers on nasal mucosa[122]. Schenk carried out a number of studies in a canine model to demonstrate that surgery on the nasofrontal duct in this area could lead to fibrosis and adhesions in this area and occlusion of the drainage pathway of the frontal sinus [123, 124]. Work has also been done to study the effect of osteoplastic flap procedures on the frontal sinus in a feline model[125, 126].

The Sheep Model

The advent of endoscopic sinus surgery during the 1980 has necessitated a change in the animal models used to investigate sinus disease. All of the work thus far described has involved animals such as dogs, cats, rabbits and mice. These animals however, are not suitable for performing endoscopic sinus surgery and evaluating its effects, due to the size and shape of their nasal cavities. Gardiner described the use of cadaveric sheep heads as a training model for endoscopic sinus surgeons due to the size of the sheep nasal cavity and sinuses, and also the similarity shown to human sinus anatomy[127]. Previous work has shown that the nasal mucosa of sheep is very similar to that of humans in terms of both its physiological and histological characteristics, and is a suitably analogous model to study the effects of intranasal medications [128-130]. Based on this work, and after carrying out anatomical studies in other animals such as cows and pigs, Shaw et al developed and validated a sheep model for endoscopic sinus surgery[65]. This model involved a surgical standardization procedure involving endoscopic removal of the middle turbinate complex in order to improve access to the nasal cavity and sinuses. Middle turbinectomy has been a somewhat controversial procedure in humans, with different surgeons both advocating and discouraging its use[131, 132]. However, in human studies, no significant adverse effects on mucociliary clearance have been demonstrated[29]. In the sheep model, Shaw et al. demonstrated no adverse effect on the mucociliary transport of the adjacent mucosa. Detailed histological studies were also performed in this model to document the temporal healing pattern of the nasal epithelium after full and partial thickness injury[76]. Using this model, McIntosh et al

then studied the effect of a hyaluronic-acid based nasal pack on mucosal healing[117], and demonstrated improved reepithelialisation of wounded tissue with the application of the pack.

Oestrus ovus

Most of the animal studies already referred to have been carried out in healthy animals. As, however, the majority of sinus surgery is carried out on patients with diseased sinuses[6, 7, 24], the use of animal models to simulate the disease process is important. The sheep model, the first one suitable for use with ESS, also has the advantage of having a naturally occurring state of sinusitis. Sheep are subject to a condition known as Oestrosis, a parasitic infestation of the nasal cavity and paranasal sinuses with *Oestrus ovus*, also known as the nasal bot fly[133]. This condition also affects goats, ibex, argali, and also humans[134]. The nasal infestation is caused by the larvae of the fly, which hatch from eggs that are laid in the nasal vestibule. The larvae migrate to the paranasal sinuses[133, 135]. The presence of these larvae induces a clinical state of sinusitis, which is characterised by rhinitis and also, marked increases in the number of eosinophils and mast cells in the nasal and paranasal sinus mucosa. [133, 136, 137]. In a study comparing sheep infected with *Oestrus ovus* with control subjects, the increase in the number of eosinophils in the mucosa was by a factor of 17 to 58 depending on the location of the biopsies within the nose and paranasal sinuses, [138]. The notable increases in the numbers of these inflammatory cells suggests that the botfly infestation induces a hypersensitivity response in the

sheep[136-138], most probably due to salivary gland proteins from the *Oestrus ovus* larvae[139].

Significance of Tissue Eosinophilia in Humans

In recent work on human sinusitis, significant tissue eosinophilia has been noted as a dominant feature, and has been linked to the production of the inflammatory mediators that may be responsible for the tissue damage in the mucosa of the nose and paranasal sinuses that characterise this condition[3, 140, 141]. There has also been shown to be a statistically significant link between the degree of tissue eosinophilia and the severity of the sinus disease, as graded by the CT scan findings of the patient[142, 143]. Thus the presence of an eosinophil driven sinusitis in the sheep would closely parallel the condition of chronic sinusitis seen in human patients. However, as with any new model, it was important to document the disease state present and compare it with healthy controls to ensure that the sinusitis present would accurately mimic the disease state in humans, and its effect on the sinus mucosa and its healing after surgery.

Assessment of the Mucociliary System

As a final but important point, it is necessary to understand the techniques that are used to assess the histological state of the nasal mucosa and the function of the mucociliary clearance system, and then to evaluate the changes wrought by therapeutic interventions.

Ham made the initial observations of cilia in 1677[144], using a microscope. However, the function observation that ciliated epithelium resulted in airway mucociliary clearance was made in the 1830s, again with the aid of the microscope[10, 145]. Since then, assessment of the integrity and function of the mucociliary clearance system has been done using two basic categories of techniques; static and dynamic studies. Static studies are essentially histological studies performed via light microscopy and electron microscopy. Light microscopy is useful for assessing reepithelialisation, epithelial height, cell density, and to some degree, reciliation[117]. Light microscopy specimens can also be used for immunohistochemical techniques, to examine the samples for the presence of collagens, growth factor receptors, etc (Unpublished Data). On the other hand, electron microscopy is useful to assess reciliation, ciliary maturity and structural abnormalities[50, 146]. Dynamic studies are those that provide physiological information about mucociliary function, such as transport rate and ciliary beat frequency, and involve the use of microscopes, photodiodes, soluble and insoluble particles and radioisotope techniques[16, 147].

Static Studies

Light Microscopy

Light microscopy is a well-established technique for the assessment of mucosal samples for many years. In his classic work in the canine model, Hilding used light microscopy to assess the state of the regenerating epithelium and the degree of fibrosis and reciliation seen[19, 74, 148]. In latter work on the effects of chronic

sinusitis on sinus mucosa in experimental models, light microscopy was used by. Jacob et al used light microscopy to characterise the nature of the inflammation in their murine model of sinusitis. They specifically assessed epithelial thickness and surface area, and also performed cell counts, by using image analysis software[28]. Marks used light microscopy again to examine the inflammatory mucosa in his rabbit model of sinusitis, but in a qualitative rather than a quantitative fashion[27]. Light microscopic techniques were used by Benninger[75] to assess the regeneration of maxillary sinus mucosa after removal, and noted inflammatory changes in the mucosa, as well as incomplete ciliary return, as did Kennedy, in his work on the maxillary sinus in a rabbit model[64]. Czaja and McCaffrey utilised light microscopy and Haematoxylin and eosin staining to examine the mucosal samples in their rabbit model of chronic sinusitis, and documented the degree of inflammation seen, and also the degree of reciliation, and then the improvement in these parameters after surgery was performed[26, 63]. In the sheep model of chronic rhinosinusitis, Shaw et al characterised the healing process of nasal mucosa after full and partial thickness injuries using light microscopy techniques. Samples underwent H&E staining, and the percentage of the surface area of the mucosa that had reepithelialized with ciliated epithelium, was calculated using image analysis software [76]. This same histological technique was also used to assess the effect of middle turbinectomy on the surrounding nasal mucosa[65], and also the effect of nasal packing on the mucosa[88]. Utilising the same sheep model, McIntosh et al examined the effect of a hyaluronic acid-based packing material on nasal mucosal healing. They assessed reepithelialisation using the same protocol as Shaw et al, but also measured the

epithelial height, again using ImageMASTER Pro image analysis software (Media Cybernetics, Inc., Silver Spring, MD), and then calculated a relative epithelial height based on the baseline epithelial height measurements taken at the time of wounding. The degree of reciliation of the mucosa was also carried out using light microscopy and the same image analysis software[117]. The same analysis protocol and healthy sheep model was also used to evaluate the effects of a hyaluronic acid-based pack impregnated with Insulin Like Growth Factor – 1 (IGF-1) on mucosal healing (Unpublished Data). Based on these studies, light microscopic analysis of H&E stained sections was utilised in the studies in this thesis for the evaluation of mucosal reepithelialisation and epithelial height.

Electron Microscopy

Owing to the limitations of light microscopy to effectively visualise structures as small as cilia, electron microscopy has been used extensively to study the structure of cilia, the degree of ciliation of epithelium and the effect of sinus disease on cilia[149]. Scanning electron microscopy is most useful for assessing the surface structure and appearance of specimens, and in this way, is essentially a light microscopy with very high magnification. Mauritzi et al. [47] and Mygind et al [150] used electron microscopy techniques to describe the ultra-structural ciliary abnormalities that occurred as a result of chronic sinusitis. In a study conducted on patients with chronic sinusitis, Toskala et al. used scanning electron microscopy to examine mucosal samples and compare them with samples from healthy patients, and noted that an increased loss of ciliated cells, the presence of non ciliated cells, ciliary disorientation

and ciliary abnormalities such as compound cilia and shortened cilia were all far more characteristic of samples from diseased mucosa than from healthy patients[50]. Benninger et al. described the effects that surgery could have on the nasal mucosa by using electron microscopy to demonstrate increased ciliary structural abnormalities and decreased numbers of cilia in the mucosa which regenerated after surgery[121]. Czaga and McCaffrey utilised transmission electron microscopy (TEM) to document the ciliary ultra structural abnormalities that occurred as a result of chronic sinusitis in a rabbit model, and the correlated these with aberrations in ciliary beat frequency (CBF). They then examined the efficacy that surgery had in reversing these abnormalities[43]. Moriyama et al used scanning electron microscopy to assess the effect that ESS and the subsequent resolution of sinus disease in human patients, had on the both the degree of reciliation of the mucosa, and also on the frequency and nature of the ciliary abnormalities[70]. Shaw utilised scanning electron microscopy and subsequent image analysis techniques to characterise the time course and degree of reciliation of nasal mucosa after full and partial thickness injuries in a sheep model [151]. McIntosh et al utilised the same SEM specimen processing and image analysis protocol to assess the effects of a hyaluronic acid-based pack on the healing of nasal mucosa after endoscopic full thickness mucosal injuries were performed, again in the sheep model[117].

Dynamic Studies

Dynamic studies of mucociliary function are those that provide information about the physiological functioning and efficacy of the mucociliary clearance system, either in part or as a whole[16].

Insoluble Particle Tests

The initial observation about the motile nature of cilia, and of mucociliary flow were made using simple light microscopy and samples of ciliated epithelium such as frog cheek[145], and this process is still used today[152-154]. From this work came the observation that the surface layer of mucous, and accompanying particles was transported over the bed of cilia. Based on this, the most basic techniques of studying mucociliary transport developed. This involved placing insoluble, visible particles on the surface of the mucosa and measuring the speed at which they moved. In the seminal work describing the patterns of mucociliary clearance in the paranasal sinus complex, Hilding used topically placed ink to visualise the patterns of clearance in a canine model[19]. Messerklinger placed India ink in the maxillary sinus of cadavers immediately post death and used his observations to document the movement of the ink from the floor of the sinus to the maxillary ostium.[22]. Shaw et al. used carbon flecks to measure the effect that middle turbinectomy had on the mucociliary clearance of surrounding mucosa in a sheep model, and also to assess the effect of partial and full thickness epithelial injury on mucociliary transport. This involved the placement of these particles on the mucosa under endoscopic guidance, and then

recording the time it took for the particle to reach a predetermined location in the nasal cavity[76, 151]. Benninger et al. utilised ink dust to assess the efficacy of the mucociliary transport system in the regenerating mucosa in a rabbit model[75]. Particle tests however, require placement of particle within the sinuses or nasal cavity, and then visualisation of their movement, and so can result in some morbidity for human subjects. In the sheep model of sinusitis, the experimental protocol that necessitated serial mucosal biopsies precluded the use of a soluble particle technique to assess mucociliary function.

Ciliary Beat Frequency

The dynamic assessment technique utilized for the studies in this thesis was the measurement of ciliary beat frequency (CBF). Although Martius, in 1844, estimated CBF using a stroboscope, the study of CBF was pioneered by Proetz in 1932[155], and Lucas in 1933[156], who observed the "metachronous beat sequences" and the phenomenon of "wave like sequences", using a beam of reflected light and a microscope. This method, however, did not provide quantifiable information. In 1962, Dalhman et al.[157] were able to accurately measure CBF using a photocell and amplification. The basic principle behind this was the interruption to a beam of light caused by the movement of cilia was converted into a measurable signal by a photoelectric cell. Further technical improvement were made to this technique over the years, and Yager et al. used this method to measure CBF in brushings of human respiratory epithelium which were obtained by brushing technique[158]. Puchelle et al. [159]introduced the use of fast Fourier transformation of the signal data. Since

then, measurement of CBF has been used to assess the effect of various nasally administered medications on ciliary function, in a chicken embryo tracheal tissue[160], and also the effect of various concentrations of saline douching fluid[161].

Wilson et al demonstrated a significant slowing of CBF in patients with chronic sinusitis, as compared to healthy subjects, and concluded that this may be responsible for the decreased mucociliary clearance observed in such patients[162]. In a study examining CBF measurements and SEM analysis of ciliation in human patients with chronic sinusitis, Joki et al. demonstrated a correlation between the degree of ciliation and ciliary orientation, and the CBF, with lower CBFs in patients who had poor ciliation or disorientated cilia[163]. Tsang et al recommended measurement of CBF as one of the simple and clinically applicable tests of mucociliary function that should be performed in patients with bronchiectasis[149]. Measurement of CBF, however, provides quantifiable data about only one aspect of mucociliary transport, namely the frequency at which the cilia are beating[16, 164], and does not provide information on the nasal mucous. Despite this, Duchateau, in a study in healthy humans, demonstrated a strong correlation between CBF and mucociliary transport time as assessed by both the saccharin test and insoluble dye techniques. This work suggested that CBF was the most important determinant for mucociliary clearance [164]. Recent work by Boek et al. examined whether changes in CBF resulted in changes mucociliary transport as measured by ^{99m}Tc scanning. They concluded that changes in CBF resulted in significant changes in mucociliary transport, and thus, CBF was a an important determining factor in mucociliary transport rate[165]. Conversely, in a study

on patients with chronic sinusitis who underwent ESS, CBF and mucociliary transport time as measured by the saccharin test, were noted preoperatively, and 6 months post-operatively. These results were also compared to normal healthy subjects. The patients were noted to have significantly lower CBFs than healthy subjects. After ESS, although there was a significant improvement in mucociliary transport time, there was no improvement in CBF. The authors concluded that patients with chronic sinusitis may have an underlying ciliary disorder which may predispose them to sinusitis, and that since the CBF did not improve after surgery, the resolution of symptoms in patients may be due to factors other than an improvement in the health of the ciliated epithelium. Those factors included mucous viscoelasticity or tethering[162].

Summary

In summary, this study had 3 aims. The first was to document and validate the sheep model of eosinophil driven sinusitis. The second was to assess the efficacy of a hyaluronic acid based nasal pack on nasal mucosal healing after ESS in sheep with eosinophilic chronic rhinosinusitis. The third aim was to assess the efficacy of IGF-1 incorporated into the hyaluronic acid based nasal packs on mucosal healing after ESS in sheep with eosinophilic rhinosinusitis. In order to assess the effect of these interventions on mucosal healing, light microscopy was the selected technique to measure mucosal reepithelialisation and epithelial height, and electron microscopy for assessment of mucosal reciliation. To perform a dynamic assessment of mucociliary function, ciliary beat frequency was measured.

Methods and Materials

All procedures received approval from the Animal Ethics Committees at both the Queen Elizabeth Hospital and the University of Adelaide. The subjects were managed under the conditions set out in the NH&MRC guidelines for the ethical treatment of animals.

We considered that a 25% improvement in reepithelialisation or reciliation due to the application of either the nasal packing or the packing impregnated with IGF-1 would be deemed clinically significant. Based on this, a power study showed that a sample size of 9 in each of these two groups, with a 5% test level would provide a power of 80%.

The subjects for this series of studies were 18 normal sheep. They were kept in an outdoor animal holding and research facility for the duration of this study, and transported to and from the animal laboratory as needed.

Standardisation Procedure

The initial standardisation procedure was performed as previously described by Shaw et al[65]. The subject was given a general anaesthetic involving IV Sodium Pentobarbitone 60mg/ml, 12 ml, to induce anaesthesia and facilitate intubation, and then Halothane 1%-4%, and Oxygen were used to maintain anaesthesia. The subject was then placed on a specially designed cradle that positioned the sheep in a supine position with the nose lying in a vertical orientation. Surgical drapes were placed over

the animal to ensure an aseptic technique. 3 puffs of Co-Phenylcaine forte spray (Lignocaine Hydrochloride 50mg/ml (5%), Phenylephrine Hydrochloride 5mg/ml (0.5%), Benzalkonium Chloride 0.1mg/ml, Paedpharm, WA, Australia) were placed in each nostril. This was followed by 2 ml of local anaesthetic solution, (2% Xylocaine, 1/80000 adrenaline) which was injected via a dental syringe, anteriorly into the ridge of attachment of the middle turbinate on the lateral nasal wall. 6 neuropatties were then soaked in a total of 2ml of 10% cocaine solution. On each side, a neuropattie was placed above the middle turbinate, one was placed below the middle turbinate and one placed on the medial surface of the middle turbinate lying between the turbinate and the nasal septum. All of the neuropatties were placed lying in a longitudinal orientation lying anterior to posterior. The patties were left in place for 10 minutes. These procedures were performed to ensure adequate haemostasis during the procedure and are analogous to the use of topical agents during ESS on human patients.

The neuropatties were then removed and the nasal cavity was inspected using a 0 degree 4mm rigid Hopkins rod and a 30 degree 4mm Richards Sinuscope, connected to a Storz Endovision camera system and Sony PVM Trinitron Monitor. A Storz Endovision light source and cable was used to provide illumination. Endoscopic instruments were custom designed to suit the sheep nasal cavity and manufactured by Micro France. Endoscopic turbinectomy scissors were then used to remove the middle turbinate complex from the lateral nasal wall via careful dissection along the ridge of attachment of the turbinate, under direct endoscopic vision. Care was taken to avoid damage to either the lateral nasal wall or the nasal septum.

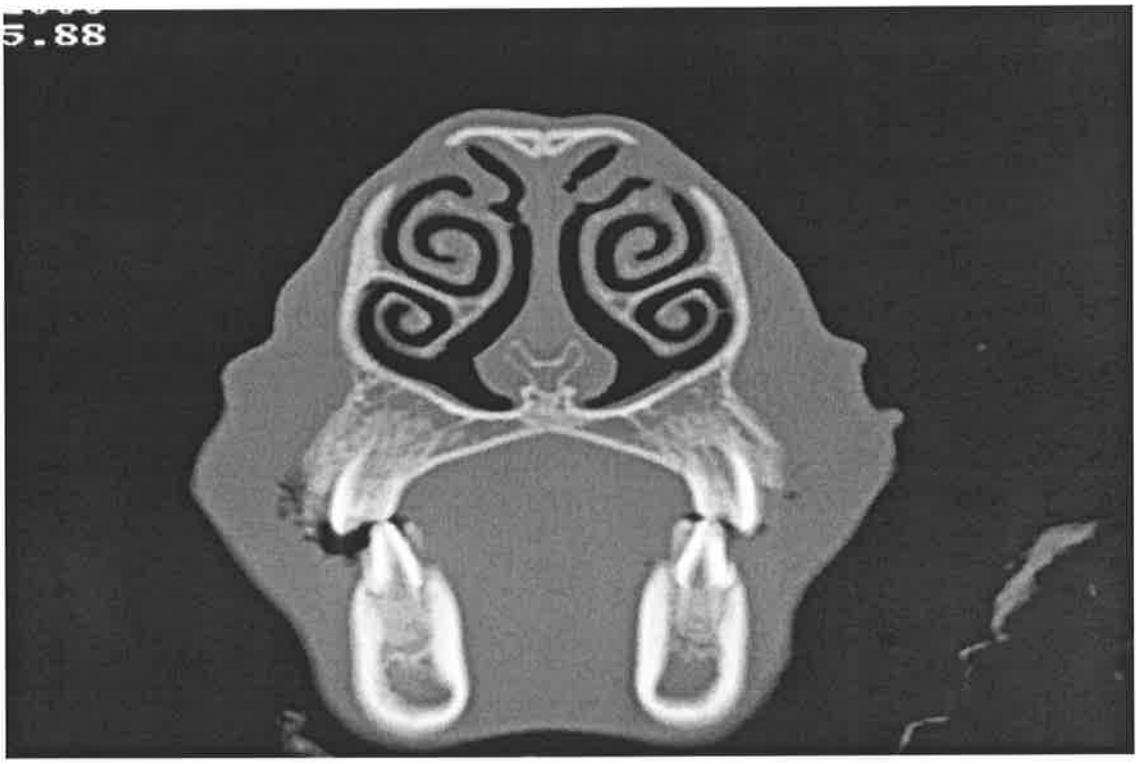

Figure 1

Figure 1 illustrates a coronal CT scan through the nasal cavity of the sheep demonstrating the large middle turbinate complex that fills the nasal cavity.

