# Evaluation of sheep model with regard to healing of nasal epithelium after endoscopic sinus surgery

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# TABLE OF CONTENTS

**Title**

**Table of contents**

**Table of figures and tables**

**Abstract**

**Declaration**

**Preface**

**Acknowledgements**

## CHAPTER 1 AIMS

## CHAPTER 2 INTRODUCTION

**2.1 History of nasal mucociliary system**

**2.2 Anatomy and physiology of the nasal mucociliary system**

*2.2.1 Phylogenesis*

*2.2.2 Embryology*

*2.2.3 Location of ciliated epithelium*

*2.2.4 Anatomy and physiology of cilia*

*2.2.5 Physiology of nasal mucus*

*2.2.6 Rheology of nasal mucus*

*2.2.7 Normal mucociliary transport*

## 2.3 Measurement of mucociliary function

*2.3.1 Measurement of ciliary form and motion*

*2.3.2 Measurement of mucus production and its rheological properties*

*2.3.3 Measurement of the combined effects of the mucus and ciliary systems*

## 2.4 Alteration of mucociliary transport in different conditions

*2.4.1 Respiratory tract infection*

*2.4.2 Allergic rhinitis*

*2.4.3 Chronic rhinosinusitis*

*2.4.4 Nasal polyps*

*2.4.5 Surgical trauma*

*2.4.6 Ultrastructural disorders*

## 2.5 Pathophysiology and pharmacology of nasal mucociliary system

*2.5.1 Influence of temperature, ph, and osmolarity*

*2.5.2 Physiological chemicals*

*2.5.3 Sympathicomimetic and sympathicolytic chemicals*

*2.5.4 Parasympathicomimetic and parasympathicolytic chemicals*

*2.5.5 Local anaesthetics*

*2.5.6 General anaesthesia*

*2.5.7 Nasal steroids and decongestants*

*2.5.8 Nasal saline irrigation*

**2.6 Endoscopic sinus surgery (ESS): concept, indications, complications, and results**

*2.6.1 Concept*

*2.6.2 Indications for ESS*

*2.6.3 Complications of ESS*

*2.6.4 Results of ESS*

## CHAPTER 3 METHODS AND RESULTS

**3.1 Standardization of the sheep as a suitable animal model for studying ESS**

*3.1.1 Introduction*

*3.1.2 Aims*

*3.1.3 Material and methods*

*3.1.4 Results*

*3.1.5 Discussion*

**3.2 Effect of packing on nasal mucosa of sheep**

*3.2.1 Introduction*

*3.2.2 Aims*

*3.2.3 Material and methods*

*3.2.4 Results*

*3.2.5 Discussion*

**3.3 Temporal healing pattern and return of cilial function in the nasal cavity after endoscopic resection of full thickness and partial thickness mucosal sections**

*3.3.1 Introduction*

*3.3.2 Aims*

*3.3.3 Material and methods*

*3.3.4 Results*

*3.3.5 Discussion*

**3.4 Evaluation of cilial regeneration by scanning electron microscopy**

*3.4.1 Introduction*

*3.4.2 Aims*

*3.4.3 Material and methods*

*3.4.4 Results*

*3.4.5 Discussion*

**CHAPTER 4 SUMMARY AND CONCLUSIONS**

**BIBLIOGRAPHY**

## Table of figures and tables

*Figure 1* ____ 53
*Figure 2* ____ 68
*Figure 3* ____ 69
*Figure 4* ____ 72
*Figure 5* ____ 81
*Figure 6* ____ 82
*Figure 7* ____ 84
*Figure 8* ____ 85
*Figure 9* ____ 94
*Figure 10* ____ 95
*Figure 11* ____ 96
*Figure 12* ____ 97

*Table 1* ..... 58
*Table 2* ..... 60
*Table 3* ..... 67
*Table 4* ..... 71
*Table 5* ..... 79
*Table 6* ..... 92
*Table 7* ..... 93
*Table 8* ..... 99
*Table 9* ..... 101

## Abstract

Four studies were performed to evaluate the influence of endoscopic sinus surgery (ESS) on the healing of nasal respiratory epithelium in a sheep model.

The first study validated the sheep as a suitable animal model. As part of the standardization of the animal model a middle turbinectomy needed to be performed. The effects of middle turbinectomy on the nasal respiratory epithelium and cilial function were studied. At 3 weeks post turbinectomy the cilial function and histology of the respiratory epithelium was unchanged. The standardized and validated model could now be used to conduct further studies on the effect of ESS on respiratory epithelium.

The second study investigated the effects of pre-operative packing with ribbon gauze and neuropatties on the nasal mucosa of sheep. Both ribbon gauze and neuropatties caused significant mucosal loss when compared with the control. The neuropatties caused less mucosal damage when compared with the ribbon gauze but this difference was not statistically significant. This damage to the epithelium is significant as it may add to the insult of ESS and may cause stasis of secretions with crusting in the post-operative phase.

The third study assessed the temporal healing process of nasal epithelium after full-thickness and partial thickness mucosal removal in sheep. On day 84 post injury there was no significant difference between partial and full-thickness injuries.

However there was a significant difference in the re-ciliation of the two types of wounds. The baseline mucociliary clearance did not differ significantly for either the partial thickness or the full-thickness wounded side. A significant interesting finding was that the healing process took much longer than expected and was still incomplete on day 84 when the sheep were sacrificed. This may account for some of the symptoms seen in the healing period in patients after ESS.

The fourth study assessed cilial regeneration post mucosal injury by scanning electron microscopy. Using a new technique, two blinded observers validated the technique with little intra- or inter-individual difference seen when assessing the specimens. This technique may be useful in further studies as re-ciliation after surgery is crucial for nasal epithelium to gain health and normal function.

This thesis has validated the sheep as a suitable animal model to study ESS and to evaluate factors that may play a role in the healing of nasal epithelium after surgery. Nasal packing was shown to cause a significant injury and should be used by ESS surgeons with caution. Full-thickness wounds should be avoided in the nose where possible as the re-ciliation was significantly reduced when compared to partial-thickness wounds. In addition the healing of nose took significantly longer than previously thought and was incomplete 84 days after surgery. Scanning electron microscopy was shown to be an important outcome measure for the healing of the nasal mucosa after ESS and a new technique for measuring the re-ciliation was validated.

# Declaration

**I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis. I further consent to the thesis being made available for photocopying and loan if applicable, if accepted for the award of the degree.**

4-5-2002

**Chi-kee Leslie Shaw**

## Preface

Part of the work described in this thesis has been published or submitted for publication. The publications are listed in the order of publication.

1. Shaw C-K. L., Dymock R.B., Cowin A., Wormald P-J. (2000) Effect of packing on nasal mucosa of sheep. Journal of Laryngology and Otology; 114: 506-509.

2. Shaw C-K. L., Cowin A., Wormald P-J. (2001) Standardization of the sheep as a suitable animal model for studying endoscopic sinus surgery. Australian Journal of Oto-Laryngology; 4 (1): 23-26.

3. Shaw C-K. L., Cowin A., Wormald P-J. (2001) A study of the normal temporal healing pattern and the mucociliary transport after endoscopic partial and full-thickness removal of nasal mucosa in sheep. Immunology and Cell Biology; 79 (2): 145-148.