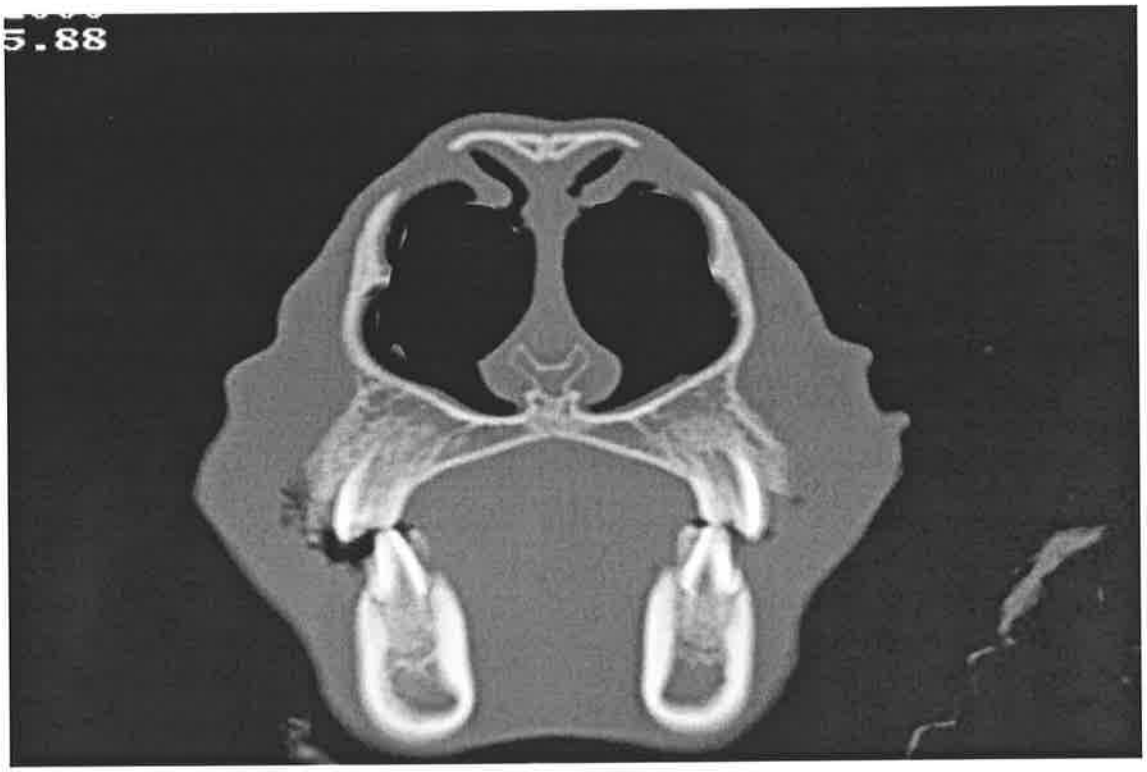

Figure 2

Figure 2 illustrates the appearance of the sheep nasal cavity after removal of the middle turbinate complex.

Initial haemostasis was then performed using local application of the previously removed neuropatties, and if this proved insufficient, unipolar suction diathermy was utilised. At the end of the procedure, the cavity was carefully inspected to ensure haemostasis, the anaesthetic agents were turned off and the animal extubated. The animals were kept in the holding facility of the animal laboratory for 3 days and carefully monitored for any evidence of pain or distress, or any postoperative bleeding. They were then returned to the animal holding facility (paddock) until their next procedure.

Full Thickness Injury and Day 0 Baseline Biopsies

After a period of 28 days had elapsed, the subjects were returned to theatre for the initial wounding procedure, again previously standardised and described [65, 76, 117]. Once again, the cavity was initially inspected with the endoscope. A pair of endoscopic biopsy forceps was then used to take samples of tissue from the lateral nasal wall bilaterally after an initial mucosal incision and elevation using a Freer's elevator. The procedures for processing these tissue samples will be discussed later. A microdebrider fitted with a 4mm cutting blade (Xomed, Jacksonville Fl) was then used to create a full thickness mucosal injury on the lateral nasal wall, in 2 longitudinal strips, above and below the ridge of previous attachment of the middle turbinate. This procedure was then repeated on the other side. The sites of mucosal injuries are marked on figure 3 with the red stars. A specially designed measuring device was used intranasally, to ensure that the injury commenced at least 15mm

from the aperture of the nostril, and extended posteriorly to about 10 mm from the anterior aspect of the ethmoid terminal ridge.

In each individual subject, the exact dimensions of the injury and their relationship to these fixed landmarks were documented. These measurements were then used to plan the sites of future biopsies on Days 28, 56, 84 and 112, and these were documented on a planning sheet for each subject. On a side selected by computer randomisation, either a 15mm x 50mm piece of hyaluronic acid based nasal packing material in 9 of the sheep, or the same sized piece of packing impregnated with IGF-1 in the other 9, was placed on the lateral nasal wall under endoscopic guidance. The other side was left unpacked as a control. The position of the nasal packing is indicated by the white crescents in figure 3.

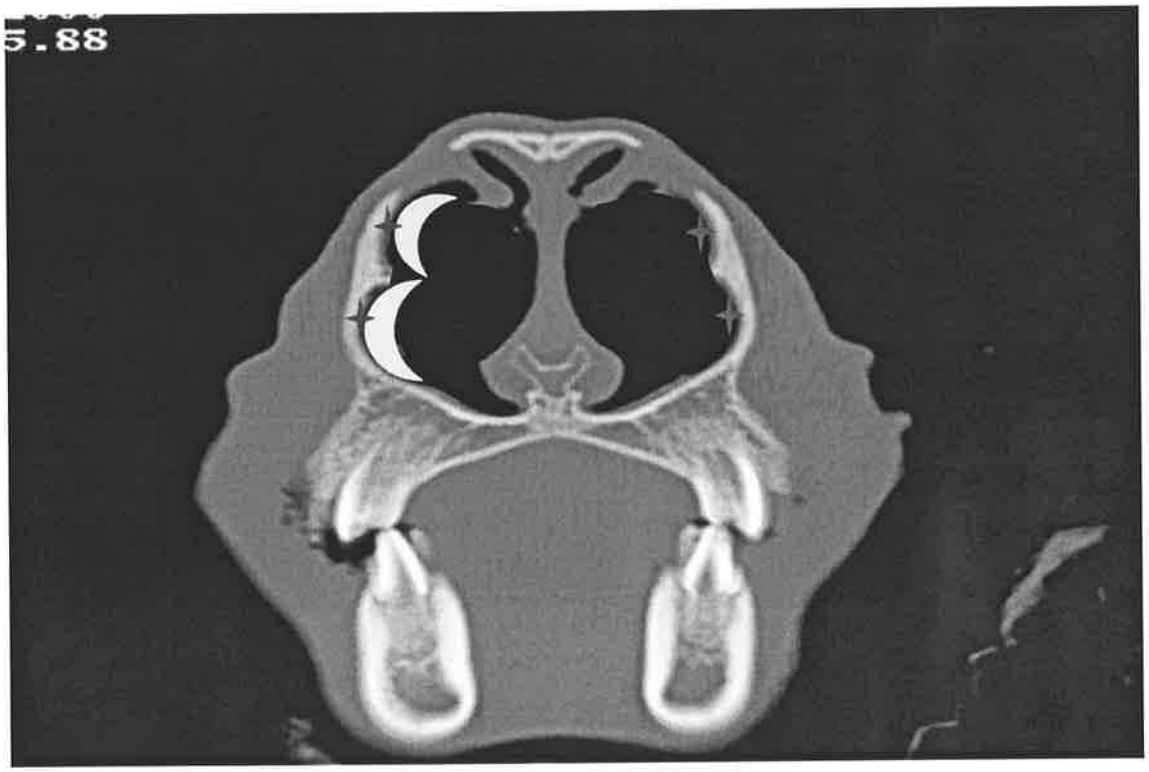

Figure 3

Figure 3 illustrates the sites of mucosal injuries and nasal packing in the sheep nasal cavity.

In order to simulate adjacent mucosal surface injuries, full thickness mucosal wounds were created between the lateral nasal wall and the adjacent ethmoid terminal with the microdebrider. Care was taken to minimise the damage to the delicate bone of the ethmoid terminal, and to avoid dislocation and medialisation of the ethmoid terminal. The ethmoid terminal or turbinate closely resembles the middle turbinate in humans. It is a similar size and has the same proximity to the lateral nasal wall as the human middle turbinate, as illustrated in figure 4, and figure 5(closer view)

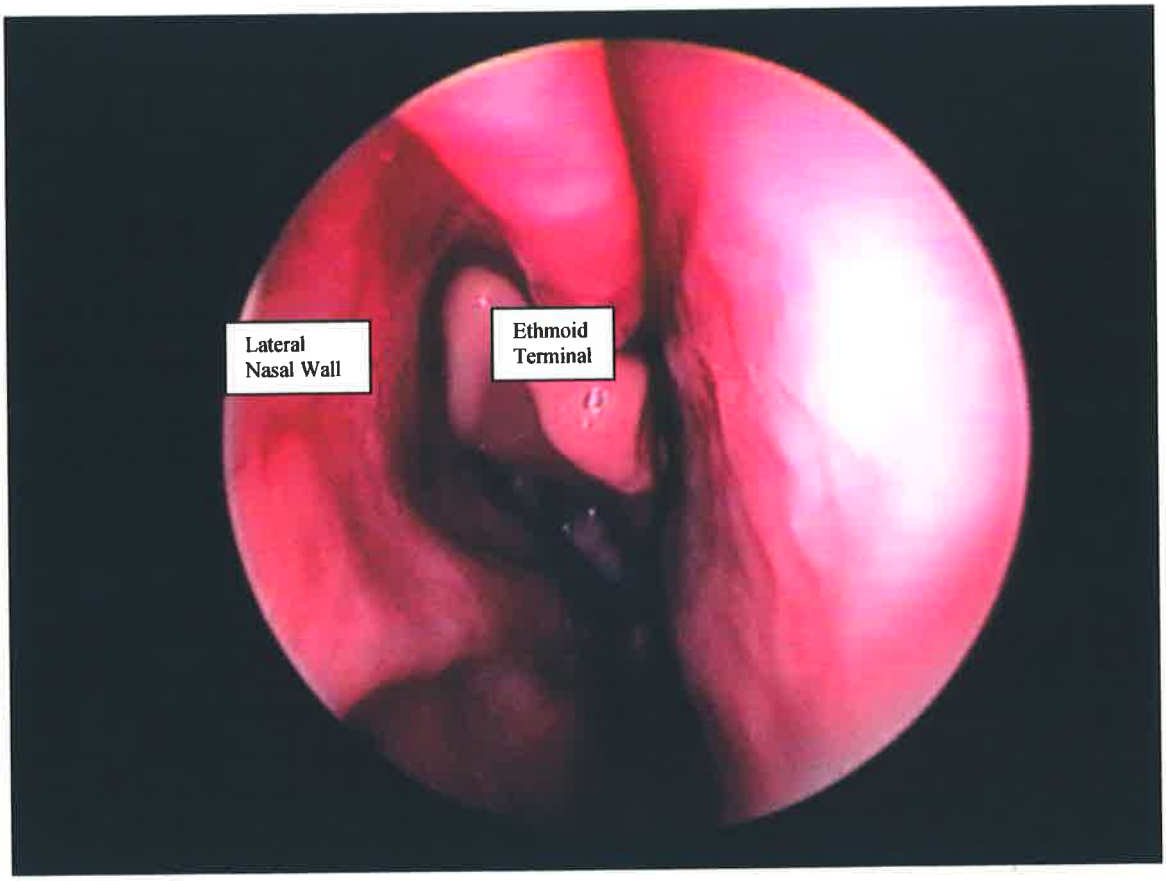

Figure 4

Figure 4 illustrates the appearance of the lateral nasal wall and the ethmoid terminal; the site where adhesions were created

Computer generated randomisation was used to select the side of the nose to be packed. In each subject, a 1cm x 1cm piece of hyaluronic acid based packing, with or without impregnated IGF-1, was placed between the two injured surfaces on the same side where packing was placed on the lateral nasal wall, while the control side was left unpacked. Packing impregnated with IGF-1 was used in the same sheep in which the IGF-1 impregnated packing was used to pack the injuries on the lateral nasal wall. The subjects were observed in the animal laboratory for a period of 3 days, and then transferred to an animal holding facility.

Day 28, 56, 84 and 112 Biopsies

After a further period of 28 days, the animals were then returned to the animal laboratory for their first post-injury biopsy. The biopsy was performed under sedation, attained using Xylazene, 20mg/ml, 0.2 ml via intramuscular injection. The nasal cavity was then inspected using the rigid endoscope. The first sample taken was a mucosal brushing for ciliary beat frequency. This technique will be described later. The nasal cavity was then sprayed with Co-Phenylcaine forte for the purposes of local anaesthesia. After allowing 2 minutes for this to take effect, a Freer's dissector and biopsy forceps were then used to raised a small mucosal flap and then take a mucosal biopsy from the area predetermined by the planning sheet. The exact site of the biopsy was determined via the use of a custom designed measuring device under endoscopic guidance. The brush cytology was taken from a separate area of the wound. This procedure was then repeated on the other side. The subjects were then examined endoscopically to ensure haemostasis. They were kept in the animal laboratory for 3 days before returning to the animal holding facility.

This biopsy procedure was then repeated on Days 56, 84, and 112 post-injury. After the final biopsies were performed, the subjects were held in the animal holding facility for a further 12 months, with the intention being to perform further biopsies at this time to assess long term healing outcomes. Those results will form the basis of a further paper and will not be discussed in this thesis.

Preparation and Analysis of Light Microscopy Specimens

The protocol for the processing of the light microscopy specimens was identical to that previously described by Shaw et al[65, 76] and McIntosh et al.[117]. After removal from the subject, the sample was placed between 2 layers of foam within a plastic cassette, each with a unique cataloguing number assigned to it and labelled. A 23-gauge needle was used to manoeuvre the specimen into an optimum position. If there was blood contaminating the specimen, it was washed off with 0.9% saline solution. The cassettes were then placed in a 10% formaldehyde solution for a period of 3 hours. After this, they were transferred to a 70% ethanol solution for storage. These cassettes were then placed in a Tissue-Tek® VIP Processor for overnight fixation. The next morning, the specimens were removed and mounted in paraffin wax. After this, the specimens were then placed in a refrigeration unit (<-4 degrees Celsius) for a period of 1 hour. The frozen mounts were then sectioned using a Leica microtome set to 4 microns, and mounted on glass microscope slides. The slides were allowed to dry overnight in a heating unit, and then were stained with haematoxylin and eosin. After being allowed to dry, cover slips were placed over the sections and secured with an appropriate adhesive.

Each slide was labelled with the specimen's catalogue number, which did not in any way identify the subject, the side from which the specimen came, or the date of the biopsy. In this way, the researcher was blinded when assessing the slides.
Using Image Pro Plus image analysis software, and a light microscope, digital images were created from the slides, which were then used for analysis.

Assessment of Reepithelialisation

The degree of reepithelialisation in the healing tissue was determined by measuring the length of visible surface tissue on the area analysed, and then the length covered by epithelium, as illustrated in figures 6 and & 7. The % reepithelialized could then be calculated. This was performed on the entire area of mucosal surface seen on the images. This method of calculating reepithelialisation has previously validated and published by Shaw and McIntosh [76, 117].

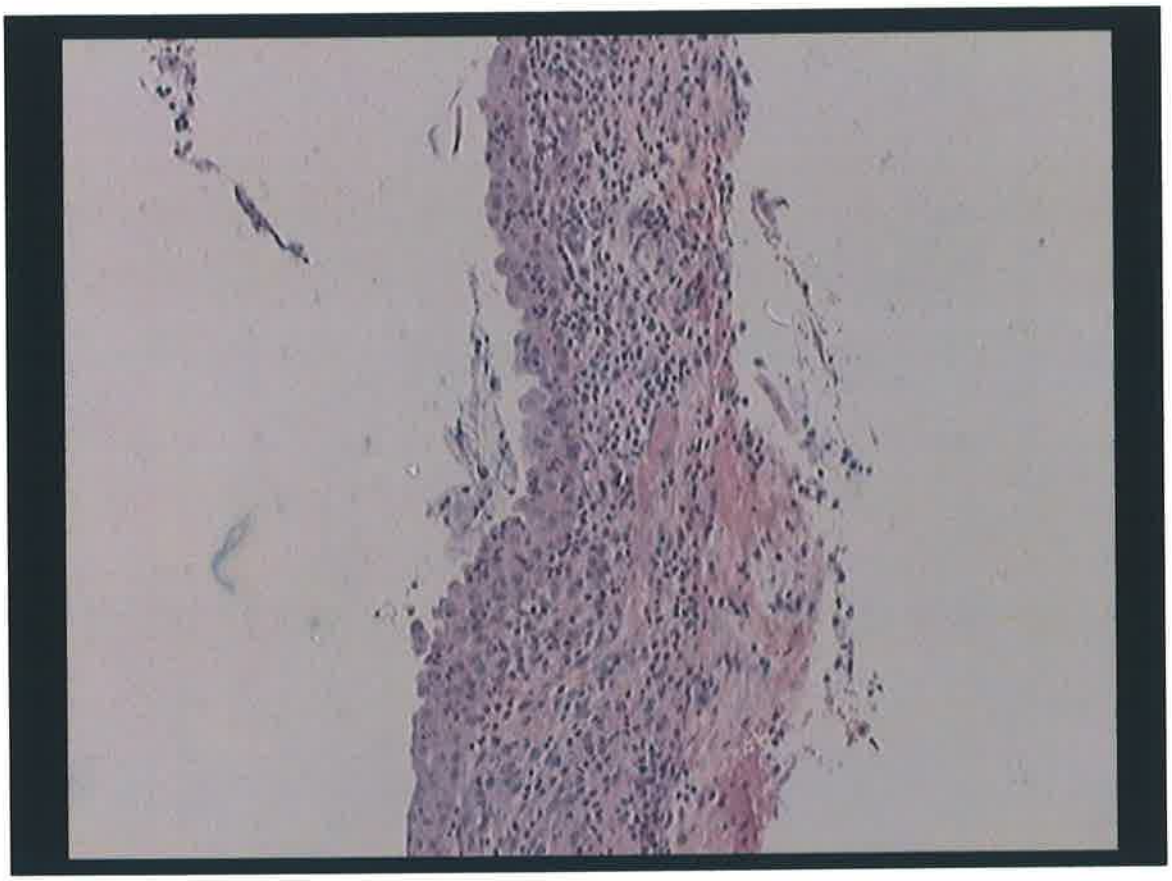

Figure 6

Figure 6 A light micrograph of the nasal mucosal epithelium

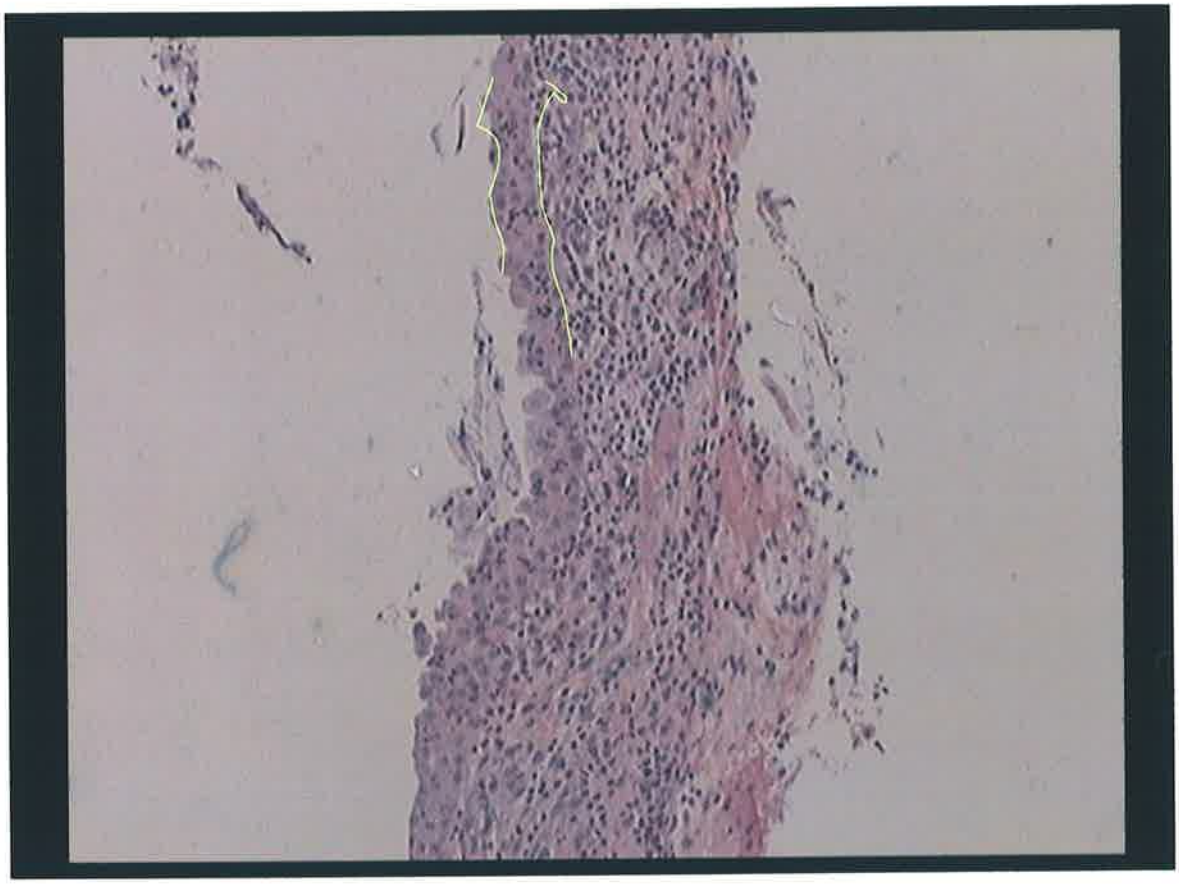

Figure 7

Figure 7 – The technique of assessing mucosal reepithelialisation.

Assessment of Epithelial Height

Epithelial height was calculated using the same digital images used for measuring re-epithelialisation. The epithelial height was the average distance between the apical surface of the epithelium and the basement membrane, over the entire length of mucosa analysed for the reepithelialisation measurements (see figure 8).