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# CHAPTER 1

# AIMS

The aims of this thesis were the followings:

1. To develop a standardized sheep model by establishing the effect of the initial surgical manipulation of the middle turbinate on the respiratory epithelium and cilial function

2. To assess the effect of preoperative packing with 2 most commonly used materials, ribbon gauze and neuropatties, on the nasal mucosa

3. To establish the temporal healing pattern and return of cilial function in the nasal cavity of the sheep after resection of full thickness and partial thickness mucosal sections

4. To evaluate cilial regeneration by scanning electron microscopy

# CHAPTER 2

# INTRODUCTION

## 2.1 HISTORY OF NASAL MUCOCILIARY SYSTEM

There has been a growing acknowledgement over the last century of the important role played by mucociliary clearance in the efficient functioning of the respiratory system. Mucociliary transport is one of the most important first line defense mechanisms of both the upper and lower airway (Quraishi et al, 1998).

The mucociliary clearance was first observed by Purkinje and Valentin in 1830s (Purkinje and Valentin, 1834). Sharpey provided the first detailed descriptions of the action of cilia in a wide range of animals as well as the observation of ciliary motion in the reproductive and respiratory systems of mammals (Sharpey, 1835). Over the last century there has been a period of active research into the mucociliary apparatus notably by such researchers as Hilding, Lucas, and Proetz (Sleigh et al, 1988). Lucas and Douglas not only confirmed the role of cilia in nasal clearance, but they also concluded that mucus is propelled by the tips of cilia, which themselves move in a low viscosity layer beneath the mucus (Lucas and Douglas, 1934). The relationship between the properties of mucus and mucociliary clearance was further studied by Dalhamn and he concluded that the composition and quality of the secretions are important factors (Dalhamn, 1956).

The development of electron microscopy provided a new impetus into understanding of the mucociliary transport apparatus. Electron microscopy provides exquisite detail of the normal structure of cilia and is also indispensable in identifying ciliary dysmorphology. Morphological abnormalities of cilia result in a spectrum of

respiratory tract pathology including chronic rhinosinusitis and recurrent bronchial infection (Greenstone et al, 1988).

## 2.2 ANATOMY AND PHYSIOLOGY OF THE NASAL MUCOCILIARY SYSTEM

### 2.2.1 PHYLOGENESIS

The biological structure of cilia is phylogenetically very old as they are found in all animal species. They are found in the respiratory tract of vertebrates especially and they serve to keep airway clean by propelling the mucus layer (Deitmer, 1989). Cilia are not only important in mucociliary clearance for airway, but they are also important in reproduction as the movement of spermatozoa is caused by a ciliary motor in the tail of the sperm (Deitmer, 1989).

### 2.2.2 EMBRYOLOGY

Cilia can be demonstrated in the human embryo from fourteenth week onward, however, it is not known if cilia are functioning at this time (Proetz, 1953; Carson et al, 1981). It is thought that cilia undertake special functions during human development. The observation that molecular defects of the cilia in the immotile cilia syndrome are associated with situs inversus in 50% of cases supporting the theory that the visceral tube was rotated by the action of cilia (Afzelius, 1979).

## 2.2.3 LOCATION OF CILIATED EPITHELIUM

It is possible that almost every cell can form cilia as each cell possesses the primary tubule apparatus in the form of a mitotic centriole which is present in the cilia (Friedman and Bird, 1971). In the human, all surfaces of the upper airways are covered by ciliated epithelium with the exception of the nasal vestibule, parts of the nasopharynx, pharynx, and larynx that are covered by squamous epithelium, and the olfactory area, which has a specialized sensory epithelium. The tracheobronchial tree is ciliated down to the nonalveolar walls of the respiratory bronchioles (Sleigh et al, 1988).

Apart from the respiratory tract, ciliated epithelium is found in the efferent ductules of the epididymis, the fallopian tube and adjacent parts of the uterus (Afzelius, 1979). Cilia are also found in tissues such as pancreas, kidney, liver, heart, connective tissue and skin, where their function is unknown (Deitmer, 1989).

## 2.2.4 ANATOMY AND PHYSIOLOGY OF CILIA

Normal cilia have a highly organized symmetrical substructure, which includes two centrally situated separate vertical tubules, surrounded by nine sets of vertically orientated paired microtubules (doublets), the so called 9+2 configuration. The doublets are linked by strands of nexin, and the A subfibers of each doublet possess

outer and inner dynein arms, projecting towards the next doublet. These radial spokes with dilated heads can attach to projections associated with central microtubules. Dynein arms are associated with ATPase activity (Sleigh et al, 1988; Deitmer, 1989).

Propulsion of liquids by cilia can be explained by two active parts in the ciliary beat cycle - the effective (or power) stroke, and the recovery (or preparatory) stroke of the cycle. In the effective stroke of the cycle cilia remains fully extended and move through an arc in a plane perpendicular to the cell surface, whereas in the recovery stroke, a bend is spread along the length of the cilia from base to tip. This allows the cilia to swing sideways near the cell surface back to the starting position for the next effective stroke. The outer nine doublets actively slide against one another to produce bending movements. The ATPase protein in the dynein arm uses energy from ATP for active sliding movements (Sleight et al, 1988; Deitmer, 1989). Ciliary movement is coordinated into a continuous metachronous wave. In a metachronous process there are three possible directions of the effective beat of the cilia, and thus of transit and the direction of the metachronous wave pattern. When the effective beat and the metachronous wave run in one direction, it is termed symplectic metachrony. On the other hand, it is termed antiplectic metachrony when the effective beat and the metachronous wave run at 180 . Diaplectic metachrony is the wave pattern whereby the effective beat and the metachronous wave run at 90 . The antiplectic metachronous wave pattern is the most common pattern, and is found in the respiratory system of vertebrates (Deitmer, 1989). During the effective stroke, the tips of cilia are in contact with the mucus. The cilia beat with a frequency

normally between 5 and 20 Hz at 37 C and this rate is dependent on temperature and viscosity of mucus layer (Deitmer, 1989). The cilia beat most efficiently at 10.8 Hz (Devalia et al, 1990). Most of the data concerning ciliary beat frequency (CBF) were collected from in vitro studies. The CBF in vitro varies from zero, when cilia remain in the rest position, to about 30 Hz. There are only scanty data concerning the CBF in vivo because of the technical difficulty of making measurements (Sleigh et al, 1988).

### 2.2.5 PHYSIOLOGY OF NASAL MUCUS

Mucus is an essential component of the mucociliary transport system. Mucus serves to trap and transport inhaled airborne particles and endogenous products and it is produced at a resting rate of 0.5 to 1 ml of mucus/$cm^2$ mucosa over 24 hours (Quraishi et al, 1998). Nasal mucus consists of a combination of glycoproteins (sialomucins, fucomucins, sulfomucins) and ions in 95% water (Boat and Mathews, 1973). The proportion of the constituents of the healthy mucus is: water 95%, proteins and glycoproteins 2-3%, lipids 1%, mineral 1% and DNA 0.02% (Mathews et al, 1963; Potter et al, 1967). The water and ions contents are produced mainly from the serous glands and from transudation from the capillaries. Mucus also contains other endogenous products including enzymes (lysozymes, lactoferrin), circulatory complements, C reactive protein, immunoglobulins (IgA, IgE, IgG, IgM, IgD), and cells (surface epithelium, basophils, leucocytes and eosinophils) (Quraishi et al, 1998). Eighty percent of the dry weight of mucus is made up of glycoproteins.

These mucins are secreted by the goblet cells and the seromucinous glands of the lamina propria. Globlet cells respond to direct stimulation whereas mucous and serous glands are under the control of the parasympathetic nervous system being stimulated by muscarinic receptors of the M1 and M3 subtypes (Mullol, 1992). The mucins are high molecular weight polydisperse glycoproteins with a large amount of sugar residues. The carbohydrate and sugar components of the mucins are thought to have a considerable influence on the rheological properties of mucus (Barton and Lourenco, 1973). The mucins can link to each other either by non-covalent bonds or by covalent bonds. And the three dimensional lattice which is created contributes to the viscoelastic properties of mucus as well (Litt, 1981; Silberberg, 1983; Carlstedt et al, 1985). As the mucins absorb water, they swell rapidly and form a gel (Quraishi et al, 1998).