The relative epithelial height was then calculated by comparing measurements at the various time points to the baseline epithelial heights as measured at day 0.Again, this technique has been previously described[117]. A student's T Test was used to analyse the relationship between reepithelialisation and relative epithelial height, and the use of nasal packing.

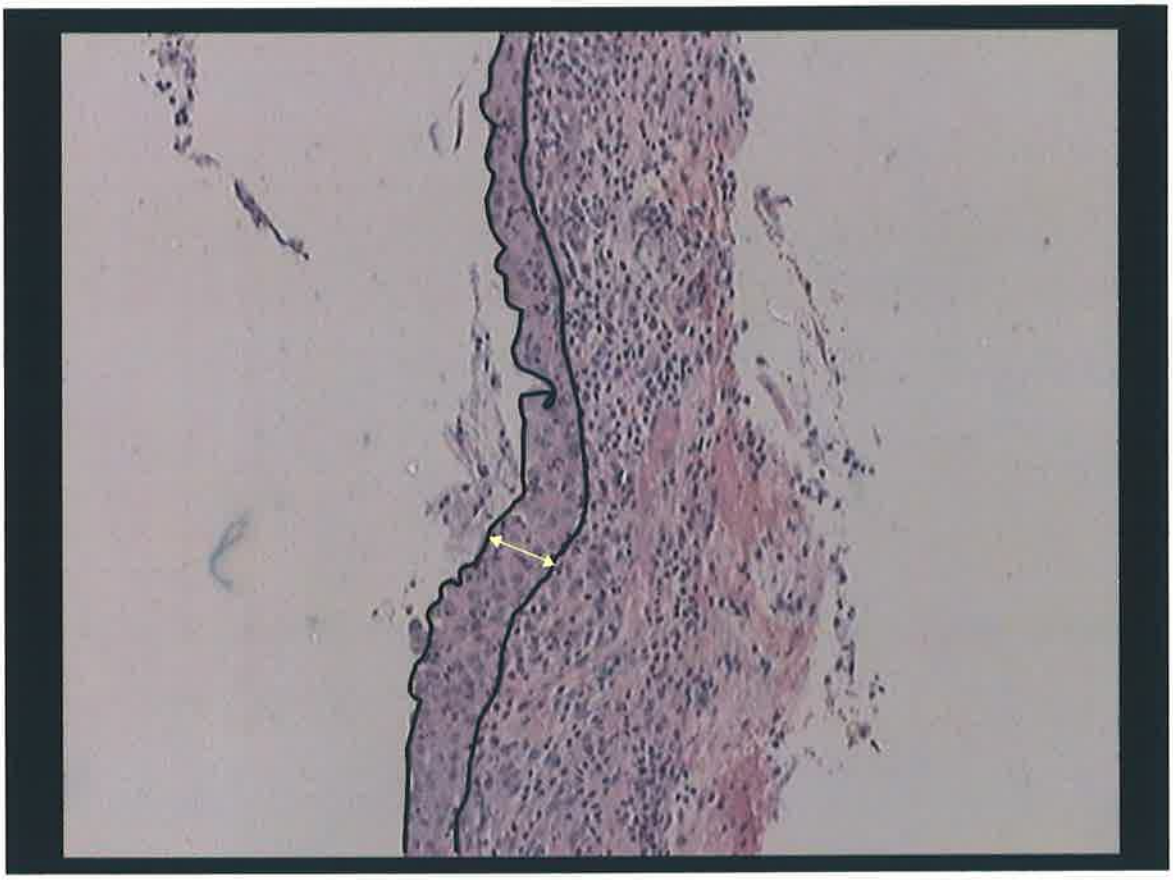

Figure 8

Figure 8 – Assessing epithelial height

Preparation and Analysis of Electron Microscopy Specimens

Specimens were taken at days 0, 56 and 112 for electron microscopic assessment of ciliary regeneration. After biopsy, the specimens were placed in a phosphate buffered saline solution, and surface blood and mucous was carefully removed using a sonication process. The specimens were then placed in a solution consisting of 4.0% paraformaldehyde and 1.25% glutaraldehyde, phosphate buffered saline pH 7.2. The specimens were then stored at 4°C. Specimen fixation was then performed using an automated processor. The specimens were dehydrated using serial strengths of acetone and alcohol. The process involved a sequence of immersions in different media; Osmium tetroxide, 70% ethanol, 90% ethanol, 95% ethanol, 100% ethanol, a mixture of 100% ethanol and 100% acetone in a proportion of 1:1, and then 100% acetone. The specimens were then dried in a carbon dioxide critical point dryer and then mounted on electron microscopy stubs. A final coating of gold and carbon was then applied.

Digital images of the surface were then obtained using a Phillips Field Emission Scanning Electron Microscope Excel 30, and these were then stored in a high resolution TIFF format. These images were taken from 4 areas of surface mucosa on each specimen, and at both 500x and 2000x magnification (see figure 9). AnalySIS Pro® (Soft Imaging Systems GmbH) (Version 3.00) image analysis software was then used to calculate the degree of reciliation in the healing mucosa.

The software allowed the images to be assessed on an intensity scale, and cilia proved to be electron dense and thus bright as compared to the background that was less intense. This intensity difference enabled calculation of the surface area of the mucosa covered by cilia. These calculation were performed at 500x magnification, and our group has previously validated this method of measurement by demonstrating minimal inter and intra observer differences between 2 blinded researchers on 2 different occasions (unpublished data). Guoet al[166], and Clark et al.[167], described a similar method of assessing ciliary density using SEM and image analysis. A Student's T-test was utilised to determine the relationship between mucosal reciliation and the presence or absence of nasal packing

Preparation and Analysis of CBF Specimens

The samples taken for CBF measurements were collected using a nasal brushing techniques, previously well established in the literature from a planned area of the wound. This non-invasive technique has been demonstrated to reliably sample the ciliated epithelium of the nasal cavity, and can be tolerated without the use of local anaesthetic. [47, 158, 162, 168, 169]. The samples were taken after the sheep were given intramuscular sedation but prior to the application of any local anaesthetic. This was due to the well known cilio-inhibitory effects that local anaesthetic preparations such as cocaine and lignocaine, and decongestants such as oxymetazoline, have been shown to demonstrate with in-vivo models[169-172]. General anaesthetic agents such as halothane have been shown to have significant effects on ciliary beat frequency[173-175]. As the day 0 baseline mucosal biopsies and lateral nasal wall

injuries were performed while the sheep were under general anaesthesia, no baseline CBF samples were taken at day 0. When the day 28 biopsies were performed using intramuscular sedation, the baseline CBF samples were taken from an uninjured area of lateral nasal wall mucosa to simulate day 0 baseline biopsies. A Cyto-brush (Crown Scientific) was moistened in Ham's nutrient solution, and a brushing was taken from the nasal mucosa. The position of these biopsies was predetermined using the planning sheet for each subject, and localised intranasally using a measuring device and endoscopic visualisation. The brush was then swirled in a specimen tube containing the nutrient medium to dislodge the samples. These tubes were then sealed and placed in a carbon dioxide incubator set at 37°C. The reason for this is that ciliary activity has been shown to be extremely sensitive to temperature, with the optimum temperature being between 30° and 40° C, essentially normal body temperature. Temperatures below this range result in significant slowing of CBF[16, 176]. Within 3 hours of sampling, CBF measurements were made. Previous work has demonstrated that cilia remain viable and motile in appropriate nutrient solutions for approximately 34 hours[168, 169]. After the specimen tubes were agitated to provide a suspension of cells, a 1 ml volume was pippetted from the tube onto a glass slide with a well. A cover slip was placed over the top of the well, and sealed with silicon grease. The slide was then placed on the stage of a Nikon Eclipse TE 30 phase contrast microscope (Nikon Corporation, Tokyo). The stage was fitted with a Series 20 Chamber, model RC-26 (Warner Instrument Corporation) warming device that was preset to 37°C. The light source was a Nikon Super High Pressure Mercury Lamp (Nikon Corporation, Tokyo), and the signal from the microscope was processed via a

Biopac Data Acquisitions System, Model MP 100 (Biopac Systems Inc., Santa Barbara, Ca) and a PC. Under microscopic vision, areas of ciliated cells were located, and CBF measurements were performed using a photodiode technique and Fast Fourier transformation. A total of 10 randomly selected areas of ciliated cells were measured for each side of each sheep, and the CBF averaged.

A Student's T-Test was used to determine the relationship between the CBF at various time points and the use of nasal packing.

Assessment of Intranasal Adhesions

At the time of the day 28 biopsy, the nasal cavity was initially inspected using the endoscope. At this time, the presence or absence of an adhesion between the ethmoid terminal and the lateral nasal wall was noted, and the image of this was captured using a Video Cassette Recorder attached to the Sony Camera system that provided the endoscopic vision. This process was repeated at each of the biopsy time points. Figure 10 illustrates an example of an adhesion, whereas figure 11 illustrates the absence of an adhesion. We did not attempt to grade the adhesions based on size due to the small sample size in this study

A Fischer's Exact Test was used to determine the relationship between the formation of adhesions and the presence or absence of nasal packing.

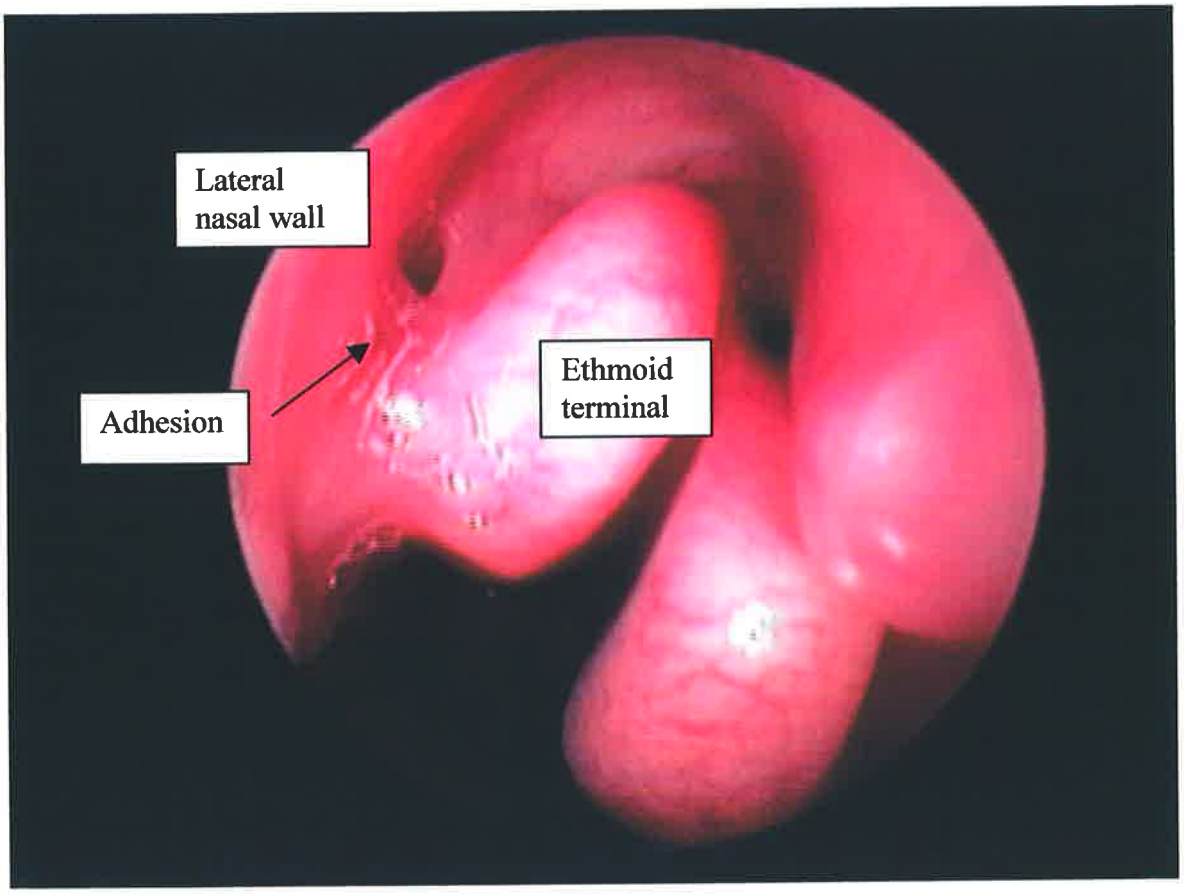

Figure 10

Figure 10 – Adhesion formation between the lateral nasal wall and the ethmoid terminal

Results

The Effect of Inflammation On Mucosal Healing

Light Microscopic Analysis of Epithelial Healing

Reepithelialisation

Light microscopic analysis was performed to determine the effect of chronic sinusitis on reepithelialisation of injured mucosa (Figure 12), by comparing the control specimens taken from the 9 sheep with sinusitis that were treated with Merogel packing, with the control specimens from a series of healthy sheep who were also treated with Merogel packing. At all time points after day 28, there was a significant difference between the degree of reepithelialisation of the two groups, with the chronic sinusitis group showing far worse reepithelialisation; day 56 (34.3% vs. ,78.4 % ($p<0.01$)) and day 84 (59.7% vs. 91.9% ($p<0.05$)). At day 112 post injury, the chronic sinusitis group showed only 65.3% reepithelialisation, whereas the healthy sheep attained 94.7% reepithelialisation ($p<0.01$).

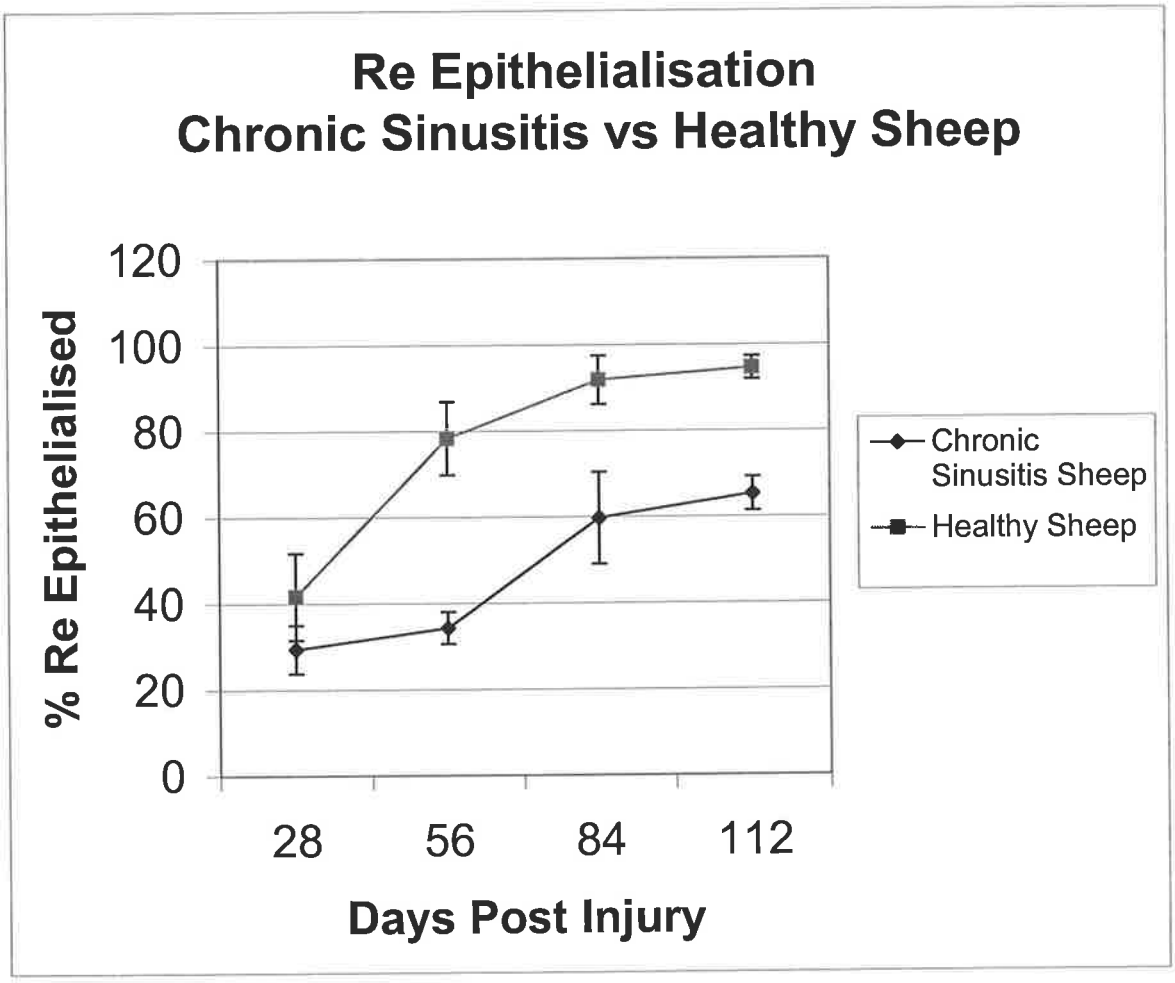

Figure 12

Figure 12 illustrates the comparison of mucosal reepithelialisation between the healthy sheep and those with sinusitis. (Mean +/- SEM)

Epithelial Height

Epithelial height was measured, and then expressed relative to the baseline epithelial height, in order to derive the relative epithelial height measurements (Figure 13). At day 28, the relative epithelial height in the infested sheep was significantly greater than that of the healthy sheep, (1.41 vs. 0.81 ($p<0.05$)). At the latter time points of days 56, 84 and 112, the values in the healthy sheep were 0.90 at day 56, 0.95 at day 84 and 0.93 at day 112, while the values in the infested sheep were 1.03 at day 56, 1.17 at day 84 and 1.02 at day 112. There was no statistically significant difference between the two groups at any of these time points when these results were analysed using a Student's T test.

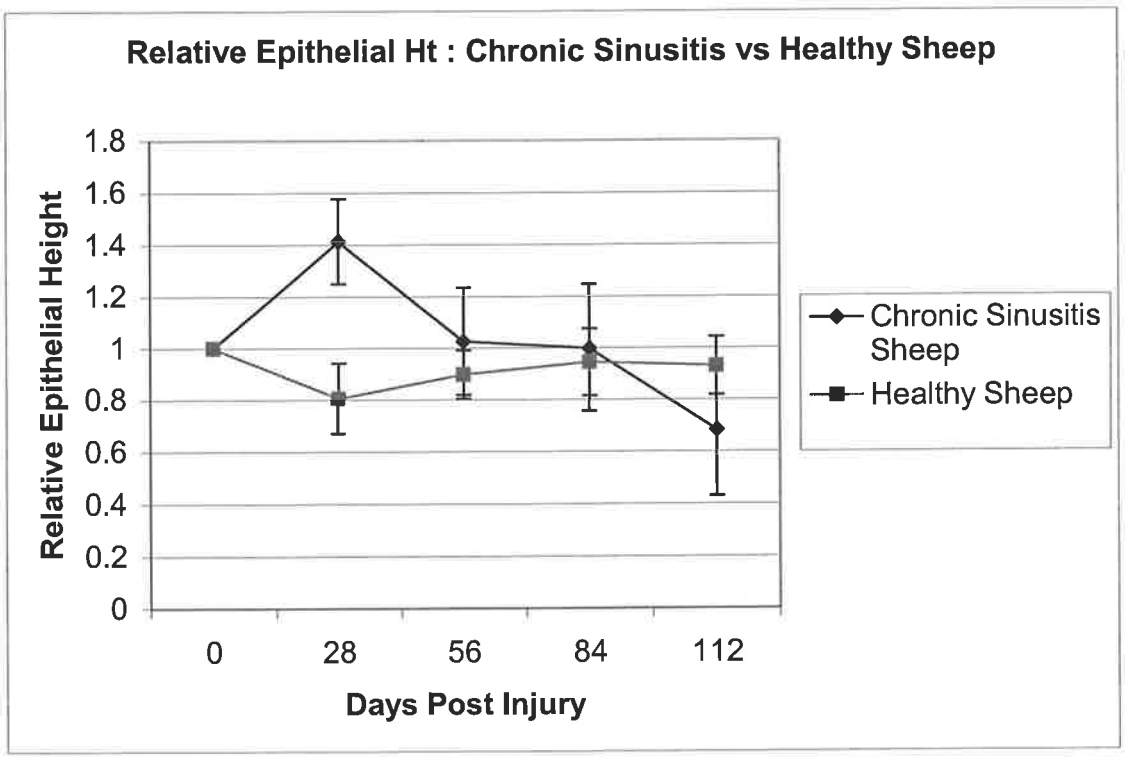

Figure 13

Figure 13 shows the comparison of relative epithelial heights (a comparison to baseline) between the chronic sinusitis group and the healthy sheep. (Mean +/- SEM)

Scanning Electron Microscopic (SEM) analysis of Reciliation

The degree of reciliation in mucosal samples from the groups was determined using SEM at 500x magnification (Figure 14). At day 56, there was no significant difference between the two groups, but at day 112, the chronic sinusitis group demonstrated 86.9% reciliation versus 76.65% reciliation in the control group ($p<0.01$). Although this was statistically significant, we do not feel that a 10% difference in ciliation would necessarily be clinically significant.