The nasal mucus is thought to consist of a double layer (Lucas and Douglas, 1934). The overall thickness of this nasal mucous layer is quite variable and lies between 0.5 and 10 μm (Reissig, 1978). The cilia move freely in the aqueous sol layer (also called the periciliary layer) close to the cells but only contact the undersurface of the superficial gel layer (Deitmer, 1989; Quraishi et al, 1998). The tips of cilia engage the gel layer during their effective stroke but lying beneath it during the recovery stroke (Sanderson and Sleigh, 1981). The gel layer with a depth of 0.5-2.0 μm is believed to originate from mucosal glands and it contains most of the glycoproteins. The sol layer has a depth of 7-10 μm and it arises either in the seromucinous glands or by transudation and resorption directly through the cell membrane (Rutland et al, 1982; Deitmer, 1989). The double layer of the nasal mucus can be explained by the

presence of a pH gradient at mucosal surface (Deitmer, 1989). These two layers of the mucous blanket are visible on both light microscopy and electron microscopy (Deitmer, 1989). The cilia tend to beat at a frequency of about 10 Hz with fast effective strokes and slow recovery strokes in this sol layer and thus produces effective mucociliary transport (Quraishi et al, 1998). However, the cilia will not be able to form a recovery stroke if the sol layer is too shallow. The mucociliary transport will also be impaired if the sol layer is too deep as the cilia cannot reach the gel layer (Proctor, 1977; Sleigh, 1990).

### 2.2.6 RHEOLOGY OF NASAL MUCUS

Mucus behaves as a non-Newtonian viscoelastic material (Sleigh et al, 1988). This non-Newtonian material behaves in a way that the viscosity of mucus decreases as the applied force is increased, so the more forcefully the cilia beat the more easily the mucus moves. The viscoelastic properties of nasal mucus are mainly derived from the glycoproteins and water. Other constituents such as immunoglobulins, ions and DNA also exert some effect (Quraishi et al, 1998). The optimal viscoelastic properties of mucus for efficient nasal mucociliary transport is determined by the ratio between viscosity and elasticity. There is a significant positive and negative correlation between the mucociliary transport rate and the viscosity/elasticity ratio at values above and below the optimal viscoelasticity (Quraishi et al, 1998). The mucociliary transport rate is proportional to the elastic recoil and is inversely proportional to viscosity of mucus (Litt, 1970; Dulfano and Adler, 1975; Adler and

Dulfano, 1976). Thus the viscosity of mucus falls with increasing rate of shear, but at the same time the elasticity of mucus increases as the rate of shear increases, so that at low frequencies viscous effects dominate and at high frequencies elastic effects dominate (Sleigh et al, 1988). In addition to the elasticity of the mucus, the cohesional forces between the molecules within the mucous layer facilitate the formation of a continuous mucus blanket. The cohesional forces have to be strong enough to overcome gravity (Quraishi, 1998). Spinnability is a property which is shared by all types of mucus and this is characterized by its ability to be drawn into long threads under the effect of traction. Spinnability has been used as a measure of cohesion and elasticity of mucus. It has been shown that as spinnability decreases the mucociliary transport increases (Quraishi et al, 1998).

In summary, the rheological properties of normal mucus are very well matched with the characteristics of the ciliary propulsion system (biorheological matching). The glycoproteins provide appropriate viscoelasticity and cohesional forces and sheets to be propelled efficiently (Silberberg, 1983; Sleigh et al, 1988; Quraishi et al, 1998).

### 2.2.7 NORMAL MUCOCILIARY TRANSPORT

Normal mucociliary transport requires an intact, metachronously beating ciliated respiratory epithelium, as well as a balanced mucous blanket of optimal viscoelasticity (Deitmer, 1989). The characteristics of mucociliary transport may be altered by changes in any of these components, and such changes may be used to

regulate mucociliary transport or changes resulting from disease may interfere with transport (Sleigh et al, 1988). It is not yet clear if nervous or hormonal influence plays a direct role in regulating the mucociliary transport. There is evidence for nervous and hormonal control of mucous secretions. An increase in mucous load in turn stimulates ciliary activity, however, there is no convincing evidence of direct nervous or hormonal control of ciliary beat frequency. It has been suggested that mucociliary clearance is regulated more by reflexes (or even direct cellular contacts) that stimulate secretions and alter ion balances than by control of ciliary beating. If there is direct control of ciliary beating, an adrenergic system is more likely to be involved (Sleigh et al, 1988).

One of the functions of the nasal mucociliary transport is to transport about 80% of the inhaled particles >12.5 μm that trapped in the mucous blanket and to move posteriorly towards the nasopharynx (Quraishi et al, 1998). The direction of mucociliary transport is the same in all humans. Even after mucosal injury the regenerating ciliated epithelium will adapt itself to the direction of beat of the surrounding mucosa. The underlying control mechanism is not clear (Deitmer, 1989). There are several characteristic features of the mucociliary transport in the nose. Firstly, there are differences in mucociliary transport rates between different sites in the nose depending on ciliary beat frequency, density of ciliated epithelial cells, length of the cilia, and mucus quality (App et al, 1993). The mucociliary transport rate at the anterior portion of inferior turbinate is 1-2mm/hour, and increases to 8-10 mm/hour on the posterior portion of the inferior turbinate (Lale et al, 1998). At areas lacking of ciliated epithelium the mucociliary transport can be

bridged there by the traction exerted by the viscous mucous layer (Giordano and Holsclaw, 1976). Thus the direction of the transit can also be changed by a change in the viscosity of the mucus when obstacles to the direction of ciliary beat are present (Deitmer, 1989). In addition, the direction of mucociliary transport in the nose is independent of body position and the transit is always directed towards the nasopharynx. Mucus is then carried from the posterior surface of the soft palate to the posterior wall of pharynx. Any obstacles such as septal spur will be bypassed and mucus will still be carried towards the nasopharynx (Deitmer, 1989). On the other hand, mucociliary transport in the paranasal sinuses is directed towards the natural ostium. For example, the mucus is still carried towards the natural maxillary sinus ostium even after the creation of inferior meatal antrostomy into the maxillary sinus. The mucociliary transport in the maxillary sinus follows a star shape originating from the floor and ascending in a spiral to the natural ostium (Messerklinger, 1966). The ethmoid and sphenoid sinuses drain directly into the natural ostium (Deitmer, 1989). There are two specific transit pathways in the frontal sinuses. The first transport pathway includes a lateral stream to the natural ostium from the lateral part of the floor of the frontal sinus, and a descending stream in the lateral part of the frontonasal duct. The second pathway involves an ascending stream in the medial wall of the frontonasal duct extending as far as the intersinus septum. Therefore, only part of the mucus is eventually transported into the nose from this circular transport pattern (Messerklinger, 1967; Deitmer, 1989).

## 2.3 MEASUREMENT OF MUCOCILIARY FUNCTION

Nasal mucociliary clearance can be studied by measuring one of the followings: (1) ciliary form and motion; (2) mucus production and its chemical and physical characteristics; and (3) efficiency of the combined effects of the mucus and ciliary propulsion system (Lale et al, 1998).

### 2.3.1 MEASUREMENT OF CILIARY FORM AND MOTION

In order to study the ciliary activity alone, mucus-free isolated cilia needs to be harvested from the nasal cavity. Nasal cilia are easy to harvested from the inferior turbinate, the floor of the nose and the anterior nasal septum with the use of nasal brushing, forceps or curettes (Lale et al, 1998).