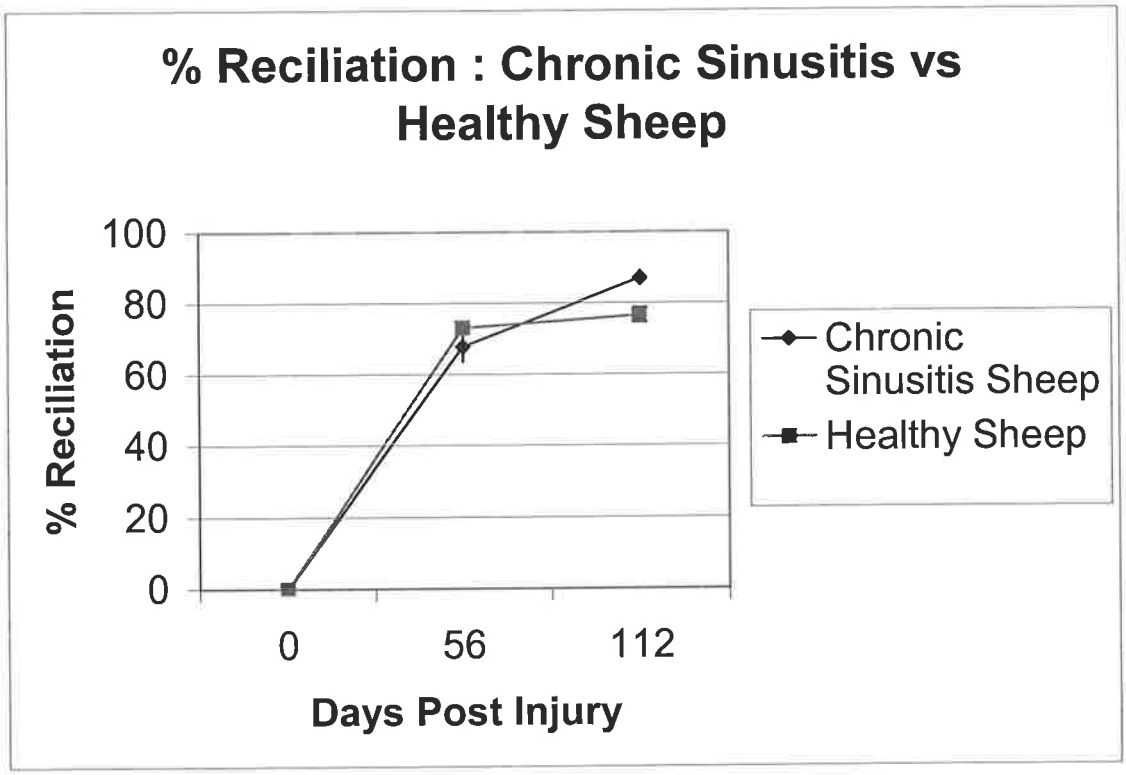

Figure 14

Figure 14 represents the comparison in reciliation as assessed by SEM between the chronic sinusitis group and the healthy group. (Mean +/- SEM)

The Effect of Hyaluronic Acid Based Nasal Packing (Merogel) on Mucosal Healing

Light Microscopic Analysis

Reepithelialisation - Merogel

Figure 15 illustrates the degree of reepithelialisation noted in the control sides compared to the sides packed with the hyaluronic acid. In the control group, the percentage of the mucosa that had reepithelialized was 29.43% at day 28, 34.28% at day 56, 62.77% at day 84 and 65.30% at day 112. In the sides packed with the hyaluronic acid/IGF-1, the degree of reepithelialisation was 20.69% at day 28, 45.93% at day 56, 58.79% at day 84 and 62.05% at day 112. There were no statistically significant differences in mucosal reepithelialisation at any time point between the 2 groups when analysed with a Student's T test, nor was there any trend, with the values for both groups being similar at each time point (Figure 15).

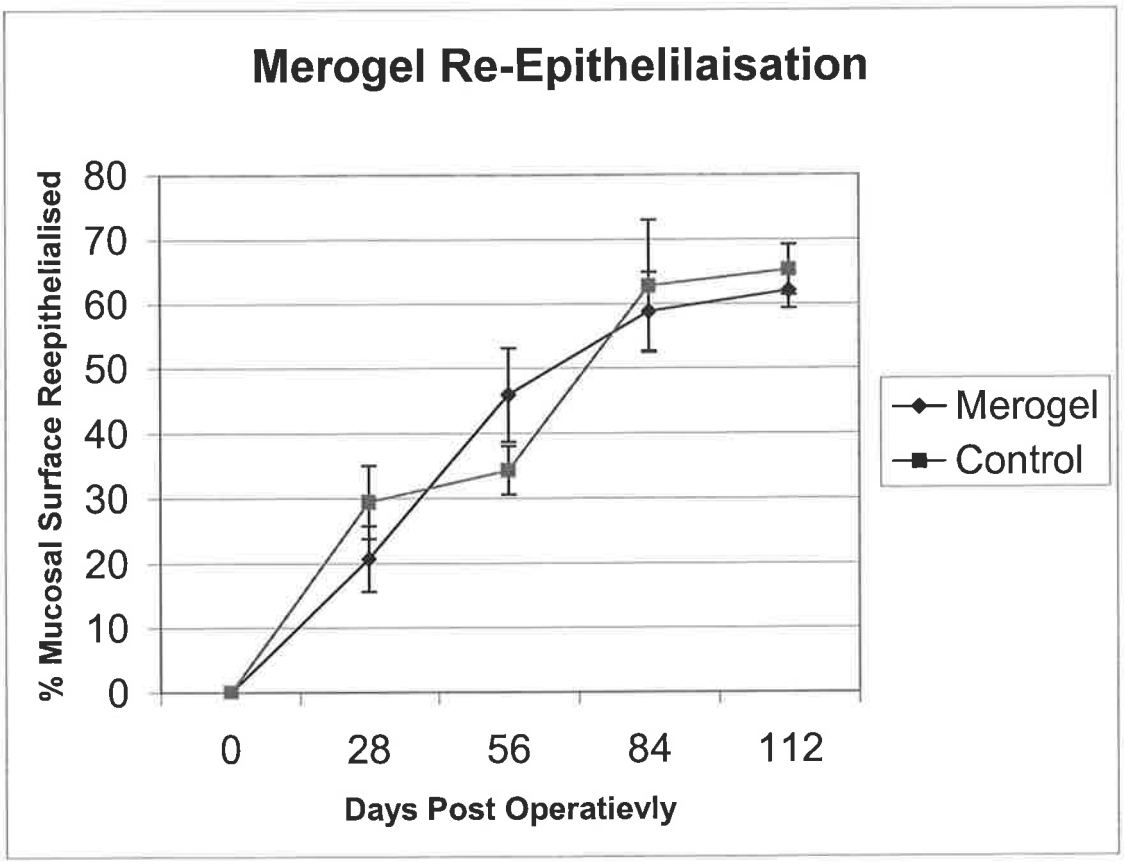

Figure 15

Figure 15 illustrates the degree of mucosal reepithelialisation in the two groups post operatively. (Mean +/- SEM)

Epithelial Height - Merogel

The relative epithelial height, as a measure of the thickness of the regenerating epithelium, is illustrated in figure 16. In the control group, the values were 1.41 at day 28, 1.03 at day 56, 1.17 at day 84 and 1.02 at day 112. In the Merogel group, the values were 1.33 at day 26, 0.92 at day 56, 1.34 at day 84 and 0.99 at day 112. There were no significant differences at any time point between the sides treated with Merogel packs and the control sides, nor was there any trend (Figure 16). Both groups returned to preoperative epithelial heights by day 112 postoperatively.

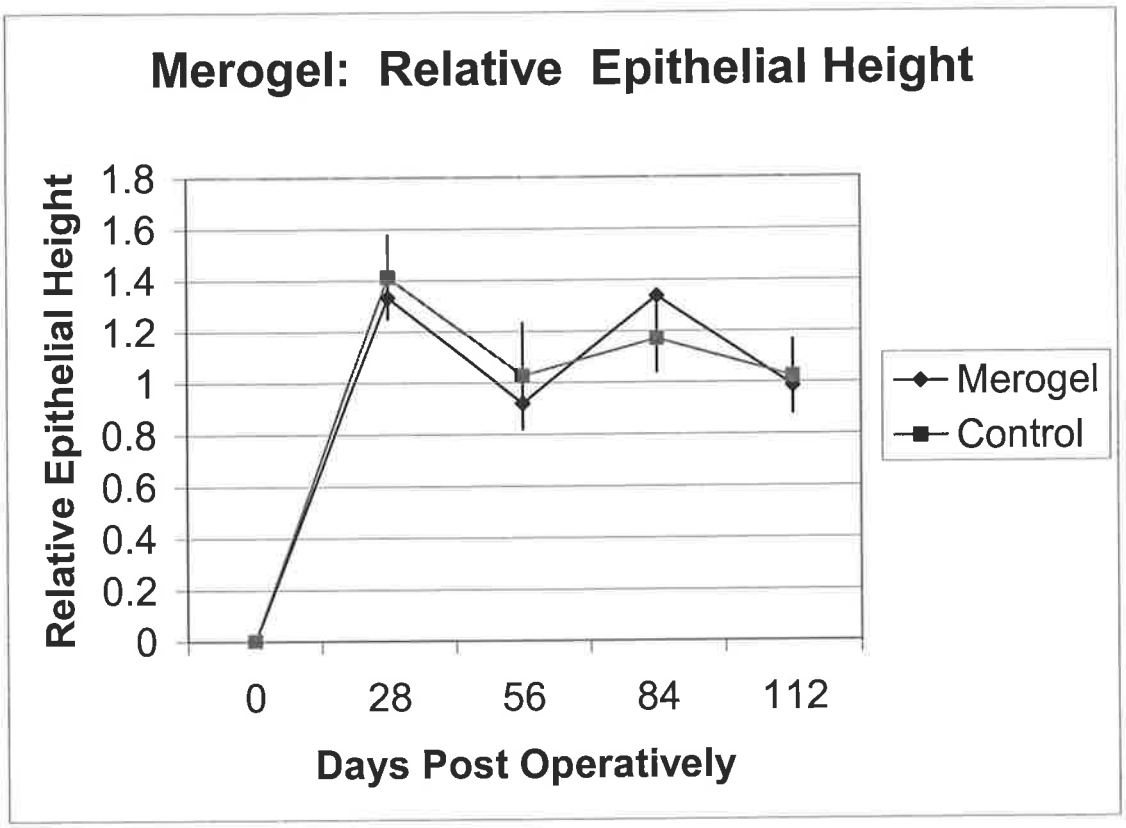

Figure 16

Figure 16 illustrates the relative epithelial height of the regenerating mucosa in the sides treated with Merogel compared to untreated controls (Mean +/- SEM)

Electronic Microscopic Analysis

Reciliation - Merogel

Electron microscopic analysis of the regenerating cilia revealed 67.77% reciliation at day 56 and 86.92% at day 112 in the control group, compared to 64.96% at day 56 and 79.60% at day 112. At day 112, there was a statistically significant difference between the Merogel group (79.6% reciliation) compared to the control group (86.9%), ($p<0.01$) (Figure 17).

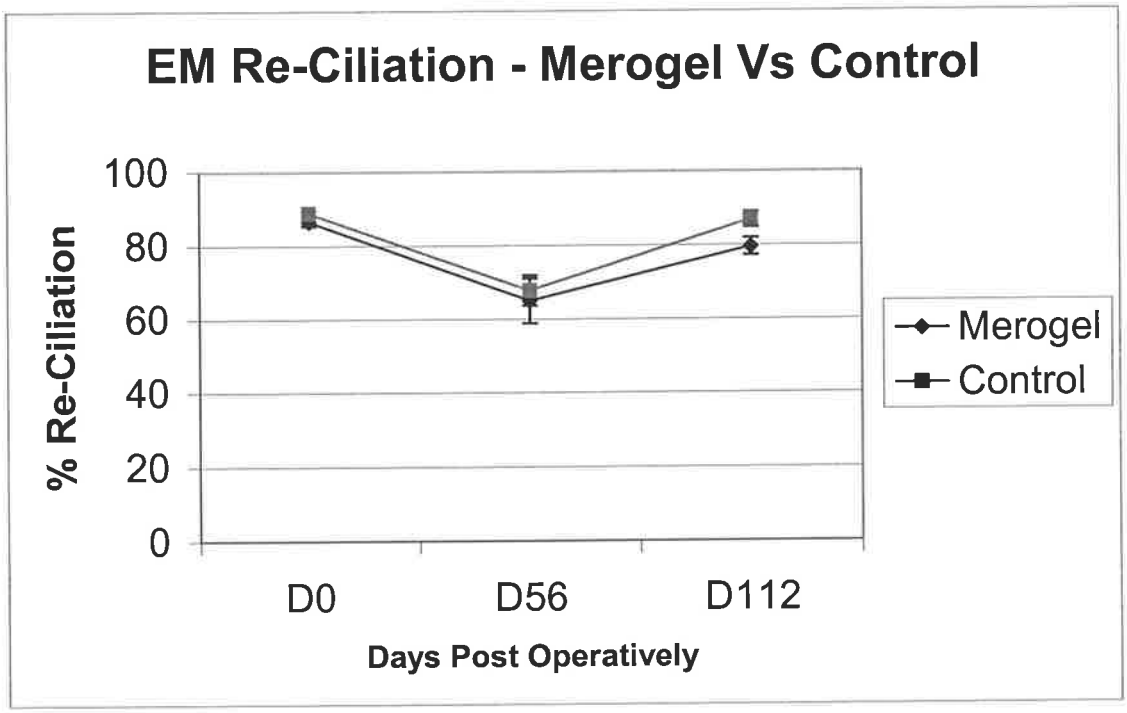

Figure 17

Figure 17 represents the degree of re-ciliation of the mucosa post operatively, as assessed by electron microscopy. (Mean +/- SEM)

Adhesion Formation - Merogel

On the control sides where adhesions were created, an adhesion rate of 22.22% was noted, and this was consistent over all time points. In comparison, adhesions were seen in 44.44% of the sides treated with the hyaluronic acid packs, but this difference was not statistically significant ($p>0.05$). (Table 1)

Adhesion Formation		
	Merogel Packing	**Controls**
Adhesion	**4 (44.44%)**	**2 (22.22%)**
No Adhesion	**5 (56.56%)**	**7 (77.88%)**

Table 1

Table 1 illustrates the rate of adhesion formation in the two groups.

Effect of Hyaluronic Acid/IGF-1 (Merogel/IGF-1) Packing on Mucosal Healing

Light Microscopic Analysis

Reepithelialisation – Merogel/IGF-1

Assessment of the degree of mucosal reepithelialisation in the sides packed with the hyaluronic acid/IGF-1 packs compared to the control sides, by light microscopy, is shown in Figure 18. For the control side, the levels of reepithelialisation at each time point were 18% on day 28, 51% on day 56, 52 % on day 84 and 65 % on day 112 For the sides packed with hyaluronic acid packs impregnated with IGF-1, the values were 17 % on day 28, 38 % on day 56, 71 % on day 84 and 63 % on day 112. There were no statistically significant differences between the two groups at any time point when analysed using a Student's T-Test. There was an obvious separation at day 84 with the sides packed with the control sides demonstrating 52 % reepithelialisation compared to 71 % in the sides packed with hyaluronic acid/IGF-1 packs but this was not statistically significant.

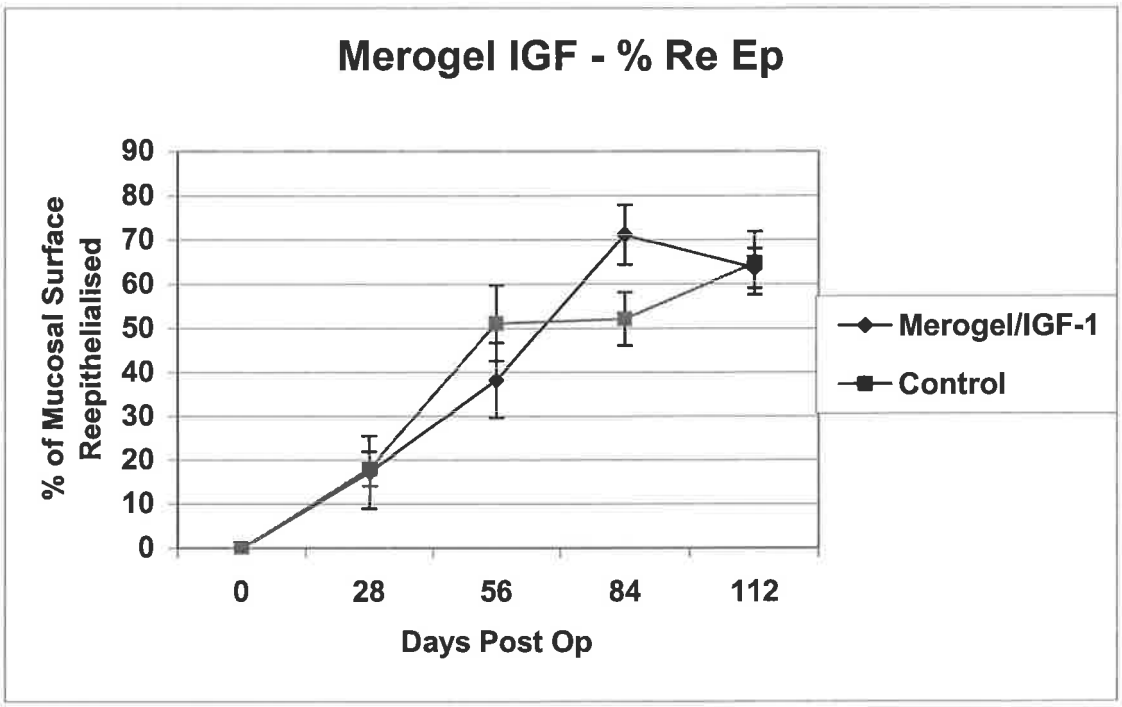

Figure 18

Figure 18 illustrates the percentage surface area of the injured mucosa that had reepithelialized at Day 28, 56, 84 and 112 post-operatively. (Mean +/- SEM)

Control Comparisons – Reepithelialisation – Merogel/IGF-1

The comparisons between the control groups for the sheep treated with Merogel/IGF-1 and those treated with Merogel alone are shown in figure 19. This comparison was performed to ensure there was no systemic or enhanced local effect of the IGF-1 on the mucosa in the opposite nasal cavity. In the Merogel control group, the values were 29.43% at day 28, 34.28% at day 56, 62.77% at day 84 and 65.30% at day 112. In the Merogel/IGF-1 control group, the values were 17.91% at day 28, 51.11% at day 56, 52.10% at day 84 and 64.60%. There were no statistically significant differences noted between the two groups at any time point when the results were analysed using a Student's T Test.

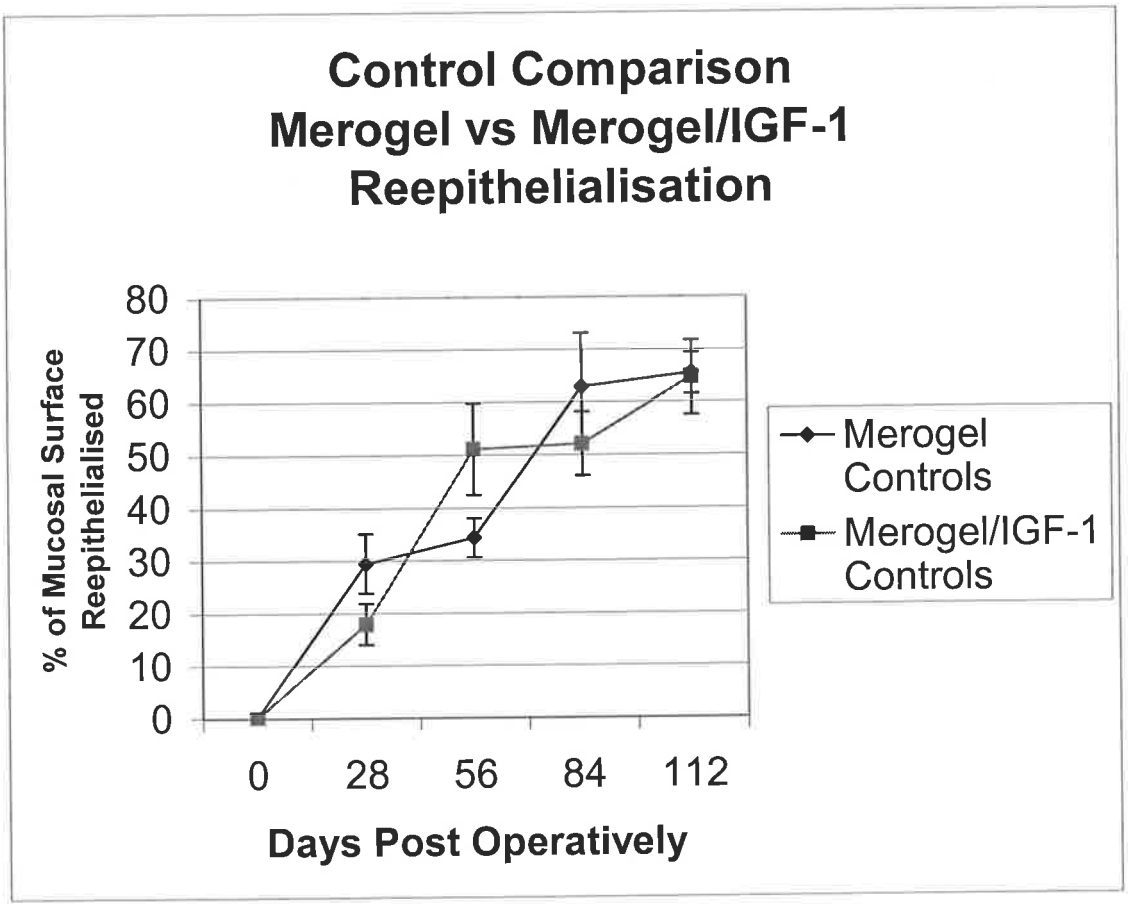

Figure 19

Figure 19 illustrates the comparison between the control sides of the subjects Treated with Merogel/IGF-1 packs, and the control sides of those treated with Merogel packs alone. (Mean +/- SEM)

Epithelial Height – Merogel/IGF-1

In figure 20, the relative epithelial heights of the control group and the group treated with Merogel/IGF-1 are compared. For the control sides, the values for relative epithelial height at each time point was 1.10 on day 28, 0.96 on day 56, 0.94 on day 84 and 1.10 on day 112. For the sides packed with Merogel/IGF-1, the values were 1.10 on day 28, 0.86 on day 56, 1.50 on day 84 and 0.98 on day 112. There were no statistically significant differences noted between the two groups at any of the time points post operatively when the data was analysed using a Student's T Test, though as with the reepithelialisation measurements, there appeared to be separation at day 84.

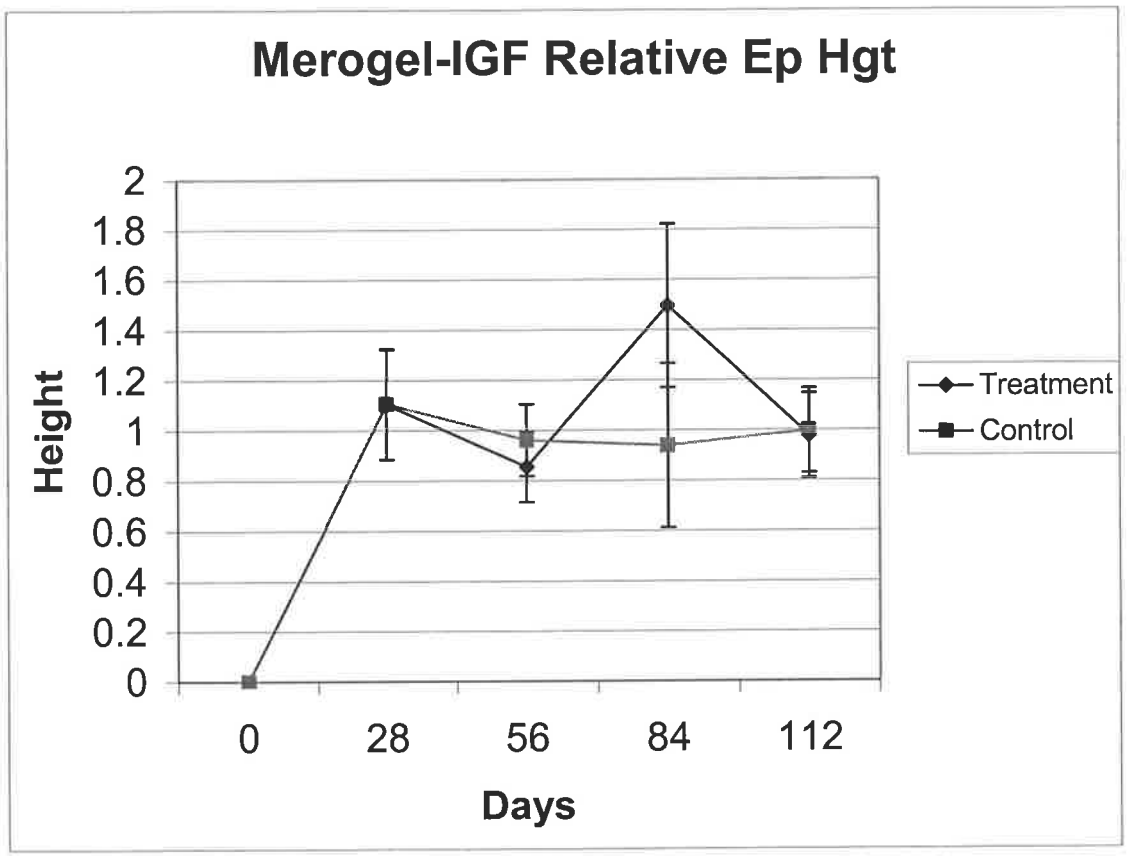

Figure 20

Figure 20 illustrates the comparison between the relative epithelial heights of the control groups and the Merogel/IGF-1 group. (Mean +/- SEM).

Control Comparisons – Epithelial Height – Merogel/IGF-1

Again, comparisons were made between the relative epithelial height measurements of the Merogel/IGF-1 control group and a Merogel control groups to ensure there was no systemic effect (Figure 21). The Merogel control values were 1.41 at day 28, 1.03 at day 56, 1.17 at day 84 and 1.02 at day 112. The comparative values of the Merogel/IGF-1 controls were 1.10 at day 28, 0.96 at day 56, 0.94 at day 84 and 0.99 at day 112. As with the comparison of reepithelialisation control values, there were no statistically significant differences between the two groups (Student's T Test).

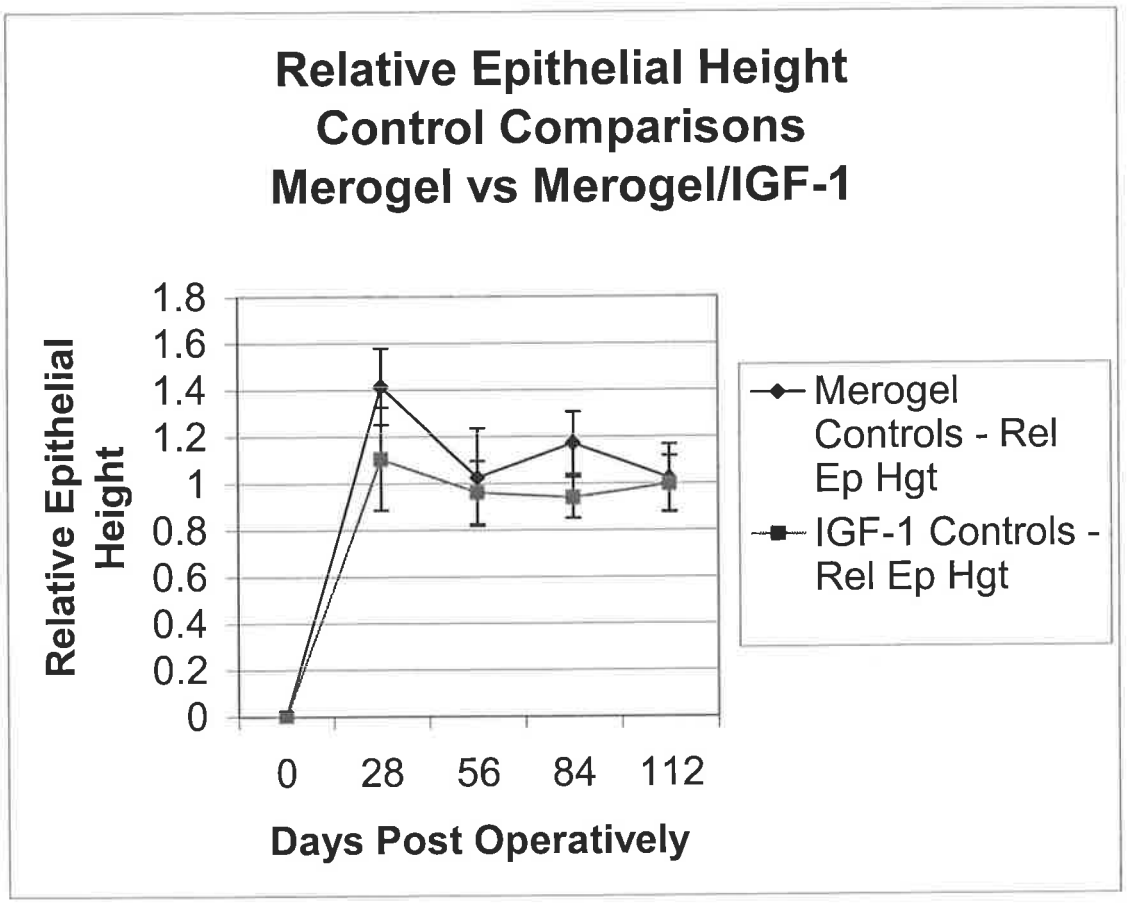

Figure 21

Figure 21 illustrates the comparison of relative epithelial heights between the Merogel/IGF- 1 controls and the Merogel controls. (Mean +/- SEM)

Scanning Electron Microscopic Analysis

Reciliation – Merogel/IGF-1

Scanning electron microscopy was used to assess the degree of reciliation of the nasal mucosa post operatively (Figure 22). At day 56, there was a statistically significant decrease in reciliation in the Merogel/IGF-1 group versus the control group, 59.20% vs. 77.68% ($p<0.01$; Student T Test). At day 112, this difference was still apparent with 69.70% reciliation in the IGF-1 group versus 87.26% reciliation in the control group ($p<0.01$).

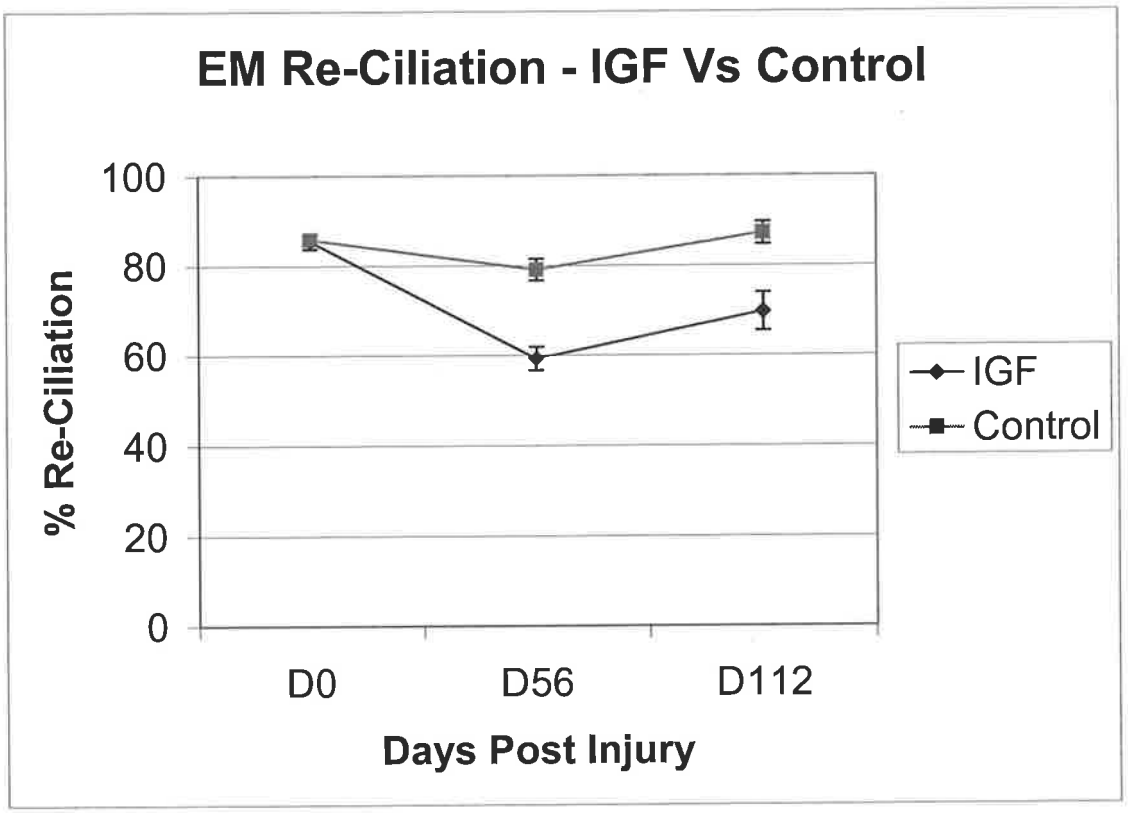

Figure 22

Figure 22 illustrates the % of the mucosa that had undergone reciliation at the various time points post operatively. (Mean +/- SEM)

Control Comparison – Reciliation – Merogel/IGF-1

A comparison of the Merogel/IGF-1 controls with a Merogel control group (Figure 23), revealed a significant difference at day 56 post operatively, demonstrating 79.07% reciliation in the Merogel/IGF-1 controls, versus 67.77% ciliation in the Merogel control group ($p<0.01$). At day 112 post operatively, there was no significant difference between the groups. Although this difference at Day 56 is statistically significant ($p < 0.05$; Student's T test), we do not feel that an 11.3% difference in reciliation of the regenerating epithelium would be clinically significant.

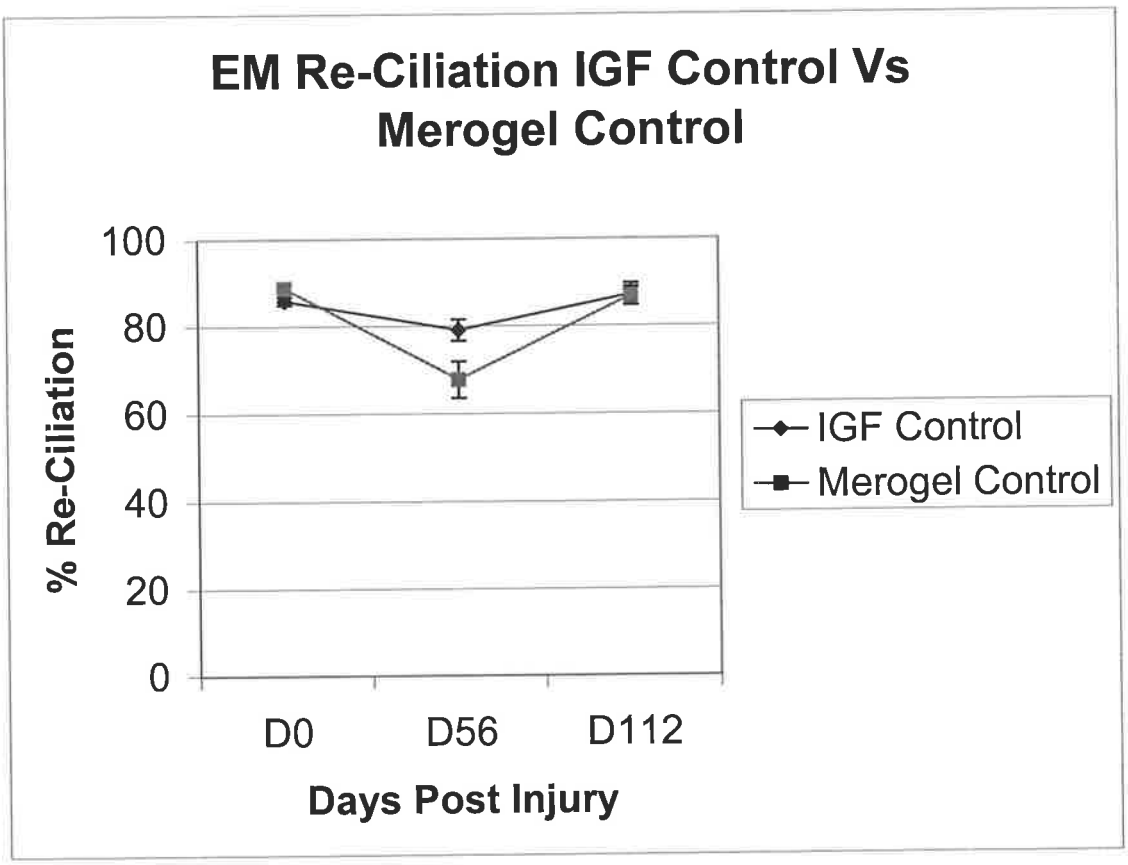

Figure 23

Figure 23 depicts the comparison between the control sides of the sheep treated with the Merogel/IGF-1 packs and those of the sheep treated with the Merogel packs alone. (Mean+/- SEM)

Adhesion Formation – Merogel/IGF-1

The use of Merogel/IGF-1 packing material had no statistically significant effect of the formation of adhesions ($p>0.05$; Student T test), with an adhesion formation rate of 55.57% in the unpacked sides, compared to 33.33 % in the packed sides (Table 2). However, there was a trend towards decreased adhesion formation in the sides packed with the Merogel/IGF-1 packing material.

Adhesion Formation		
	Merogel/IGF-1 Packing	**Controls**
Adhesion	**3 (33.33%)**	**5 (55.56%)**
No Adhesion	**6 (66.67%)**	**4 (44.44%)**

Table 2

Table 2 illustrates the rate of adhesion formation in the two groups.

Comparison of Merogel and Merogel/IGF-1

Light microscopic analysis

Reepithelialisation – Merogel vs Merogel/IGF-1

A comparison or mucosal reepithelialisation was made between the sides treated with Merogel and those treated with Merogel/IGF-1 (Figure 24). The reepithelialisation values for the Merogel sides were 20.69% at day 28, 45.93% at day 56, 58.79% at day 84 and 62.05% at day 112. The values for the sides treated with Merogel/IGF-1 were 17.10% at day 28, 38.13% at day 56, 71.06% at day 84 and 63.49% at day 112. There were no statistically significant differences between the two groups at any time point ($p > 0.05$; Student T test).

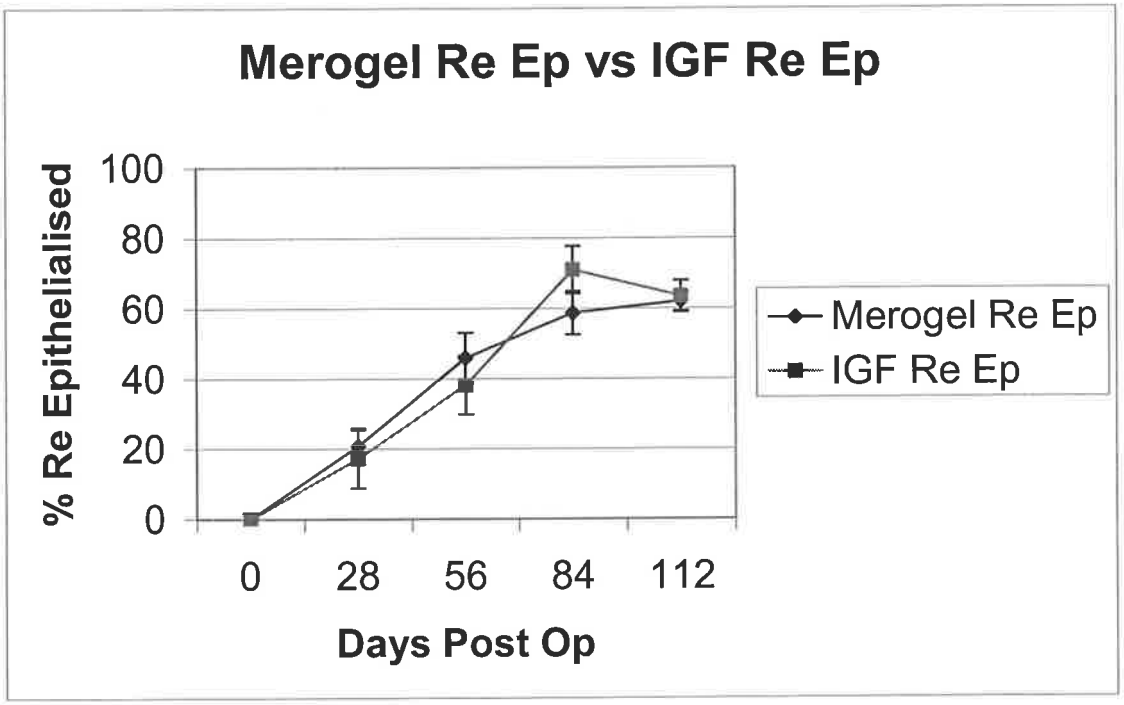

Figure 24

Figure 24 – A comparison of the reepithelialisation % in the sides treated with Merogel compared to those treated with Merogel/IGF-1 (Mean +/-SEM)

Comparison – Merogel vs Merogel/IGF-1 – Epithelial Height

Figure 25 illustrates the comparison of relative epithelial heights in the sides treated With Merogel versus those treated with Merogel/IGF-1. The values for the sides treated with Merogel were 1.33 at day 28, 0.92 at day 56, 1.34 at day 84 and 0.99 at day 112. For the sides treated with Merogel/IGF-1, the values were 1.03 at day 28, 0.86 at day 56, 1.50 at day 84 and 0.98 at day 112. There were no significant differences between the two groups at any time point ($p>0.05$; Student's T test)

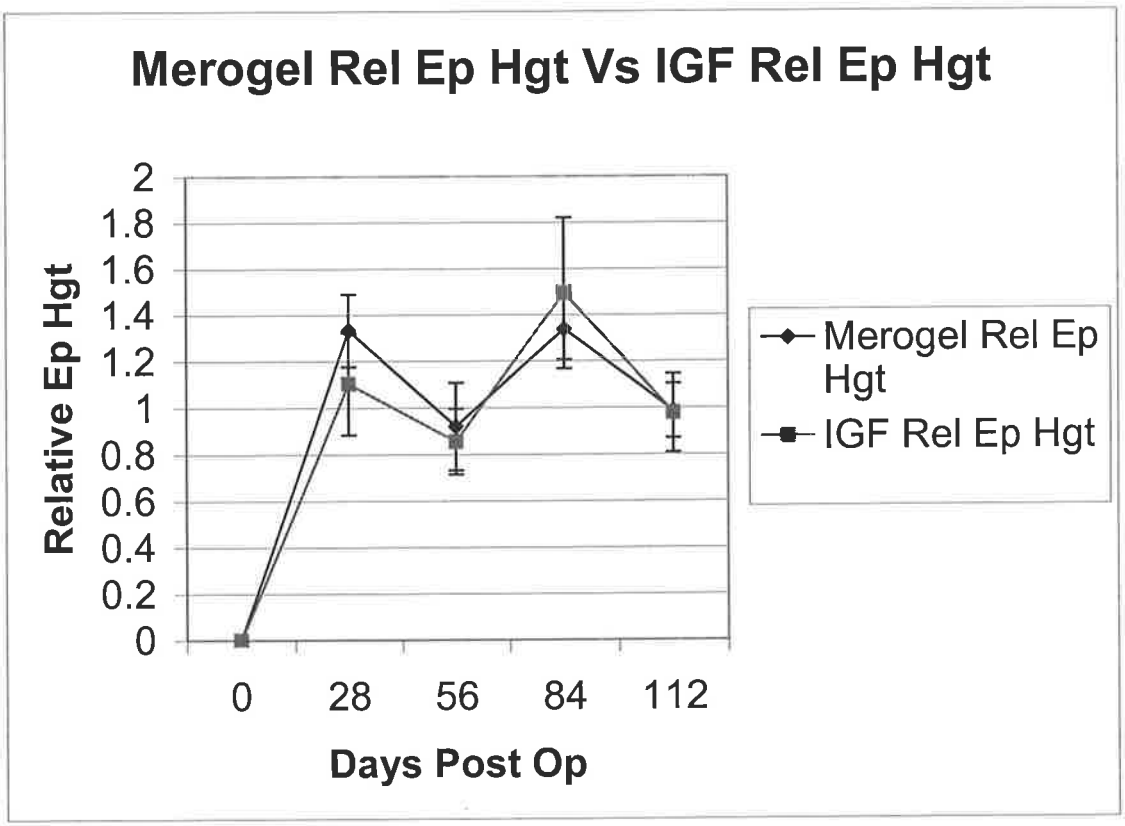

Figure 25

Figure 25 illustrates the comparison in relative epithelial height between the sides treated with Merogel and those treated with Merogel/IGF-1 (Mean +/- SEM)

Electron Microscopic Analysis

Comparison – Merogel vs Merogel/IGF – Reciliation

Figure 26 illustrates the comparison of reciliation values in the sides treated with Merogel, compared to those treated with Merogel/IGF-1. For the sides treated with Merogel, the % of ciliated mucosa was 64.96% at day 56 and 79.60% at day 112. This was compared to values of 59.20% at day 56 and 69.20% at day 112. There were no statistically significant differences between these values at any of the time points examined ($p > 0.05$; Student T test). However, there was a non-significant trend towards decreased mucosal ciliation in the sides treated with Merogel/IGF-1.