The ciliary beat frequency (CBF) was first measured by Martius with stroboscopy in 1884 (Deitmer, 1989). However, this is unreliable at frequencies around 6-20 Hz because of the phase difference between groups of cells and metachronous movement (Bleeker and Hoeksema, 1971; Torremalm, 1975). Moreover, the entire preparation can never be brought to virtual immobility by the stroboscopy. Stroboscopy must only be regarded as a psychosensorial method of measurement (Deitmer, 1989).

In 1933 Lucas first reported that it was possible to observe the ciliary activity of respiratory mucosa under reflected light with relatively minor magnification (Lucas, 1933). In 1962 Dalhamn and Rylander were first to carry out measurements of CBF with this method using a photosensitive cell that converts the reflections of light from beating cilia into an electric current and then an oscilloscope display via an amplifier (Dalhamn and Rylander, 1962). This is the most popular applied method for measurement of CBF (Lale et al, 1998). Since then various modifications have been described with the advent of newer electronic developments. Scattering of a laser beam by the cilia or interruptions of a light source can all be transduced to an electric signal which can be amplified, filtered, and then digitized. A fast Fourier transformation using computer software can then be used to calculate, and display the CBF results with statistical analysis (Lale et al, 1998). When studying preparations with beating cilia in vitro, it is important to maintain the specimens at constant temperature, pH and osmolality in a physiological range. CBF shows consistent readings in the temperature range 32-40 C. Between 19 C and 32 C CBF increases in a linear fashion, and above 40 C CBF declines (Lale et al, 1998).

Various ultrastructural abnormalities of cilia can impair the mucociliary transport. The ciliary structural abnormalities can be detected by transmission electron microscopy. Precautions must be taken during preparation of the mucosa as the mucosal surface is usually covered by mucus or blood so that ciliated epithelium itself remains concealed (Deitmer, 1989). The mucus can be washed away from the mucosal surface by rinsing the specimen in physiological saline. The orientation of the specimen can be facililated by stretching the mucosa on a surface, and by doing

so it helps to prevent contraction of the specimen. Finally rapid fixation of the specimen is also important during the specimen preparation since autolysis can rapidly change the ultrastructural appearances (Deitmer, 1989).

The biopsy of nasal cilia is relatively straightforward, but the equipments required to measure CBF or to examine ultrastructures of cilia is complex and expensive. This equipment is only available in a few research centers (Lale et al, 1998).

### 2.3.2 MEASREMENT OF MUCUS PRODUCTION AND ITS RHEOLOGICAL PROPERTIES

Several methods of measuring nasal mucus rheology in vitro have been described, but these in vitro studies do not give a true picture of in vivo conditions as only small mucus samples can be obtained from normal healthy nasal mucosa. In addition, the proportion of mucus sample which is derived from the gel layer or from the sol layer cannot be determined. Different machines have been built to measure certain properties of mucus. The main ones in use are the magnetic microrheometer, the controlled stress technique, the capillary viscometer, and the coaxial cylinder sensor system (Lale et al, 1998; Quraishi et al, 1998). However, the properties of mucus these machines measured do not correspond precisely to a definite physiological function. Thus a reliable test for measuring rheological properties of mucus with a definite clinical application is yet to be found (Lale et al, 1998).

## 2.3.3 MEASUREMENT OF THE COMBINED EFFECTS OF THE MUCUS AND CILIARY SYSTEMS

**Saccharin test**

This test is a simple and widely used clinical test for measuring mucociliary transport. It was first described by Andersen in 1974, and the test was subsequently modified by Rutland and Cole (Andersen et al, 1974; Rutland and Cole, 1981). The size of the saccharin crystal used has varied from a diameter of 0.5 mm up to a diameter of 3 mm with a weight of 14mg (Deitmer, 1989). Usually a 1mm diameter or quarter fragment of a saccharin tablet is placed at the anterior end of inferior turbinate, and the patient is asked sit quietly with their head forward, not to sniff, sneeze, eat or drink. The time taken to the first perception of the sweet taste is the saccharin clearance time (Rutland and Cole, 1981; Lale et al, 1998). It is believed that the saccharin dissolves in the entire mucous layer and presumably the periciliary fluid layer, and saccharin is transported to the nasopharynx and the base of tongue (Deitmer, 1989; Lale et al, 1998).

Saccharin clearance time is a useful screening test. Various definitions have been proposed of the end point at which the test should be regarded as negative (Deitmer, 1989). The average saccharin clearance time for a healthy adult free of nasal disease is between 7-15 minutes (Lale et al, 1998). Most researchers accept that saccharin clearance time greater than 20-30 minutes should be considered as abnormal (Deitmer, 1989; Lale et al, 1998). When using the saccharin clearance test as a screening tool, it must be taken in conjunction with the patient's presenting

symptoms and complete physical examination of the nose. If the saccharin clearance time is greater than 60 minutes, it is likely that there is a significant abnormality of cilia or mucus. It is also likely that patients who have a saccharin clearance time greater than 20 minutes have disturbed nasal mucociliary transport. And if these patients who have treatable nasal disease with saccharin clearance time greater than 20 minutes, their impaired mucociliary transport might be improved by the appropriate treatment of the nasal disease (Lale et al, 1998).

**Insoluble inert markers**

Charcoal powder is an inert insoluble substance which has been used as an inert tracer for measurement of mucociliary transport rate. And charcoal powder was reported as the most reproducible method of assessing nasal mucociliary clearance (Passali et al, 1984). Charcoal powder was transported more rapidly than saccharin by the mucociliary clearance. This suggests that the mucus layer is the most effectively transported layer (Armengot et al, 1990; Lale et al, 1998).

**Dye**

A dye such as indigo carmine can be placed on the anterior nasal mucosa and measuring the time taken to appear in the oropharynx (Duchateau et al, 1985). This is relatively time consuming for the investigator to watch the oropharynx and requires the subject to keep his mouth open for up to 20 minutes. The results obtained from this method are similar to that provided by saccharin clearance time (Lale et al, 1998).

**Radioisotope method**

With the advent of technology, the nasal mucociliary clearance can be measured by gamma scintigraphy. A particle of known size is first radiolabelled with $^{99m}Tc$ or $^{111}I$. A gamma camera is then used to track the movement of many of these radiolabelled particles that are inhaled, sprayed or insufflated into the nose (Lale et al, 1998). The fall in detected radioactivity is proportional to the square of the distance from the nasal tip to the moving particle. The mucociliary transport rate using this method was between 3.3-8.2 mm/minute (De Espana et al, 1986). Different tracer used will affect the speed of transport in the nose. For example, seroalbumin labeled with $Tc^{99}$ was transported at 8 mm/minute but pertechnetate labeled with $Tc^{99}$ was transported at a speed of 5 mm/minute (Ewert, 1965). This radioisotope method provides the most physiological information about the deposition, dispersion and clearance of particles in the nose. However, this method remains a research tool for the study of intranasal drug delivery as the equipments involved are not readily available to most of the research units and the subject is exposed to radiation (Davis et al, 1992; Lale et al, 1998).

## 2.4 ALTERATION OF MUCOCILIARY TRANSPORT IN DIFFERENT CONDITIONS

### 2.4.1 RESPIRATORY TRACT INFECTION

Viruses responsible for the common cold not only disrupt the ciliary propelling system, but they also alter the viscoelasticity of the mucus leading to impairment of the mucociliary clearance (Deitmer, 1989; Lale et al, 1998). In viral infections ciliated epithelium undergoes extensive acute desquamation with only 10% of the surface is covered by ciliated epithelium (Deitmer, 1989). The resulting damage to the cilia is the primary cause for the reduction in mucociliary clearance (Quraishi et al, 1998). Disorientation of ciliated cells may occur in regeneration after infections, and this may lead to abnormal mucociliary clearance with stasis of mucus (Sleigh et al, 1988). Viral infection also causes an increase in vascular permeability and an increase in the volume of nasal secretions. There is a reduction in the elasticity of mucus and nasal secretions tether to the gland openings. The released inflammatory mediators (histamine, prostaglandins, leukotrienes C4 and D4, and bradykinin) increase the volume and reduce the viscosity of nasal secretions while leukocytes, immunoglobulins and DNA, increase the viscosity of mucus (Quraishi et al, 1998).