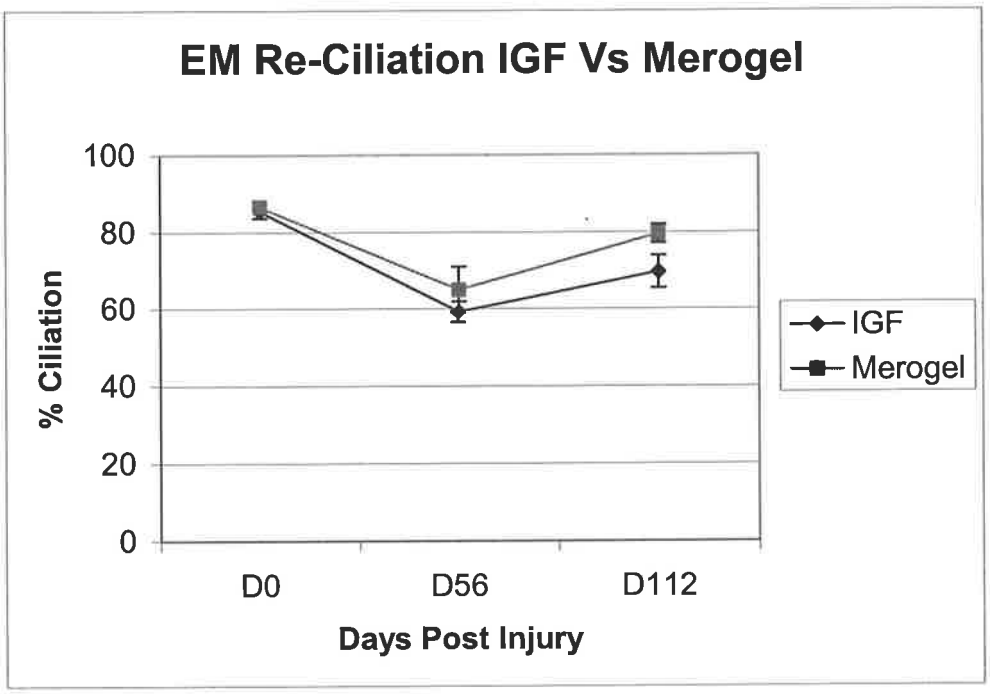

Figure 26

Figure 26 demonstrates the comparison of mucosal reepithelialisation in the sides treated with Merogel and those treated with Merogel/IGF-1

The Effect of Nasal Packing on The Ciliary Beat Frequency of The Regenerating Nasal Mucosa

Ciliary Beat Frequency – Merogel

Figure 27 illustrates the comparison between the mean ciliary beat frequency measurements in the sides treated with hyaluronic acid packs and the control sides. The baseline CBF value for the control group was 7.51Hz and for the Merogel group, 6.61Hz. The values for the control sides were 4.80Hz at day 56, 4.17Hz at day 84 and 5.16Hz at day 128, compared to 5.28Hz at day 56, 4.05Hz at day 84 and 6.28Hz at day 112. At no time point was there a statistically significant difference between the two groups when the results were analysed using a Student's T test. At day 28, there was no measurable ciliary activity from any sample in either group. At day 112, neither group had returned to the preoperative ciliary beat frequency values.

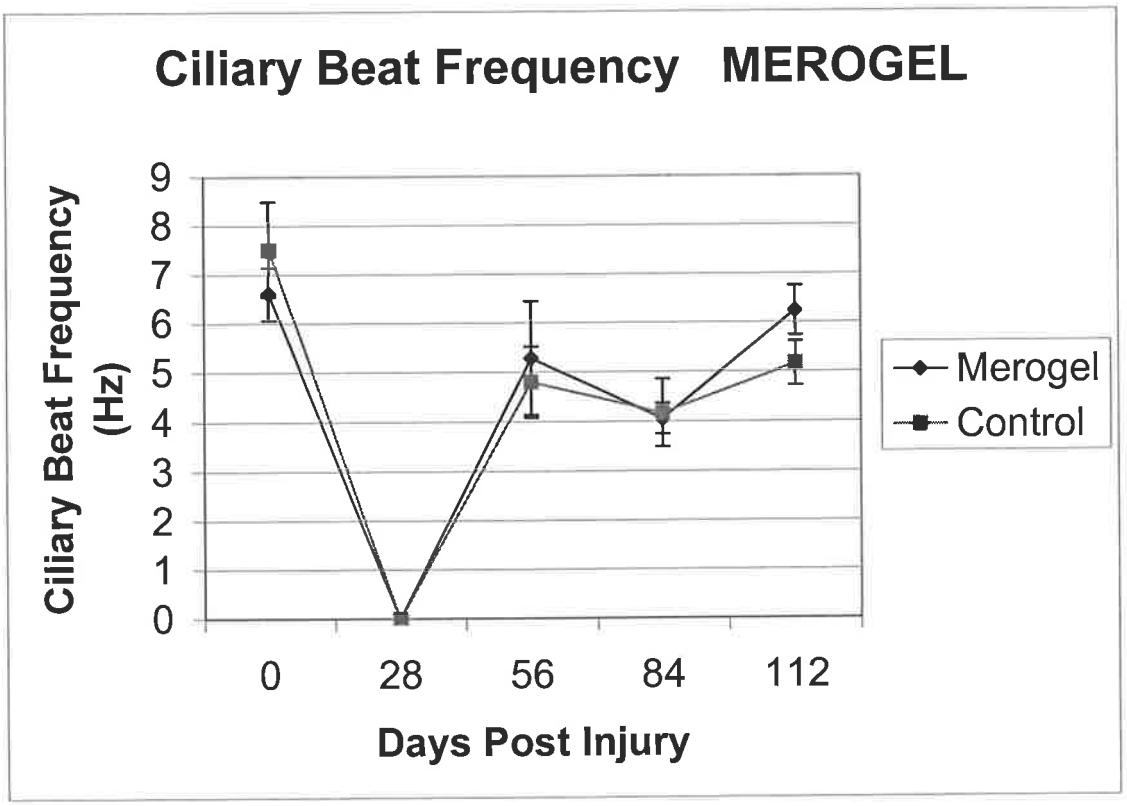

Figure 27

Figure 27 illustrates the effect of Merogel nasal packing on the CBF of the nasal mucosa post operatively. (Mean +/- SEM)

Ciliary Beat Frequency – Merogel/IGF-1

With regards to the Merogel/IGF-1 packing, figure 28 illustrates the comparison with the control sides. In the control sides, the value at day 56 was 3.94Hz, 1.35Hz at day 84 and 6.92Hz at day 112. In the sides treated with Merogel/IGF-1, the values were 3.34Hz at day 56, 2.70Hz at day 84 and 5.44Hz at day 112. At day 112, this difference was statistically significant ($p<0.05$; Student's T test), with the hyaluronic acid/IGF-1 sides demonstrating a lesser CBF than the control sides, suggesting a detrimental effect of the packing (Figure 28). There was no measurable ciliary activity at day 28 in either group, and both groups had returned to their respective pre operative CBF values of 6.78Hz in the control group and 5.55Hz in the Merogel/IGF-1 group by day 112.

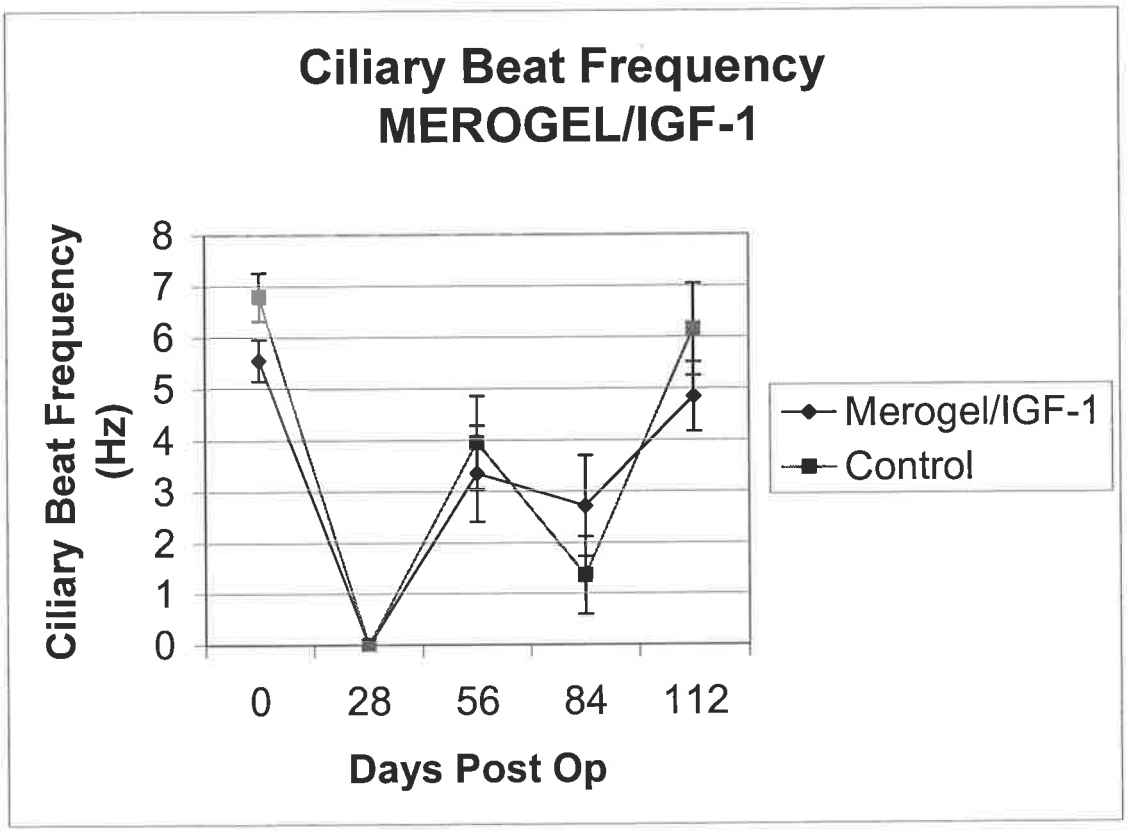

Figure 28

Figure 28 illustrates the effect of the Merogel/IGF-1 packing on the CBF of the regenerating nasal epithelium. (Mean +/- SEM)

Ciliary Beat Frequency – Merogel vs Merogel/IGF-1

The sides treated with hyaluronic acid packs were compared with those treated with Merogel/IGF-1 in figure 29. The values for the hyaluronic acid sides were 5.28Hz at day 56, 4.05Hz at day 84 and 6.28Hz at day 112, compared to 3.34Hz at day 56, 2.70Hz at day 84 and 5.44 Hz at day 112. There were no statistically significant differences between the two groups at any time points when the data was analysed using a Student's T test, though there appeared to be a consistent but non significant trend in favour of increased CBF in the Merogel group (Figure 29).

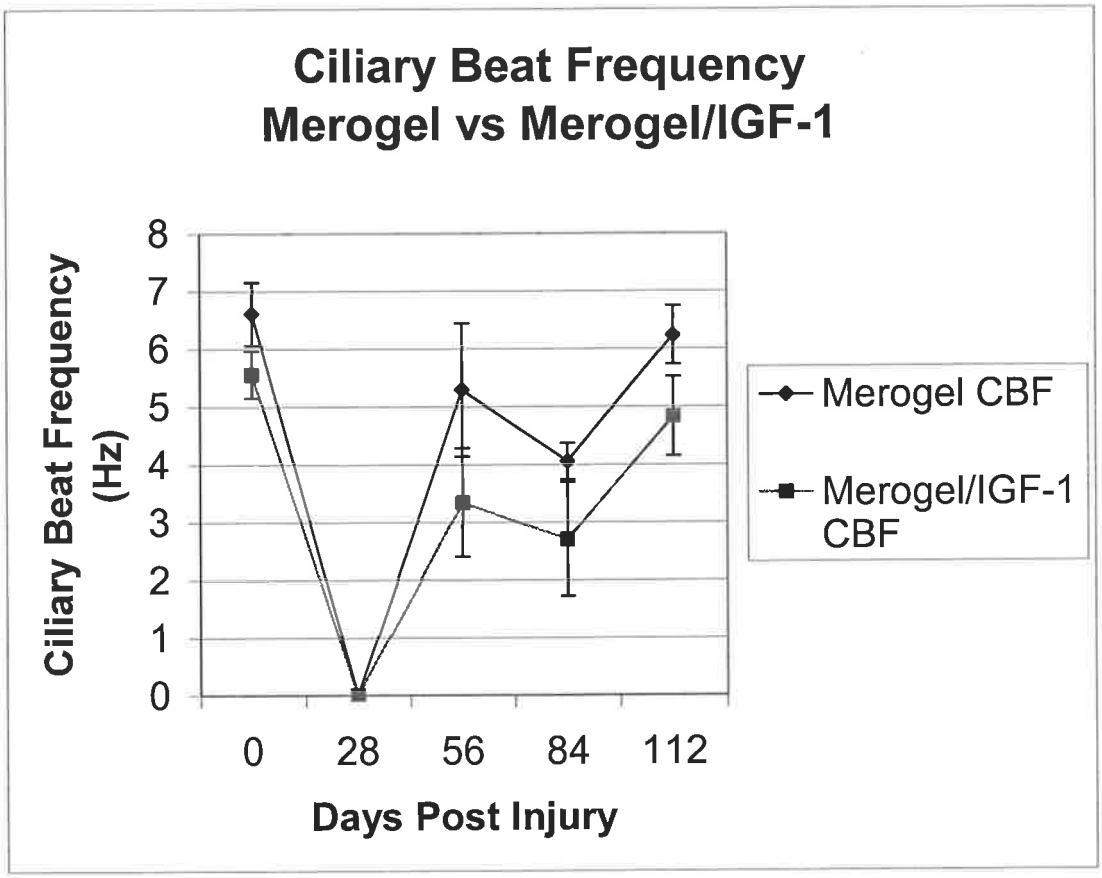

Figure 29

Figure 29 illustrates the comparison between the effects of the hyaluronic acid-based packs, and the hyaluronic acid/IGF-1 packs, on the CBF of the healing mucosa. (Mean +/- SEM)

Ciliary Beat Frequency – Control Comparisons

In order to exclude a systemic or extended local effect of the IGF-1, comparisons were made between the two control groups. The Merogel control values were 4.80Hz at day 56, 4.17Hz at day 84 and 5.16Hz at day 112, compared to 3.94Hz at day 56, 1.35Hz at day 84 and 6.92Hz at day 112 in the Merogel/IGF-1 sides. When the CBF values were compared (Figure 30), there was a significant difference at day 84, with the Merogel controls demonstrating increased CBF compared to the Merogel/IGF-1 controls ($p<0.05$; Student's T Test). However, the Merogel/IGF-1 controls returned to their approximate pre-operative values of 6.78Hz by day 112, while the Merogel controls fell slightly short of their preoperative level by day 112 (5.16Hz vs 7.51Hz).

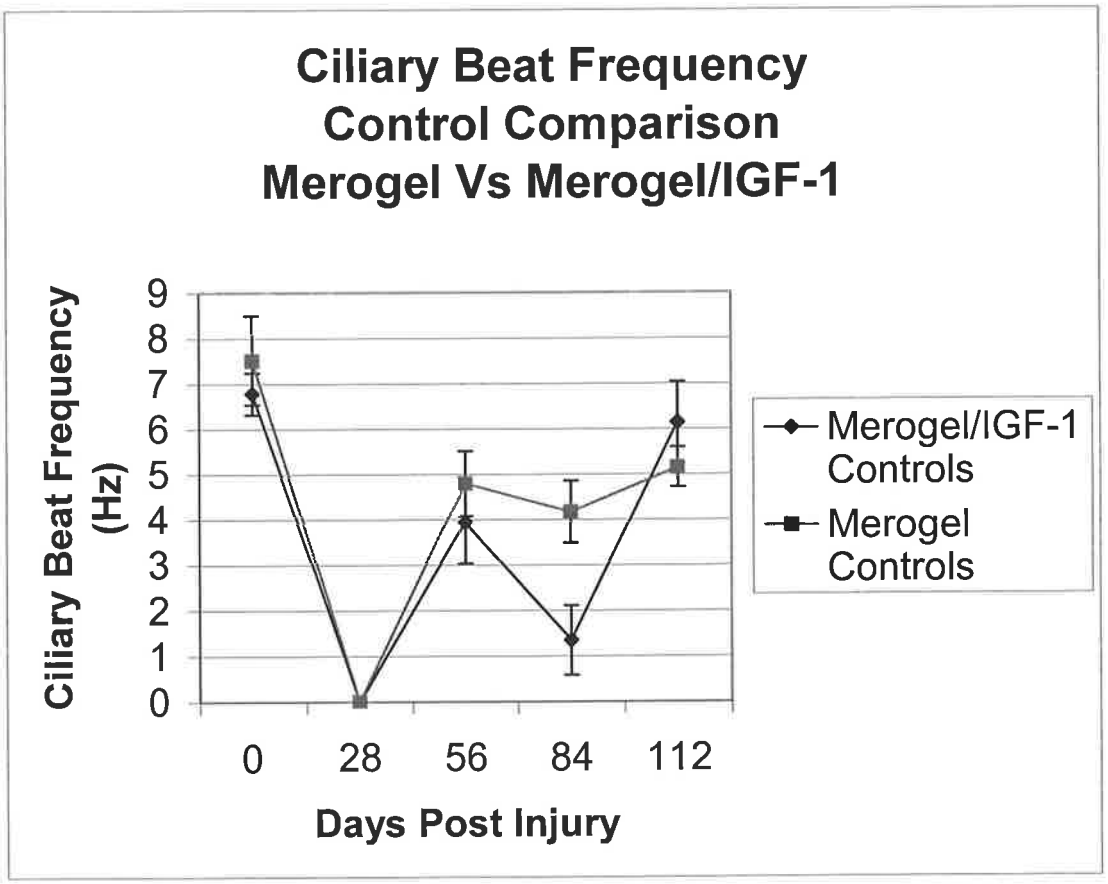

Figure 30

Figure 30 illustrates the comparison between the CBFs of the control sides of the Merogel group and the Merogel/IGF-1 group. (Mean +/- SEM)

Discussion

The Effect of Eosinophil Driven Sinusitis on Nasal Mucosal Healing

In the sheep model, the presence of inflammation secondary to the *Oestrous ovus* nasal parasite results in impaired mucosal healing. The injured mucosa showed significantly slower reepithelialisation after day 28 as compared to healthy subjects; day 56: 34.3% vs. 78.4% ($p<0.01$), day 84: 59.7% vs. 91.9% ($p<0.01$). 5. At Day 112, reepithelialisation is incomplete and significantly worse in inflamed tissue than in the healthy sheep, with only 65.3% reepithelialisation compared to 94.7% in the healthy sheep.