Bacterial infections caused by Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus and pseudomonas will lead to protracted cell damage. These bacteria produce specific toxins that damage epithelial cells with the loss of ciliated

epithelium. Neutrophils which gather at the sites of infection also produce an elastase that is directly toxic to ciliated respiratory epithelium (Lale et al, 1998).

### 2.4.2 ALLERGIC RHINITIS

The results from experiments on the patients with allergic rhinitis are controversial. Some studies suggested that there is an increase in nasal mucociliary clearance while other studies suggested a reduction in response to allergen challenge (Lale et al, 1998). During acute allergic nasal reactions there is an alteration in the rheological properties of mucus and an increase in CBF as a result of released inflammatory mediators. Therefore, the nasal mucociliary clearance might be improved. However, there are also damages to the nasal cilia in patients with allergic rhinitis with absence of dynein arms and radial spokes, ciliary membrane damage, compound cilia and disorientation of central tubules (Lale et al, 1998). All these ciliary changes can adversely affect the nasal mucociliary clearance.

### 2.4.3 CHRONIC RHINOSINUSITIS

This chronic inflammatory disease of nasal and sinuses mucosa results in abnormal mucus and cilia. The mucosa becomes oedematous with shedding of ciliated epithelial cells and squamous metaplasia. Ciliary abnormalities have also been

demonstrated such as compound cilia (Lale et al, 1998). In chronic sinusitis there is a mucus hypersecretion as a result of hyperplasia and hypertrophy of nasal acinar cells (Quraishi et al, 1998). The high viscosity and elasticity of retained sinus secretions is due to the absorption of sodium and water with released DNA from damaged host and bacterial cells. Levels of serum and local proteins also rise during inflammatory process. This rise in local proteins contributes to the high viscosity of mucus as well (Quraishi et al, 1998). Thus excessive viscous fluid with ciliary abnormalities impairs the nasal mucociliary clearance in chronic rhinosinusitis.

### 2.4.4 NASAL POLYPS

Nasal polyps are oedematous swelling of the nasal or sinus mucosa and are commonly found in chronic rhinosinusitis. The ciliated surface of the polyps can undergo squamous metaplasia. The direction of mucus flow may be altered due to the pedunculated swelling of mucosa (Lale et al, 1998). It has been shown that patients with nasal polyposis have impaired mucociliary clearance as measured by saccharin clearance time and gamma scintigraphy (Lale et al, 1998).

## 2.4.5 SURGICAL TRAUMA

Any form of nasal or sinus surgery involves certain degree of trauma to the ciliated respiratory epithelium. In particular endoscopic sinus surgery involves removal of varying amount of respiratory mucosa in order to widen the natural sinus ostium for ventilation and drainage. Currently little is known about the healing of the nose. The time taken for epithelial regeneration varies, and it depends on the type and extent of damage (Deitmer, 1989). It is generally believed that mucosal regeneration is possible if the remaining basement membrane is left intact (Deitmer, 1989). The regenerated nasal or sinus mucosa usually resumes mucociliary transport in the same direction as before (Proetz, 1953).

## 2.4.6 ULTRASTRUCTURAL DISORDERS

Ultrastructural disorders can be genetically determined or acquired. Genetically determined defects including defects in the dynein arms and radial spokes, are often found in both respiratory tract cilia as well as in the tail of the sperm of the same individual (Deitmer, 1989). On the other hand, the acquired ultrastructural changes of cilia are caused by physical or chemical injury or by chronic inflammation (Deitmer, 1989). About 5-10 percent of the cilia show abnormalities in children and adults who are free of nasal disease (Lale et al, 1998). There are several congenital anomalies of respiratory tract cilia which affect nasal mucociliary function. In the

well-known Kartagener's syndrome (dextrocardia, recurrent sinusitis and bronchiectasis) the immotile cilia are due to the absence of dynein arms. The mucociliary clearance is severely impaired in these patients as they have only 40% of their ciliated cells working and they also lack coordination or metachronicity (Greenstone et al, 1988; Lale et al, 1998). Kartagener's syndrome is only a subgroup of a wide spectrum of disease termed primary ciliary dyskinesia (Greenstone et al, 1988). Primary ciliary dyskinesia is characterized by life-long sinusitis, recurrent bronchial infection, and impaired mucociliary clearance as a result of structural defects of the ciliary axoneme such as deficient dynein arms (Greenstone et al, 1988). Nasal mucociliary clearance, as measured by saccharin clearance time, is grossly abnormal in these patients. Therefore, saccharin test is a useful, sensitive screening test for impaired nasal clearance. However, it is difficult to perform in children due to lack of cooperation. In addition, this test is not particularly specific in that it measures the effects of mucus as well as ciliary function (Greenstone et al, 1988). Comparable recurrent sinobronchial infection and male infertility can also be found in cystic fibrosis and Young's syndrome. However, these conditions differ from primary ciliary dyskinesia in that the infertility in both cases is due to azoospermia and problem with mucus while ciliary ultrastructure is normal (Greenstone et al, 1988; Lale et al, 1998).

## 2.5 PATHOPHYSIOLOGY AND PHARMACOLOGY OF NASAL MUCOCILIARY SYSTEM

### 2.5.1 INFLUENCE OF TEMPERATURE, PH, AND OSMOLARITY

Physiological investigations of ciliary activity were first studied by Engelmann in 1877 (Deitmer, 1989). It has been shown that the optimal CBF lay between 30 and 40 C, and above and below these temperatures the CBF drops (Deitmer, 1989; Lale et al, 1998). Mucociliary system is very sensitive to excessive drying but within the usual atmospheric conditions there is little alteration in nasal mucociliary transport rate (Proctor et al, 1973). The optimal relative humidity is about 90% (Deitmer, 1989). Neutral pH values are crucial for optimal ciliary activity. Ciliary activity is not affected with pH values between 7 and 10, whereas ciliary stasis rapidly set in outside these pH values (Deitmer, 1989). Appropriate osmolarity is necessary for normal ciliary activity. It is a controversial area as to whether hyper- or hypo-osmolar solutions lead to loss of ciliary activity (Deitmer, 1989).

### 2.5.2 PHYSIOLOGICAL CHEMICALS

Extensive investigations have been carried out in a search for a suspected physiological regulation of nasal mucociliary clearance. Histamine, gamma-

aminobutyric acid (GABA), vasoactive intestinal peptide (VIP), encephalin, and calcitonin-gene related peptide, have been shown that they have no influence on ciliary activity (Deitmer, 1989). On the other hand, prostaglandin E1, E2 and F2α, ATP, capsaicin, neurokinin A, bradykinin and substance P were found to stimulate ciliary activity (Deitmer, 1989).