Numerous animal models of chronic sinusitis have been developed and described. In some of Hilding's early work in a rabbit model, when bands of mucosa were removed in the vicinity of the maxillary sinus ostium, they were replaced with scar tissue, which the author speculated resulted in interruption to mucociliary flow and subsequent suppuration[19]. However, numerous further studies have been performed with the specific aim of deliberately inducing a state of sinusitis, with ensuing mucosal inflammation, in the experimental subjects. Hinni et al. created a rabbit model of sinusitis by accessing the maxillary sinus via an external approach and

instilling a bacterial solution into the sinus on one side after obstructing the sinus ostium, and leaving the other side obstructed but uninfected. The presence of obstruction alone did not lead to pathological changes, but the presence of infection and obstruction lead to significant losses of viable ciliated epithelium, with over 86% of the ciliated cells being non viable within 5 days[42]. Forsgren et al. also utilised a rabbit model, and sacrificed the subjects at various time points and then studied the entire sinus complex including the bone. They noted that after denudation of the sinus epithelium, there was extensive replacement of this mucosa with connective and scar tissue within a week of the injury[119]. Rabbit models with sinusitis induced by inoculation of the maxillary sinus with various bacterial agents have also been used to study the effect of corticosteroids[177], fibrin sealant containing antibiotics [45], the nature of the immune response to the infection [178], and comparing computerised tomography scanning and magnetic resonance imaging, in subjects with acute sinusitis[179]. Jacob et al described a murine model created by the use of bacteria and nasal packing into the nasal cavity[28]. This model utilised one side of the nose as a control, and the other as the site of the nasal packing and bacterial inoculation. The mucosal samples were analysed for epithelial height changes using image analysis software and reported qualitative increases in epithelial height and cell counts as a results of sinusitis. This study demonstrated that inoculation with *Bacteroides fragilis* and obstruction of the maxillary sinus ostium with an insoluble nasal pack; Merocel Nasal Sponge (Xomed Jacksonville, Fl.), resulted in a localised chronic rhinosinusitis. A similar model had been described previously but this involved only intranasal inoculation of bacterial solution via droplets placed in the outer nares, and

then sacrifice of the animals at various time point and subsequent histological study[180]. This work revealed that intranasal administration of *Streptococcus pneumoniae* induced an acute bacterial rhinosinusitis when assessed by tissue culture and histological evidence of neutrophil infiltration. All of these animal models allowed evaluation of the sinus mucosa at various time points via sacrifice, but unlike the sheep model, did not permit serial biopsies to be taken from the same subject.

These sinusitis models have then been utilised further to study the effect of surgical procedures. Benninger et al. induced sinusitis in a rabbit model via occlusion of the maxillary sinus ostia, and then studied the effects of inferior antrostomy versus middle meatal antrostomy on the progress of the sinusitis by measuring ostial patency, and gross and histological assessment of the sinus mucosa for evidence of inflammation. They noted that chronic inflammation lead to a loss of ciliated epithelium but there was no significant difference between the two procedures with regards to patency of the ostia or progression of the sinus disease[44]. Czaja et al. demonstrated that middle meatal antrostomies performed in rabbits with *Streptococcus pneumoniae* induced sinusitis, resulted in a return to normal in ciliary beat frequency, and a reversal of the histopathological changes which characterised the infected mucosa[63].

Our model differs from previous discussed animal models of chronic sinusitis in two fundamental ways. The sheep model is the only model described which is suitable for the study of endoscopic surgical interventions on the nose and paranasal sinuses, and the model in its healthy state has now been well described and studied [65, 76, 88, 117].

Secondly, we have relied on a naturally occurring parasitic infestation, which induces an eosinophilic driven inflammation of the nasal mucosa. Other sinusitis models described have required the direct inoculation of infective organisms such as *Streptococcus pneumoniae* into the maxillary sinus, which is then occluded [26], or packing of the nasal cavity with a non-absorbable pack[28]. As our model requires no such manipulations, it closely parallels chronic sinusitis seen in human patients.

An interesting finding in our model was that at Day 28 post injury, the infested subjects had a statistically significant increase in the relative thickness of the regenerating epithelium as compared to the healthy subjects (1.41 vs. 0.081, ($p<0.01$)). It is possible that this epithelial hyperplasia represents a derangement in the normal healing process, possibly due to the various inflammatory mediators present as a result of chronic sinusitis. Chronic sinusitis has been described as not only being an infectious condition, but also a complex inflammatory disease with multiple cytokines such as IL-4, IL-13 and IL-5, histamine, and leukotrienes[81]. If we examine work performed in other animal models of chronic sinusitis, specifically the rabbit model, it has also been proposed that inflammatory processes secondary to infection may impair tissue healing after injury [42]. Czaja et al. [26] revealed loss of ciliated epithelium, and the analysis of mucociliary function showed a decreased ciliary beat frequency and disordered patterns of mucociliary transport[26]. Similar findings regarding the mucosa and ciliary function have been demonstrated by other authors in rabbit models[42] [44]. However, they did not comment on the epithelial

thickness of the sinus epithelium during the healing process. Jacob et al. noted significant epithelial thickening in their murine model of chronic sinusitis [28], though in their study, mucosal injuries were not performed and the thickening was due to the presence of bacterial infection alone. Schlosser et al. also noted goblet cell hyperplasia in their rabbit model of sinusitis but this again was due to the infective state rather than healing after surgery[45]. In our subjects, a significantly thickened regenerating mucosa seen in the infested sheep at day 28 post injury. We feel that the increase in epithelial height in the infested sheep represents a derangement in the normal healing process that is probably due to the pre-existing inflammatory state in the injured tissue. It has been well documented that multiple bioactive factors are present in the presence of active inflammation. These include interleukins, ICAM molecules and growth factors. These substances may interact with the normal regenerative process resulting in the increased epithelial thickness noted in our study [4, 181-185] [81].

With regards to the degree of reciliation seen in the regenerating mucosa, there was no difference between the two groups at day 56, but at day 112, there was a significant difference between the two, with the chronic sinusitis group showing 86.9% reciliation versus 76.64% reciliation in the healthy group ($p<0.01$). As the difference between the two groups is just over 10%, this result it is not likely that this represents a clinically significant difference. Nevertheless, it is interesting that the presence of inflammation did not have the same deleterious effect on reciliation that it did on reepithelialisation, as the sheep showed far better degrees of reciliation than

reepithelialisation at day 112; 86.9% reciliation, as compared to 65.3% reepithelialisation. This would suggest that although only 2/3 of the injured surface has reepithelialized, the epithelium present is well ciliated.

In a rabbit model of sinusitis, Czaja and Montgomery utilised SEM to observe improvements in epithelial ciliation after middle meatal antrostomies were performed, but they did not quantify their results[63]. Benninger et al. noted the presence of normal ciliated epithelium in the maxillary sinuses of rabbits after a Caldwell-Luc procedure was performed. This analysis was performed using light microscopy but the degree of reciliation was not quantified[75]. Keles et al used transmission electron microscopy (TEM) to document the changes in the ciliated epithelium in patients undergoing ESS. They noted "complete recovery" of ciliary structural abnormalities in the epithelium after surgery, but again, did not quantify the degree of reciliation[71]. Moriyama et al used SEM to observe the changes in ciliation after ESS in human patients, but once again, did not quantify their results and described only the presence or absence of normal ciliated cells[70].

We cannot compare the functionality of the new epithelium in terms of mucociliary clearance, as this was not assessed in both groups. However, measurements of CBF were made in the chronic sinusitis sheep model and these can be discussed. At day 28 post operatively, there was no measurable ciliary activity in any sample, suggesting that mucociliary clearance at that time would be severely impaired, if functional at all. By day 56, there was measurable activity, but the relative CBF values indicated

that from day 56, there was an increase in CBF up to day 112, where the CBF values approached only 60% of their preoperative levels. This is consistent with previous work in human subjects, which showed that it took 6 months for CBF values to reach normal, healthy levels after ESS, with the most rapid improvements being from months 4 to 6[68]. This was despite the mucosa appearing to be grossly normal and healthy when subjected to endoscopic examination. As previously discussed, the studies by Moriyama et al. and Keles et al. did not involve any analysis of ciliary function at 6 months post op[70, 71]. Guo et al. demonstrated recovery of ciliated epithelium after ESS but also commented that further work was needed to assess the functionality of the ciliated epithelium[166]. Czaja and McCaffrey examined the time course of healing after middle meatal antrostomies were performed in a rabbit model of chronic sinusitis, and importantly, measured CBF as well as mucociliary clearance times as a functional assessment of the sinus mucosa. They noted that 6 weeks after surgery, the mean CBF in their infected models, which had previously been significantly lower than that of controls, had recovered to a mean CBF that was not statistically different to healthy controls. The increase in CBF correlated significantly with an increase in the mucociliary clearance transport time[63]. Unfortunately, there has been no previous work performed to directly measure the CBF of nasal mucosa that has been wounded and then allowed to heal.

Conclusion

In summary, the presence of the nasal parasite *oestrous ovus* incites an eosinophilic driven sinusitis in the sheep model. In this model, there is significantly impaired epithelial healing after full thickness mucosal injury as assessed by reepithelialisation and compared to healthy sheep. There also appears to be a derangement in the healing process that results in an increased epithelial height in the regenerating mucosa. The presence of inflammation does not however result in a clinically significant difference in mucosal reciliation when compared to healthy sheep.

The Effect of Hyaluronic Acid Based Nasal Packing (Merogel®) on Nasal Mucosal Healing

In this sheep model of chronic rhinosinusitis, a dissolvable hyaluronic acid-based pack (Merogel) did not confer any significant improvement in mucosal healing after full thickness injury at time points from day 28 up to day 112 post operatively when assessed by reepithelialisation, and epithelial height. Both groups showed a steady increase in surface reepithelialisation over the post-operative period with separation apparent at each time point but no significant differences or consistent trend. By day 112, neither group had exceeded 65% reepithelialisation, demonstrating that even 4 months after surgery, there was poor mucosal healing apparent. After an initial increase in relative epithelial height in both groups at day 28, the relative heights returned to an approximate value of 1.0 by day 112.

When the reciliation of the injured mucosa was considered, there was no significant difference seen between the two groups at day 56. However, at day 112, there was a statistically significant difference in reciliation between the packed group and the control groups (76.9% vs. 86.9%, $p<0.01$) but the difference was small enough to not be considered clinically significant.

The use of the hyaluronic acid based nasal packing had no significant effect on the formation of intranasal adhesions in the model.

These results are not consistent with previous studies that have investigated the effect of hyaluronic acid on tissue healing. Numerous animal studies have been performed which have demonstrated the benefit of hyaluronic acid on aspects of tissue healing. In one study involving the harvest of foetal mouse forelimbs, wounds were created and then sutured. The limbs were then kept in a cell culture medium, half with a high concentration of hyaluronic acid and half with plain medium, for 7 days, and the wounded tissue was then examined for its histological features. The wounds treated with hyaluronic acid demonstrated no typical scar morphology and resembled healthy tissue, while the control group demonstrated the characteristics of scar formation[186]. In other work, the hyaluronic acid content in wounded tissue in foetal rabbits was reduced using a hyaluronidase, and compared to controls, and the depleted wounds were shown to have increased evidence of collagen deposition, fibrosis and neoangiogenesis[91]

Although there is strong evidence, described above, that hyaluronic acid can be applied to attain a beneficial effect on wound healing, questions have raised as to whether the nature of foetal wound healing is also dependent on the foetal environment, especially amniotic fluid, or to factors specific to foetal tissue such as the concentration of hyaluronic acid in the extracellular matrix. An elegant study was performed in which adult skin was grafted onto mouse foetuses in utero, and then those areas were wounded 40 days later. The wounded areas demonstrated adult type

healing, suggesting that the amniotic fluid environment was not solely responsible for foetal wound healing, and that intrinsic foetal tissue factors, including the extracellular matrix, were important. Work was also described where foetal tissue transplanted to adults and then wounded, demonstrated foetal type healing despite being in an adult environment [187] [96]. The authors concluded that their findings supported previous work that showed that application of exogenous hyaluronic acid could not result in totally scarless healing. Despite these controversies, many further studies in animal models have been performed to investigate the effect of hyaluronic acid on aspects of healing.

King et al. created wounds within hamster cheek pouches, and treated them serially with either topical application of Hyaluronic acid plus a carrier vehicle, or the carrier vehicle alone. The wounds treated with hyaluronic acid healed more quickly and with less extravasation of inflammatory compounds[188]. Utilising a rat model, Hagberg et al. used a topically applied hyaluronic acid solution to influence healing and adhesion formation after flexor tendon surgery. After tenotomy and tendon repair, either Sodium hyaluronate solution in 3 different concentrations, or 0.9% saline was injected to fill the tendon sheath. Each animal acted as its own control. After 15 days, the harvested tendons were tested for range of motion and the force required for such motion, the degree of adhesion formation and limitation to function were calculated. The highest concentration sodium hyaluronate solution tested demonstrated a significant decrease in the extent of adhesion formation, and importantly, no decrease

in the tensile strength of the repaired tendon[189]. A similar concentration dependent effect was seen when the abdominal cavity of rats was pre coated with hyaluronic acid solutions of varying concentrations (0.1,0.25 and 0.4%, a control phosphate buffered solution, and no solution at all), prior to a controlled caecal injury. When the animals were examined 1 week later, the mean incidence of adhesions were significantly decreased in proportion to the increasing hyaluronic acid concentration, and the number of rats with adhesions decreased from 89% in the control group to 50% in the 0.4% hyaluronic acid group[190]. A concentration dependent benefit was seen when hyaluronic acid was applied to rat tympanic membrane perforations. Perforations treated with hyaluronic acid healed more rapidly and with less scarring than controls, and the higher the concentration of hyaluronic acid applied, the more rapid the closure of the perforation[191].

There has only been one previous comparable animal study examining the use of hyaluronic acid based packs in the nasal cavity. In work performed in a healthy sheep model, McIntosh et al. demonstrated that a dissolvable hyaluronic acid-based pack (Merogel®) had a significant beneficial effect on aspects of mucosal healing after full thickness mucosal injuries[117]. At day 84-post injury, there was a significant improvement in reepithelialisation of the mucosa treated with hyaluronic acid nasal packs, as compared to unpacked controls, suggesting more rapid healing. There was also a significant improvement in epithelial maturity as assessed by epithelial height, at Day 28 and day 56 post injury. This more rapid return to mature epithelium was attributed to the presence of the hyaluronic acid its effects on cell differentiation and

migration[117]. However, no difference in re-ciliation was seen. In our sheep model of eosinophilic driven chronic sinusitis, the benefits of the hyaluronic acid pack were not apparent. There was no significant difference in re-epithelialization, or epithelial height between the packed and controlled sides. In this study, these parameters were analysed with the same methodology used by McIntosh et al[117] in the healthy sheep model. We theorise that the lack of efficacy of the hyaluronic acid packing in this study was due to the presence of sinusitis and inflammation at the time of the injury and during the subsequent healing process. The effect of this inflammation on mucosal healing has previously been addressed in this discussion, and the same factors that lead to poor healing in the presence of inflammation may also interact with the hyaluronic acid to prevent it having a significant effect on healing. The studies attesting to the efficacy of hyaluronic acid based substances in enhancing healing, which have previously been referred to in this discussion, were not carried out in the presence of infection and inflammation.

Reciliation was examined in addition to reepithelialisation and epithelial height as cilia represent an essential component of the mucosa in regards to the function of mucociliary clearance. The reepithelialisation of mucosal surfaces would be less important in terms of function with the absence of ciliary return [70, 117]. Again, the only comparable work was the study performed by McIntosh et al. in the healthy sheep model. In that work the use of hyaluronic acid-based packing did not result in any significant difference in reciliation post operatively[117]. In this model of eosinophil driven sinusitis, there was a statistically significant difference in reciliation

at day 112, this difference of 7.3% was too small to be deemed to be clinically significant. Thus, as with McIntosh's work[117], the hyaluronic acid packing had no clinically significant effects on reciliation.

With the advent of endoscopic sinus surgery, the use of packing has become more controversial. Improvements in visualisation of the operative field, as well as technological advances in haemostatic cautery devices have facilitated intraoperative haemostasis. Although haemostatic packs are still used, there is also an increasing use of smaller or dissolvable packs that are targeted to a specific site.

Brennan[80] examined the use of polyurethane packing for the specific purpose of splinting open the middle meatus after ESS to prevent adhesions forming between the lateral nasal wall and middle turbinate. If adhesions form, they can lead to obstruction of sinus drainage and further disease. The specific pack was called the Boomerang Turbinate Glove and was designed with a splint portion to lie against the nasal septum and separate it from the middle turbinate, and a glove portion that fitted over the middle turbinate and prevented its contact with the lateral nasal wall. This was left in place for 9 to 14 days post operatively in a series of 234 consecutive patients, and oral antibiotic cover was given. The timing of the removal of the pack was based on patient tolerance and comfort, and the traditional time period for which nasal packing has been utilised post operatively. There was no control group, but the

author reported an overall adhesion incidence of 1.3%, compared to his historical incidence rate of 5% in patients prior to this series[80].

Toffel described a series of 3000 patients in which he used the Toffel Merocel (polyvinyl acetate) Sinus-Nasal Pak ®system. A small wedge of the material was endoscopically placed between the middle turbinate remnant and lateral nasal wall, and a larger antibiotic coated Merocel Sponge was placed lower down in the nose to decrease septal oedema and swelling. Oral antibiotic cover was also given, and no episodes of toxic shock syndrome were reported. The packing system required removal 1 week post operatively with the patient given intravenous sedation, as this was the amount of time that most patients were able to tolerate the packing. Again, there was no control group for comparison. The study reported very high success rates in terms of symptom control, but this was attributable to the entire surgical procedure and follow up protocol, rather than just the packing system[56].

If expansile nasal packs are left in situ for a number of days post-operatively the expansion of the pack combined with the post-operative swelling of the tissues after surgery may exert considerable pressure on the tissues. Granulation tissue may also grow into the pores of the pack. Removal of such a pack may cause considerable mucosal damage, pain and bleeding[82], as well as damage to the underlying epithelium[88]. These concerns with non-absorbable packing have resulted in increased interest in the use of dissolvable packing material. Fanous reported excellent results with oxycel and surgicel packing after conventional nasal surgery, in terms of both patient comfort and haemostasis[89]. More recently, a dissolvable

hyaluronic acid-based pack (Merogel®, Medtronic Xomed, Jacksonville, Fl) has been developed for use as nasal packing material. As well as the pro-healing activities of hyaluronic acid that have previously been discussed, hyaluronic acid is also believed to possess bacteriostatic effects which may prove beneficial to prevent postoperative infections[192] and reduce the need for prophylactic antibiotics. It has the advantages of being highly biocompatible as it is a naturally occurring substance in the body and it dissolves in the post-operative period, which makes it ideal for use as a nasal pack[101].

In an attempt to reduce adhesion formation in other areas of the body, a number of solid hyaluronic acid based substances have been developed for both animal and human trials. In a randomised blinded control trial in a rabbit model, Seprafilm, a hyaluronic acid and carboxymethylcellulose film, was topically applied at the time of surgery to the injuries, a myotomy, an unrepaired myotomy, or a repaired enterotomy, created on the surface on the bowel. At a period of 14 days post operatively, after which time the Seprafilm had been completely absorbed, a surgeon blinded to the previous use of Seprafilm performed a repeat laparotomy. There was both a clinically and statistically significant decrease in the incidence of adhesion formation in the subjects treated with Seprafilm as compared to controls (6.6% vs. 30 % and 6.6% vs. 33% for repaired and unrepaired myotomies respectively)[193]. Similar significant benefits for a hyaluronic acid carboxymethylcellulose membrane have been shown in an equine model involving small bowel injury (100% adhesions in controls vs. 20% in treatment group)[194], and also in a rat model examining adhesion formation after

laser induced injury to the uterine horn[195]. In a similar rat model involving uterine horn injuries, a hyaluronic acid solid film significantly reduced adhesion formation (43%(HA) vs 88%(Control) after excisional but not electrocautery injuries[102]. None of these studies reported any adverse incidents attributable to the use of hyaluronic acid or its derivative compounds. There have been a number of recent animal studies that have examined the effect of Merogel on mucosal healing in the nasal cavity. Maccabee et al. studied the effect of Merogel packing in a rabbit model, where the maxillary sinuses were completely stripped of mucosa. They noted extensive fibrosis within the regenerating epithelium, loss of the normal mucociliary surface epithelium and Merogel fibres being incorporated into the new mucosa. Their conclusion was that mucosal healing may be impaired by Merogel. However, their biopsies were taken 14 days after the mucosal injury was created, and may represent and early tissue response[196]. As our initial biopsies were taken at day 28, we cannot comment on the early phases nasal mucosal healing in the presence of Merogel In a murine model, Jacob et al. noted that implants of Merogel resulted in new bone formation in the nasal cavities of their subjects, as well as on calvarial bone after subcutaneous implantation. These results suggested that Merogel may induce neo-osteogenesis when in contact with damaged, regenerating bone[197]. This may possibly have clinical implications when Merogel is used in the clinical situation in the presence of exposed bone in the sinonasal region after full thickness mucosal injuries. There have however, been no previous studies in an animal model of intranasal adhesions, nor of the effects of Merogel on healing after ESS, and in this respect, our work represents the first study of its type.

In addition to the animal studies described, there have also been numerous clinical trials in human patients. These have been in gynaecological and abdominal surgery, as well as in nasal surgery. In a blinded, randomised, prospective and multicentre clinical trial involving 127 female patients, the efficacy of Seprafilm at preventing adhesions after gynaecological surgery was examined. All patients underwent the procedure of myomectomy, and baseline grading of adhesions present was made. At the end of surgery, the patients were randomised to either the Seprafilm group or the control group. The Seprafilm group had a sheet of Seprafilm placed on the posterior surface of the uterus and wrapped anteriorly to ensure that all incisions were covered. All patients underwent a 2nd look laparotomy at a mean of 23 +/- 2 days after the initial surgery. A video recording was made of this procedure. An independent gynaecologic surgeon who was blinded to the treatment group then viewed the recordings and graded the adhesions according to a scale. This grading was then compared to that at the initial surgery. The number of sites of adhesion to the uterus was significantly lower in the Seprafilm treatment group than the control group (4.98 vs 7.88), and the mean adhesion severity score and mean area of adhesions were also significantly higher in the control group. Importantly, no adverse effects or increases in adhesion formation were seen after the use of Seprafilm [106]. In another prospective study involving 183 patients undergoing colonic resection and ileal pouch-anal anastomosis with diverting loop ileostomy, subjects were randomised into receiving a hyaluronic acid/carboxymethylcellulose film placed under the incision and above the peritoneum, or having a standard closure. At the ileostomy closure

procedure 8 to 12 weeks later, the abdominal cavity was examined for incisions under the incision. Of the subjects treated with Hyaluronic acid, 49% had adhesions compared to 94% of the controls. Dense adhesions were observed in 58% of the controls, compared to 15% of the treated subjects. In the control group, adhesions involved a mean of 63% of the incision length, as opposed to 23% of the treatment group. These differences were all statistically significant. There were also no statistically significant differences in adverse effects between the two groups[198].

With specific regards to nasal surgery, in a clinical trial on 10 human subjects, 1 randomly chosen side of the nose was left unpacked while the other side was packed with a hyaluronic acid-based pack (Supragel Sinus (hylan B)). The packing was shown have significant beneficial effects in terms of synechiae, stenosis of the middle meatus, mucosal regeneration and subjective measures such as nasal pain and congestion, when compared to no packing[199]. There has also been a human trial conducted in the use of a hyaluronic acid based cream (Rhinogen) to enhance post op mucosal healing after ESS. This was compared to H.E.C. ointment and demonstrated to have a significant benefit in improvements in nasal airflow, crust formation and the condition of the nasal mucosa[200]. In a study comparing the use of Merogel toGelfilm(Upjohn Corp'), an absorbable collagen based material, for middle meatal stenting in 100 patients after ESS, Catalano and Roffman demonstrated a significantly decreased occurrence of middle meatal adhesions. They also noted that Merogel remained in situ for a significantly longer period of time than Gelfilm. There

was, however, no control group with which to make comparisons, but no adhesions were seen in the sides treated with Merogel. In contrast to these three studies, Miller et al. examined the effect of Merogel nasal packing on wound healing in the nasal cavity after ESS, in a blinded randomised controlled trial in 37 patients. The patients were followed up for 8 weeks after their surgery, and the use of the Merogel packing was not found to confer any significant benefits in terms of either reduction of adhesion formation, infection or oedema[201].

In our study, the Merogel® nasal packing was not intended to be haemostatic or to provide support, but rather, was being used to promote healing by producing an environment rich in hyaluronic acid and also, to deliver a growth factor to the regenerating tissue. It was hoped that the use of this pack would result in faster and more complete healing, and as a consequence, decrease the formation of adhesions between the injured surfaces. In contrast to three of the human trials discussed above, and the numerous animal studies and human clinical trials conducted with other forms of hyaluronic acid, in the sheep model of chronic eosinophil driven sinusitis, the hyaluronic acid based packing demonstrated no beneficial effect with regards to adhesion formation. Our results however, concurred with study by Miller et al.[201] that demonstrated no beneficial effect of Merogel on adhesion formation. We did not note any incorporation of Gelfoam fibres into the regenerating mucosa, as noted by Maccabee et al.[196], and as we did not take samples to study neo-osteogenesis, we cannot comment on this, nor make comparisons with the work by Jacob et al[197]. The adhesion rate in the control sides was 22.22% (2/9), while in the sides packed

with hyaluronic acid based packs, the rate was 44.44%(4/9). Although these results suggest that the use of the hyaluronic acid based packs was detrimental in terms of adhesion formation, the difference was not statistically significant so this conclusion cannot be drawn. The small number of subjects in this study may have contributed to this result, and so meaningful conclusions cannot be reached about the efficacy of hyaluronic acid packs in preventing postoperative adhesions in this model until a larger series has been performed.

Ciliary beat frequency (CBF) was also examined as a means of functional assessment of the ciliated epithelium. At no time point post operatively was there any significant difference between the control sides and the sides packed with the hyaluronic acid based packing. At day 28, there was no measurable ciliary activity from any specimen in either group, suggesting that the regenerating mucosa possessed no active cilia, and thus an absence of mucociliary clearance function. At day 112, there appeared to be a separation between the two groups in favour of the sides treated with the packing, but this was not statistically significant. Neither group had attained their preoperative CBF values by day 112. Relative CBFs were calculated by comparing the CBF values at each time point to the baseline preoperative CBF values, and again, there were no significant differences at any time point. There appeared to be a trend toward increased relative CBFs in the sides treated with the hyaluronic acid based packing. In a study using cultured sheep tracheal cells, topical application of hyaluronic acid resulted in a significant increase in CBF after 7 days in culture, though it had no effect prior to that time point. The authors hypothesised that this was

due to the binding of the hyaluronic acid to a cell surface receptor[202]. There has been no other work conducted to examine the effect of hyaluronic acid on the mucociliary clearance system in either an animal model or in human patients. However, its well documented beneficial effects on tissue healing, which have previously been discussed, would suggest that it would also have beneficial effects on nasal mucosal healing, and this has in fact been demonstrated in a healthy sheep model[117]. It would seem logical that improved epithelial healing would also result in improved function of that epithelium and thus a beneficial effect on ciliary function would be expected. However, in both the healthy sheep model, and in this sheep model of sinusitis, the hyaluronic acid packing conferred no benefit in terms of reciliation of the mucosa[117]. Studies on CBF in a healthy sheep model have not been performed, so it may be that the presence of ongoing inflammation in the sinusitis model prevented the hyaluronic acid from having an effect on CBF. Further work needs to be performed to determine whether the lack of efficacy on CBF is an inherent property of the hyaluronic acid, or as a result of its inability to work effectively in a chronic inflammatory state.

We attribute the lack of efficacy of the hyaluronic acid-based packs in influencing postoperative healing, to the ongoing presence of the *oestrous ovus* infestation and the ensuing inflammatory response. Abnormal histological features[26, 28, 44] have been documented in the presence of infection and inflammation, and section 1 of the results in this thesis has discussed the effects of inflammation on mucosal healing. The milieu of inflammatory factors and bioactive substances may interact with the

hyaluronic acid and interfere with its possible beneficial healing effects, and indeed, there is some evidence for this. In a murine macrophage culture model, hyaluronic acid induced the production of IGF-1 via its interaction with the CD44 surface activation model. This IGF-1 is thought to play a role in pulmonary fibrosis, both by directly promoting fibroblast proliferation, and via its action on tumour necrosis factor alpha (TNF-α), which in turn is involved in fibrosis and granuloma formation[203]. On the other hand, the clinical state of sinusitis has been shown to decrease the relative concentration of hyaluronic acid in the extra cellular matrix[204]. In this study, the nasal bots were not removed at the time of initial surgery, nor were the sheep given appropriate pharmaceutical treatment for their infestation, thus resulting in an ongoing inflammatory state during the healing process.

Conclusion – Merogel

In the sheep model of chronic sinusitis, the use of a hyaluronic acid based nasal pack (Merogel) conferred no beneficial effects on mucosal healing after ESS, as assessed by mucosal reepithelialisation, relative epithelial height, reciliation, measurement of CBF or prevention of adhesion formation

The Effect of a hyaluronic acid-based pack impregnated with Insulin-like growth factor 1, on nasal mucosal healing.

This study has demonstrated that in a sheep model of chronic sinusitis, a Merogel pack impregnated with IGF-1 had no beneficial effects on nasal mucosal healing after full thickness injury, as assessed by reepithelialisation of the injured areas, or in regards to the relative epithelial height of the regenerating epithelium. The mucosa treated with Merogel/IGF-1 also demonstrated no significant improvement in healing when compared to the mucosa treated with Merogel alone.

Numerous studies have demonstrated beneficial effects of topically applied IGF-1 on tissue healing. In a murine model, Menetrey et al. created wounds in muscle and treated them topically with IGF-1. They noted a significant improvement in healing and increased tetanus and fast twitch muscle strengths when compared to controls[205]. In a rat model, Toung et al. used topically applied IGF-1 in a collagen medium to attempt to improve healing of bony nasal defects. In comparison to controls treated with the collagen medium alone, the regenerating bone in the rats treated with the IGF-1 demonstrated significantly increased bone density[206]. Accelerated healing due to topical IGF-1 application was noted in a cell culture model of rabbit gastric epithelium[207], and similar effects were seen in the healing of colonic anastomoses in a rat model[208], both in terms of rate of repair and anastomotic strength.

Conversely, some authors have demonstrated no definite beneficial effect from the topical application of IGF 1. In a porcine model, topical IGF-1 had no significant effect on the healing of partial thickness excisional wounds[209]. Further studies on partial thickness skin wounds in a porcine model demonstrated that recombinant IGF-1 alone had no significant effect on epidermal thickness, though in combination with recombinant platelet-derived growth factor (PDGF), it did have a significant positive effect on connective tissue content and collagen maturity, though this was in the absence of inflammation[108]. Based on this work, which suggested that IGF-1 alone did not influence wound healing, IGF-1 and IGF Binding protein 1, in an emulsified collagen medium, were applied locally to wounded muscles in a rat model. In the subjects treated with the combination, there was a significant increase in the peak breaking strength of the healing muscle at 7 days post wounding, as compared to control subjects, which were treated with IGF-1 alone. A similar significant difference was seen in rats treated with the combination treatment compared with those treated with the collagen medium alone. In subjects treated with IGF-1 alone, and compared to controls that were treated only with the collagen medium, there was no difference in peak breaking strengths[210]. This work suggested that for effective biological activity, an appropriate binding protein was needed if IGF-1 was topically applied. The authors theorised that binding proteins may protect the IGF-1 from the degrading effects of proteases, which are present in significant quantities in the wound environment, by modulating such protease activity. They also commented that slow release formulations of growth factors such as epidermal growth factor are required for clinical efficacy when applied topically to wounds, due to a steady state

model of growth factor receptor occupancy, and this model may well apply for optimal activity of IGF-1[210]. The availability of the therapeutic growth factor is very important, as it has been shown that a major problem with the use of growth factors to enhance wound healing has been poor delivery and retention of the growth factors at the desired site of action[211]. This would be another potential advantage of delivery of growth factors via the hyaluronic acid-based pack as we have shown this pack remains adherent to the wounded nasal mucosa and dissolves slowly over a period of 14 days[117]. Our group has characterised the time course of the release of IGF-1 from the Merogel packing over a 5-day period, and demonstrated a release of 51% of the total amount impregnated (Unpublished data). Other authors have also demonstrated that an esterified derivative of hyaluronic acid, in a microsphere form, was a useful and efficacious method of drug delivery via the transmucosal route [118].

In work conducted in a healthy sheep model, using an identical experimental protocol, McIntosh et al. showed that IGF-1 incorporated into a hyaluronic acid-based nasal pack had a beneficial effect on tissue healing in the normal sheep without sinusitis. At day 28 post operatively, 89% of the injured surface treated with the hyaluronic acid/IGF-1 packing had reepithelialized as compared to 44% in the control areas ($p<0.05$) (Unpublished Data). This contrasts sharply with our findings that the hyaluronic acid/IGF-1 packing had no effect whatsoever on reepithelialisation or epithelial height. As the only difference between the two studies was the presence of the eosinophil driven sinusitis in our model, we must conclude that the presence of the inflammatory state and its associated mediators lead to the lack of efficacy of the

IGF-1. If this situation is compared to that found in patients undergoing surgery for chronic sinusitis, the inflammatory stimulus is usually removed at the time of surgery with removal of the pus and infected material from the sinuses and by the administration of antibiotics at the time of induction of anaesthesia. In the sheep model used for this study, the infection with *oestrous ovus* continued during the healing process thereby simulating the healing of nasal epithelium in an ongoing environment of continued inflammation. This may have had a detrimental effect on the possible benefits of application of the IGF1 impregnated hyaluronic acid.

With regards to reciliation of the epithelium as assessed by scanning electron microscopy, the hyaluronic acid/IGF-1 packs had a detrimental effect on reciliation as compared to controls at both time points post operatively; 59.20% vs. 77.68% at day 56 ($p<0.01$), and 68.70% vs. 87.26% at day 112 ($p<0.01$). Interestingly, there was no significant difference in reciliation between the sides treated with Merogel and those treated with Merogel/IGF-1.

A vital issue in the discussion of wound healing after ESS is reciliation of the nasal and sinus mucosa. Ciliated epithelium is necessary for the function of mucociliary clearance, which is vital for the natural drainage system of the paranasal sinuses[28]. Ciliary regeneration in the healing mucosa has been described as the "most important

requirement for successful surgery"[70]. Using scanning electron microscopic analysis of samples from human patients undergoing ESS, Moriyama et al demonstrated a return to normal ciliated epithelium 6 months after surgery if only mucosa was excised, but minimal reciliation at 18 months post operatively if mucosa was stripped to bone. Effectively, this meant that the healed mucosa, though appearing normal when inspected clinically, was in fact non functional due to the lack of cilia[70]. In this study, at all time points post operatively, there was significantly worse mucosal reciliation in the areas treated with IGF-1 as compared to controls. This is in sharp contrast to McIntosh et al's results in a healthy sheep model. They demonstrated no significant difference between controls and mucosa treated with the hyaluronic acid/IGF-1 packs in terms of reciliation as assessed by electron microscopy. This is also in contrast to the in vitro work performed by McIntosh et al. which demonstrated that topical IGF-1 had a beneficial effect on respiratory epithelial wound healing in a cell culture model and scratch wound assay (Unpublished Data). In the earlier section of this study, we examined the effect hyaluronic acid-based packs had on mucosal healing in the sheep model of chronic sinusitis, and found a statistically significant worsening of reciliation at only one time point post operatively, day 84, but felt that the difference was so small, approximately 10%, as not to be clinically significant. McIntosh et al. did not observe this difference when they utilised an identical experimental protocol with hyaluronic acid-based packs in a healthy sheep model[117]. Based on these studies, we conclude that an interaction between the IGF-1 and the bioactive factors associated with an eosinophilic inflammatory state, was responsible for the deleterious effects on reciliation. IGF-1

has been noted to interact with vascular smooth muscle cells and endothelial cells during inflammatory states and augments the pro-inflammatory activities in these cells that are controlled by tumour necrosis factor 1 alpha (TNF1-α)[212]. In the inflammatory state associated with pulmonary fibrosis, IGF-1 has been implicated in promotion of fibroblast proliferation, and in acting on TNF-α to encourage fibrosis and granuloma formation[203]. We have previously noted and discussed the significantly worsened and slower healing in this sheep model of chronic sinusitis as compared to a healthy model, most likely due to the inflammatory milieu of cytokines, growth factors, etc, which may promote healing by fibrosis and scarring, rather than a regeneration of the normal mucosa, as well as degradative enzymes which may denature the IGF-1.

An important area to consider, when examining the effects of IGF-1, is the concept of cellular differentiation and ciliogenesis. Differentiation is the process where poorly differentiated intermediate cells transform into ciliated epithelium, and ciliogenesis is the process where cilia develop. Mitogenesis, however, describes an increase in the number of cells, which do not necessarily pass down the path of differentiation[16, 213]. One reason that IGF-1 may not have had a beneficial effect on reciliation is that its actions may encourage cell growth rather than cell differentiation. IGF-1 has been shown to have a role in mitogenesis in olfactory epithelium and nasal epithelium[214]. In work carried out in cultured rat tracheal cells, the withdrawal of mitogenic growth

factors such as EGF (epidermal growth factor) resulted in cell differentiation[167], and the authors concluded that high levels of mitogenic growth factors may act to prevent cell differentiation. In sheep tracheal epithelium, EGF application resulted in the growth of basal and undifferentiated cells, with a loss of ciliated cells and goblet cells[215]. In a murine model, IGF-1 was shown to increase bowel length and mass, with an overall increase in cell numbers, but was believed to encourage mitogenesis, in comparison to growth hormone, which promoted cell differentiation[114]. While none of this work has defined with certainty the exact role of IGF-1 in mitogenesis and cell differentiation, there is little evidence that IGF-1 promotes cell differentiation and ciliogenesis. Thus, although IGF-1 demonstrated promising results in a wound scratch mode (Unpublished Data), that work did not specifically examine reciliation, and conclusions cannot be drawn regarding reciliation.

Measurement of CBF was performed as a functional assessment of the regenerating ciliated epithelium. There has been no previous work documenting the effect of IGF-1 on CBF, but other cytokines such as interleukin 1β, tumour necrosis factor α have been shown to cause an increase in CBF in human nasal epithelial cells[216].
The CBFs at each time point were recorded, and then a relative CBF value was also calculated using the day 0 baseline values. At day 112 post operatively, the control sides showed significantly higher CBF than the sides treated with the Merogel/IGF-1 packing, ($p<0.05$). Although some points of separation were noted at other time points, these were not statistically significant. When the relative CBF values were compared, there appeared to be a difference at day 84 in favour of the sides treated

with the Merogel/IGF-1 but this was not statistically significant. The CBF values of the sides treated with the Merogel/IGF-1 were compared with those treated with Merogel alone, to separate the effects that the two materials may be having on CBF. At all time points after day 28, the CBF values of the sides treated with Merogel alone were consistently higher than those treated with Merogel/IGF-1. Despite this obvious trend, none of the differences were statistically significant.

One concern when incorporating bioactive agents into packing materials is the activity of these agents beyond the target region. In previous work done in the healthy sheep model, no systemic or extended local effect was noted in the control samples (Unpublished Data). When a comparison was made between the control sides in the sheep treated with hyaluronic acid/IGF-1 packs and the control sides from the group of sheep treated with hyaluronic acid-based packs, at day 56 post operatively there was a significant difference between the two groups with the IGF-1 controls showing 67.77% reciliation versus 79.07% reciliation in the hyaluronic acid control group ($p<0.01$). This result, though perhaps not clinically significant in terms of reciliation, was suggestive of a possible systemic or extended local effect of the IGF-1 in the packs. There appeared to be no systemic or extended local effects of the IGF-1 with regards to reepithelialisation or relative epithelial height. One could conclude that in the presence of inflammation and its associated cytokines and other bioactive agents, the local absorption and spread of the IGF-1 may be different, possibly due to expression of IGF-1 binding proteins

In rat models, nasally applied IGF-1 has been shown to influence olfactory neurogenesis[217], and also to bypass the blood brain barrier and deliver IGF-1 to

cerebral tissues[218]. The inference from this is that the intranasal IGF-1, which we administer via the hyaluronic acid-based packs, may enter the brain, bypassing the usual protection of the blood brain barrier. Further work would have to be carried out in the sheep model, and then possibly in human trials to confirm this, and then to investigate the possible effects of this.

Conclusions

The use of a hyaluronic acid-based pack impregnated with IGF-1 does not confer any beneficial effects on mucosal healing after ESS in a sheep model of chronic sinusitis, when assessed by reepithelialisation or relative epithelial height. The use of these packs resulted in significantly worse reciliation of the healing mucosa at both days 56 and 112 post operatively. The IGF-1 impregnated packing also had a deleterious effect on CBF, with significantly worse CBF compared to controls at day 112. There may also be an extended local or systemic effect of the IGF-1, evident by significantly worse reciliation in control samples when compared to control samples from a previous study where the packing used was the plain hyaluronic acid based packs. With regards to CBF, again there appeared to be a harmful systemic effect of the IGF-1, with controls demonstrating significantly lower CBF at day 84 compared to the controls for the sheep treated with the hyaluronic acid packs alone. The deleterious effects on reciliation and ciliary function may be due to an interaction between the IGF-1 and the bioactive substances associated with an inflammatory state, and also due to the IGF-1 acting to promote cell growth rather than cell differentiation and the associated ciliogenesis.

Conclusions

The work discussed in this thesis has demonstrated a number of important points. The presence of chronic eosinophil driven sinusitis, induced by the infestation with the oestrous ovus nasal parasite in the sheep model, results in impaired and slower mucosal healing after ESS when compared to healthy sheep. In the presence of this inflammation, the use of a hyaluronic acid based nasal packing, Merogel®, confers no beneficial effects on mucosal healing when assessed by mucosal reepithelialisation, relative epithelial height, reciliation, measurement of CBF or prevention of adhesion formation. When IGF- 1 is impregnated into this packing material, again there are no beneficial effects on mucosal healing when assessed by the same parameters. There is however, a significant slowing of mucosal reciliation when the IGF-1 is used, and a suggestion that the IGF-1 has an extended local, or systemic effect.

While this sheep model of chronic sinusitis is analogous to the disease state in human patients, it differs in that when patients are treated for their sinus disease, the source of their inflammation is usually removed and treated at the time of surgery, whereas in this model, the inflammatory state was allowed to continue on during the healing process. This has demonstrated the importance of removal of all inflammatory tissue and debris at the time of surgical management, as any material left behind which may prolong the inflammatory process will have implications on the healing process. Certainly, it appears that the ongoing inflammatory state is responsible for the lack of efficacy of the hyaluronic acid packing and the IGF-1 in this model.

Further work with this model should involve the treatment of the nasal parasite at the time of surgery to halt the inflammatory process, which would then allow the tissue to heal in a fashion analogous to human patients, and allow the evaluation of the therapeutic interventions such as hyaluronic acid based pacing and IGF-1 in a situation similar to that in which they would be used in human patients. This sheep model of chronic sinusitis will prove useful for this, and for the evaluation other commonly used therapeutic interventions in ESS such as antibiotics and steroids.

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