### 2.5.3 SYMPATHICOMIMETIC AND SYMPATHICOLYTIC CHEMICALS

For a long time it has been a controversy about the effects of sympathomimetic drugs on the mucociliary system. Some studies showed that mucociliary transport was accelerated by sympathomimetics in vivo experiments, whereas other studies showed no such effects, or only brief effects were found (Deitmer, 1989). Recent evidence indicated that α-adrenergic stimulation causes secretion from serous cells of the submucosal glands, whereas β-adrenergic agonists stimulate the mucous cells (Nadel, 1983; Basbaum, 1984). Several β-adrenergic drugs such as isoprenaline, terbutaline, and fenoterol have been shown to increase mucociliary clearance but these effects have been questioned by others (Sleigh et al, 1988). Adrenergic compounds also increase the CBF probably by directly altering the $Ca^{2+}$ balance of ciliated cells. However, noradrenaline, an α-adrenergic agent, appears not to have an effect on the mucociliary clearance (Sleigh et al, 1988). The effects due to sympathicolytic agents such as propanolol and reserpine were variable (Deitmer, 1989).

## 2.5.4 PARASYMPATHICOMIMETIC AND PARASYMPATHICOLYTIC CHEMICALS

Parasympathicomimetic drugs such as acetylcholine and pilocarpine, have been shown to increase the secretion of mucous cells, whereas parasympathicolytic drugs (atropine and scopolamine) reduce ciliary activity indirectly by decreasing this secretion (Deitmer, 1989; Sleigh et al, 1988). The cholinergic drugs stimulate mucociliary transport indirectly by increasing mucous secretion, which in turn stimulates the cilia (Sleigh et al, 1988). However, there is no evidence to show that human respiratory cilia is under direct cholinergic control (Sleigh et al, 1988).

## 2.5.5 LOCAL ANAESTHETICS

Cocaine and lignocaine are both widely used local anaesthetics in nasal and sinus surgery. Their systemic effects are well documented, however, little is known about their effects on nasal mucociliary clearance.

**Cocaine**

Cocaine has an excellent anaesthetic as well as vasoconstrictive properties. The anaesthetic property of cocaine is due to the competition of cocaine with calcium ions for a site in the nerve membrane that controls the passage of sodium ions. This blockage of sodium current interferes with the generation of nerve impulse and

therefore, produces anaesthesia (Jackson and Hersey, 1991). On the other hand, cocaine produces vasoconstriction by blocking the reuptake of endogenous noradrenaline and thus, enhancing the action of noradrenaline on alpha receptors of the vascular smooth muscle resulting in a prolonged vasoconstriction (Jackson and Hersey, 1991). The effect of cocaine on nasal mucociliary clearance is controversial and inconclusive. Lierle and Moore found that 5% cocaine solution did not affect ciliary activity, whereas a 10% and a 20% cocaine solution produced a distinct but reversible impairment in mucociliary clearance in their in vitro study (Lierle and Moore, 1934). Ingels et al also demonstrated in their in vitro experiments that cocaine decreased CBF and ciliary beat harmony at concentrations of 1.75% and higher (Ingels et al, 1994). On the other hand, data obtained by in vivo experiments may differ from in vitro effects. Ukai et al found that a 5% cocaine solution decreased mucociliary transport, whereas in their in vitro experiment, a 20% cocaine solution was needed to decrease CBF (Ukai et al, 1985). Many of the results were measured in vitro. The in vitro and in vivo measurements cannot be compared since cocaine affects mucosal blood supply (Ingels et al, 1994). The effect of cocaine on mucociliary clearance should be ideally studied in vivo as these studies reflect the actual behaviour of the mucociliary system. The intact nasal mucosa possesses an intact neurovascular supply and secretion with buffer that facilitate adaptation to varying osmotic pressures or pH values different to that of nasal secretions. The in vitro nasal mucosa does not possess this adaptation mechanisms, and therefore, the results obtained in vitro cannot be compared with those obtained in vivo (Armengot et al, 1989).

**Lignocaine**

Lignocaine is an amide local anaesthetic and it acts by stabilizing the cell membrane, reducing permeability to sodium ion entry to nerve fibers and avoiding membrane depolarization (Armengot et al, 1989). The results on the effect of lignocaine on mucociliary clearance are conflictive. In vitro studies showed that ciliary motility is profoundly reduced and even eliminated by lignocaine (Dudley and Cherry, 1978; Mostow et al, 1979). Mostow et al also showed additive ciliotoxic effects due to the stabilizer substance methyl-hydroxy-benzoate (Methylparaben) (Mostow et al, 1979). However, many in vivo studies reported no influence of lignocaine on mucociliary transport, even at concentrations of 4% (Ewert, 1967; Rutland et al, 1981; Armengot et al, 1989). Therefore, objections to the clinical use of lignocaine are not valid (Deitmer, 1989).

## 2.5.6 GENERAL ANAESTHESIA

The oxygen supply to the nasal mucosa can be provided via the blood circulation and also via the luminal air. Pure oxygen has no ciliotoxic effect (Toremalm et al, 1977). There is controversy about the effect of pure nitrogen and carbon dioxide (Deitmer, 1989). Gaseous anesthetic agent such as halothane reduces ciliary transit (Patrick and Stirling, 1977). However, nitrogen dioxide has no effect on ciliary function, even in overdoses (Proetz, 1953). Thiopentone and pentobarbital have been shown to slow tracheal transit whereas morphine and fentanyl did not have any effect on mucociliary transport (Deitmer, 1989).

## 2.5.7 NASAL STEROIDS AND DECONGESTANTS

Topical nasal steroids such as budesonide and betamethasone are first line medical therapy for chronic rhinosinusitis (Forer and Ananda, 1999). These steroids have been shown that they do not inhibit ciliary function in vivo; however, they inhibit the cilia in vitro (Deitmer, 1989).

Topical nasal decongestants such as phenylephrine and oxymetazoline, are sometimes used as part of the short term treatment for rhinosinusitis. Long term use of topical decongestants may result in rhinitis medicamentosa or rebound rhinitis (Min et al, 1998). They are essentially vasoconstrictors with effect on local blood flow. Phenylephrine is an $\alpha_1$-adrenergic agonist. An in vitro study has shown that CBF of human nasal ciliated epithelium decreased significantly after 12-hour incubation in 0.125% phenylephrine solution and after 8-hour incubation in 0.25% phenylephrine solution, both clinically used concentrations. There were significant decreases in CBF after incubation in 0.5% phenylephrine for 2 hours, in 1% for 1 hour, and in 2.5% for 30 minutes (Min et al, 1998). One must realize that nasal mucosa is not incubated with this decongestant and the ciliated cells are only exposed to this decongestant for a very short period of time when the decongestant is used in vivo in clinical setting for treatment of rhinosinusitis. Another popular decongestant, oxymetazoline, is an $\alpha_2$-adrenergic agonist. An in vivo study has shown that 5 mg oxymetazoline progressively increases nasal mucociliary transport time, although this slowing is fully reversible 30 minutes after application

(Armengot et al, 1989). However, topical nasal decongestants should be used circumspectly and they should be used for a very short period of time only.

### 2.5.8 NASAL SALINE IRRIGATION

Nasal saline douching plays an important part in the medical management of chronic rhinosinusitis. It is also beneficial in the postoperative period after sinonasal surgery (Homer et al, 2000). Nasal douching removes excess crust, debris, or mucopus, and therefore, it provides some symptomatic relief as well as better topical drug delivery to the nasal mucosa (Homer et al, 2000). There is a lot of controversy as to whether hypertonicity of nasal douching solution confers any advantage. It has been shown that hypertonic saline solution causes ciliostasis in vitro. A small effect was noted with normal (0.9%) saline, complete but reversible ciliostasis with 7% saline and complete and irreversible ciliostasis with 14.4% saline (Boek et al, 1999). These effects are thought to be due to extracellular sodium inhibiting the function of ciliary calcium channel (Ma et al, 1999). However, other studies have shown that hypertonic saline solutions improve mucociliary clearance, and the effect is probably brought about by changes in mucus rheology (Homer et al, 2000). Talbot et al showed that 3% hypertonic alkaline buffered saline improves nasal mucociliary clearance whereas isotonic alkaline buffered saline does not (Talbot et al, 1997). The other randomized double-blinded crossover trial has shown that there was no difference between 0.9% and 3% saline administration. However, 5% hypertonic saline douching solutions significantly improved mucociliary clearance in normal

subjects (Homer et al, 2000). Homer et al showed that the alkalinity of saline douching solutions has no effect on mucociliary function (Homer et al, 1999). One must realize that effects on CBF may offset any beneficial effect on mucociliary clearance caused by effects of saline on CBF. Further randomized controlled trials with larger sample size will be required to ascertain whether hypertonicity conferred any advantage.

## 2.6 ENDOSCOPIC SINUS SURGERY (ESS): CONCEPT, INDICATIONS, COMPLICATIONS, AND RESULTS

### 2.6.1 CONCEPT

The mucus produced in any sinuses is transported out of the sinuses by the mucociliary apparatus and drains into the nasal cavity via the natural sinus ostium. Messerklinger demonstrated that the frontal and the maxillary sinuses communicate with the nasal cavity via a complicated system of very narrow clefts which provide drainage and ventilation (Stammberger and Posawetz, 1990). The mucus from the frontal sinus passes through the frontal recess into the middle meatus whereas maxillary sinus drains into the ethmoidal infundibulum and into the middle meatus. These narrow clefts are parts of the anterior ethmoid system and can be seen as ethmoidal 'prechambers' of the larger sinuses (Stammberger and Posawetz, 1990). These prechambers play a key role in providing physiological conditions for the dependent larger sinuses. Any pathological conditions or anatomical variants can stenose the prechambers and thus predispose to recurrent sinus infections. Obstruction of the ostium results in retention of secretions, poor ventilation of the major sinuses with secondary infection (Stammberger and Posawetz, 1990). In order to make this diagnosis diagnostic endoscopy of the lateral nasal wall with computed tomography is required. Based on the observation that all the major sinuses always drained to their natural ostium irrespective of whether other holes were made in the sinus, an endoscopic surgical concept was developed by Messerklinger aimed at

opening the natural ostia of the diseased sinus (Stammberger and Posawetz, 1990). Thus technique of endoscopic sinus surgery (ESS) was popularized by Kennedy and Stammberger in 1980s and has been widely adopted as the best surgical technique for the treatment of chronic rhinosinusitis (Kennedy et al, 1985; Stammberger and Posawetz, 1990; Senior et al, 1998).

## 2.6.2 INDICATIONS FOR ESS

The spectrum of indications for ESS includes the followings (Stammberger and Posawetz, 1990; Kamel and Zaher, 1991; Mackay and Lund, 1997; Wormald and McDonogh, 1997; El-Guindy, 1998; Sham et al, 1998):

- Chronic rhinosinusitis resistant to medical treatment
- Fungal sinusitis
- Resistant vasomotor and allergic rhinitis
- Nasal polyposis
- Antrochoanal polyps
- Mucocoeles or retention cysts of paranasal sinuses
- Recurrent or persisting sinusitis following conventional intranasal sinus surgical procedures or more radical external surgical approaches
- Refractory posterior epistaxis
- Dacryocystorhinostomy
- Septal and turbinate surgery

- Orbital and optic nerve decompression
- Repair of blow-out fractures
- Choanal atresia
- Hypophysectomy
- Cerebrospinal fluid leak
- Nasal or paranasal sinuses tumour such as inverting papilloma

### 2.6.3 COMPLICATIONS OF ESS

In the hands of the experienced surgeons, the reported complications are very few. The major complications include cerebrospinal fluid (CSF) leak and visual loss with reported incidence of these between 0.005 to 0.5% for CSF leak and less than 0.01% for visual loss (Stammberger and Posawetz, 1990; Mackay and Lund, 1997). The minor complications of ESS include bleeding, adhesion formation and orbital haematoma with the reported incidence of these between 0.1 to 2.2% for bleeding, 8% for adhesion formation and 0.5% for orbital haematoma (Stammberger and Posawetz, 1990; Mackay and Lund, 1997). In a series of over 4500 cases, Stammberger and Posawetz reported only 3 cases of CSF leak, 1 case of pneumatocephalus, and no ophthalmic complication (Stammberger and Posawetz, 1990). The complication rate decreases with increasing experience and this learning curve was well illustrated by Stankiewicz who reported a complication rate of 29% in the first 90 cases he performed compared with only 2.2% in the next 90 cases.

Most of the complications were minor but there were 2 cases of CSF leak and one case of temporary blindness (Stankiewicz, 1989).

### 2.6.4 RESULTS OF ESS

Excellent results following ESS have been reported by many otolaryngologists (Stammberger and Posawetz, 1990; Wigand, 1990; Mackay, 1992). Overall, more than 85% of the patients regarded themselves as cured or improved, 11% were unchanged and 2% worse following ESS (Wigand, 1990; Stammberger, 1991; Lund and Mackay, 1994). Wigand reported that facial pain and headache was subjectively assessed by patients as better by 93.4%. Certain groups such as patients with cystic fibrosis, bronchiectasis, and asthmatics, particularly aspirin-sensitive asthmatics, are associated with a poor prognosis (Wigand, 1990). Those polyp patients with cystic fibrosis, especially those who had undergone heart-lung transplants, rated the operation a success in only 54% (Lund and Mackay, 1994). The poor results are probably related to the impaired mucociliary clearance in these patients. Poor prognostic factors for ESS were the combination of diffuse polypoid sinusitis with multiple allergies resistant to anti-allergic therapy, previous operation with resultant loss of anatomical landmarks, and extensive scarring (Stammberger and Posawetz, 1990).

There are several studies comparing ESS with conventional surgery (inferior meatal antrostomy and a Caldwell-Luc procedure) (Mackay and Lund, 1997). When

comparing the results of inferior meatal antrostomy with ESS for chronic rhinosinusitis, similar overall rates of subjective improvement are obtained (84% versus 87% respectively). However, when the percentage of symptoms which are the same or worse following the surgery are compared, the ESS patients do significantly better in all cases (Lund, 1988; Lund and Mackay, 1994). At present results of ESS are difficult to compare with that of the conventional approaches because of the lack of the matched groups. Long term clinical trials of carefully matched groups followed up for a long time, will be required to ascertain whether ESS is more superior to the conventional approaches (Mackay, 1992).

# CHAPTER 3

# METHODS & RESULTS

## 3.1 STANDARDIZATION OF THE SHEEP AS A SUITABLE ANIMAL MODEL FOR STUDYING ESS

### 3.1.1 INTRODUCTION

ESS involves the removal of varying amounts of nasal and sinus respiratory epithelium in order to widen the natural sinus ostia. Little is known about the healing of the mucosa and the effect of the healing process on the regeneration of normal mucociliary function (Ikeda et al., 1997). In order to study this healing process, a suitable animal model needs to be found. The following animals have been used to study sinus surgery in the past: Pasturella-free New Zealand white rabbits, dogs, pigs and sheep (Hilding, 1932; Benninger et al., 1991; Benninger et al., 1993; Mair et al., 1995; Gardiner et al., 1996). Rabbits have the disadvantage that it is very difficult to ensure that they are Pasturella-free and Pasturella infections may confound scientific findings (Friedman and Toriumi, 1989; Kennedy and Shalaan, 1989). In addition, their sinuses have to be approached through an external incision and not through the nares (Benninger et al., 1991; Benninger et al., 1993). Consequently it is not possible to perform ESS through the nares as would be the case in the human. Dogs are not readily available. In order to assess the suitability of the pig, a sagittally cut pig's head was examined. The nasal cavity was found to be unsuitable for repeated ESS due to sinus alignment and turbinate structure. In the literature the sheep's nasal cavity has been shown to be suitable for nasal endoscopy

and surgery (Gardiner et al., 1996) and was therefore studied regarding its suitability as a model for further investigation. Gardiner et al (1996) showed that the sinus anatomy (including the placement of nasal cavity, turbinates, frontal and maxillary sinuses) in sheep is analogous to humans. Histological study of the sheep's nasal mucosa showed it to be identical to that of humans (Illum, 1996). The other advantage of the sheep model is that it allows endoscopes and instruments to be used as they would in adult sinus surgery and therefore simulates the conditions found in human sinus surgery. The middle turbinate of the sheep is extensive and almost fills the nasal cavity. In order to access the middle meatus and consequently the maxillary and frontal sinuses, the obstructing middle turbinate needs to be removed. A sagittally cut sheep's head illustrating the anatomy of the nasal cavity is shown in Figure 1.

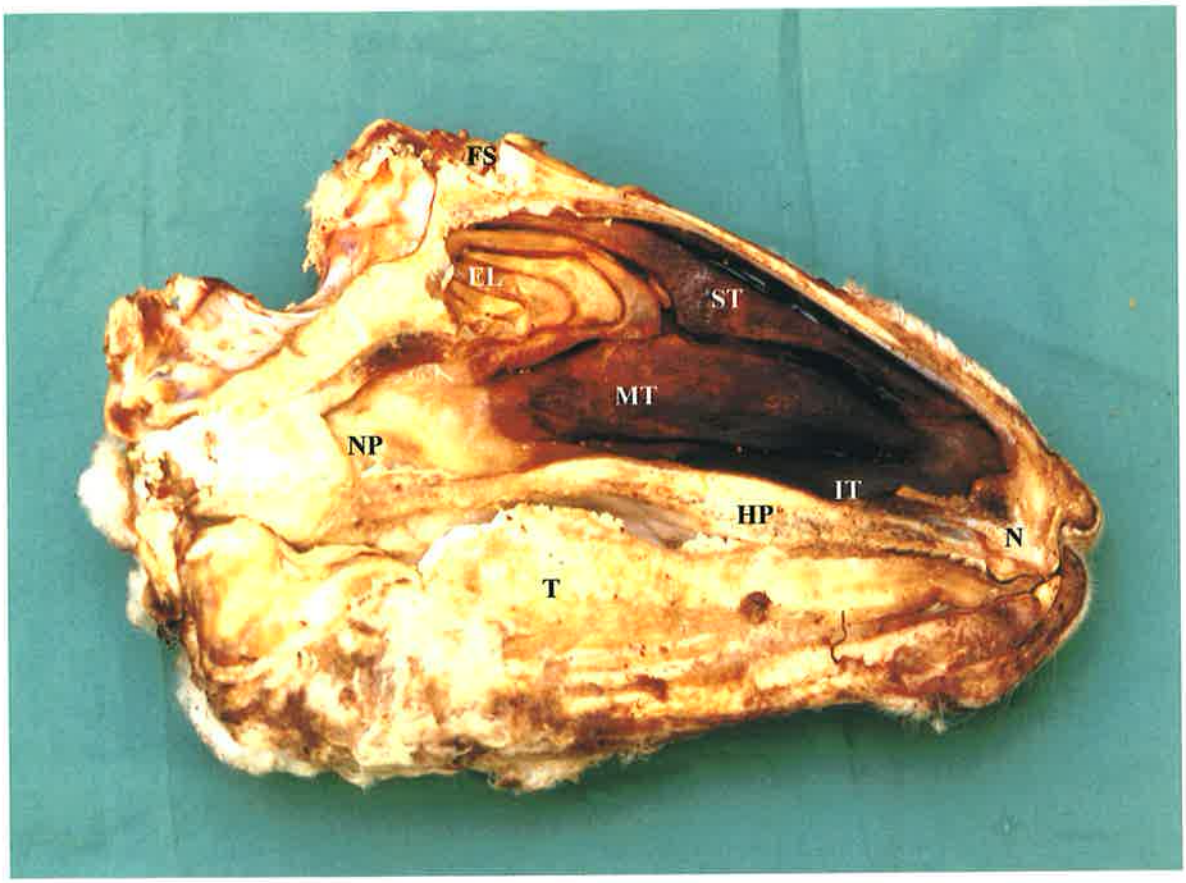


**Figure 1**

A sagittally cut sheep's head demonstrating a left sided lateral nasal wall with an extensive middle turbinate obstructing the nasal cavity. The structures of nasal cavity of sheep are very similar to that of humans. N = nostril; IT = inferior turbinate; MT = middle turbinate; ST = superior turbinate; EL = ethmoid labyrinth; FS = frontal sinus; HP = hard palate; NP = nasopharynx.

While resection of middle or inferior turbinate are common in humans as a surgical treatment for nasal obstruction, little is known whether this procedure affects the mucociliary clearance or histology of the nasal mucosa in any way (LaMear et al., 1992; Lawson, 1994; Martinez et al., 1983; Morgenstein and Krieger, 1980). Inferior turbinectomy has a success rate of around 60 per cent for improvement of nasal patency (Dawes, 1987; Martinez et al., 1983). However, this procedure has been considered by many surgeons as an undesirable operation because of the associated complications (haemorrhage, crusting, adhesions, infection and atrophic rhinitis) (Dawes, 1987). There has been one study on the effect of partial middle turbinectomy on mucociliary clearance in humans (Waguespack, 1995). This study showed that the mucociliary clearance pattern following partial middle turbinate resection was variable but did not result in any meaningful adverse effect on mucociliary transport. However, the effect of middle turbinate resection in sheep is unknown and needs to be established as part of the standardisation of the sheep as an animal model.

### 3.1.2 AIM

The aim of the study was to develop a standardized sheep model by establishing the effect of the initial surgical removal of the middle turbinate on the respiratory epithelium and cilial function of the residual nasal mucosa.

### 3.1.3 MATERIAL AND METHODS

The study was approved by the animal ethics committees of the Queen Elizabeth Hospital, Adelaide and the University of Adelaide. The power study was done by selecting an effect size of 25% with an 80% power and 5% significance level. That is a change in mucociliary clearance rates of more than 25% or a histological change in nasal mucosa is considered significant. This study showed that 10 sheep were needed. The sheep were given a general anaesthetic (GA) by intra-venous pentobarbitone, intubation and ventilation. Carbon flakes were placed 1 cm behind the anterior end of the middle turbinate. Repeated nasal endoscopic examinations were performed every 5 minutes to observe the mucociliary transport pattern and distances traveled by the carbon flakes over 15 minutes. Each sheep's middle turbinate was then decongested prior to middle turbinectomy with 2 mls of 10% Cocaine on packing. The lateral side of the middle turbinate was not in contact with any packing. The pack was left in place for 10 minutes before removal. A total of 2 mls of 2% lignocaine with 1:80,000 adrenaline was injected into the middle turbinate. The middle turbinate was removed under endoscopic control with ESS scissors. The turbinate was removed by cutting the insertion of the turbinate into the lateral nasal wall. During the procedure attempts were made to limit as far as possible the damage to the lateral or medial surfaces of the turbinate. Haemostasis was achieved by applying suction diathermy or oxycel dressing to bleeders on the stump of the middle turbinate. Once the middle turbinate had been removed, a 1x1 cm full thickness strip of mucosa was taken from the lateral side of the middle turbinate (unpacked surface) as a control. This strip was for histological

examination as a control specimen as it was not in contact with the nasal packing. The first 4 sheep that were operated on did not have these specimens taken as specimens were taken only from the medial (packed aspect) of the turbinate. We did not initially anticipate damage to the medial surface by the pack. The packed surface showed damage to the mucosa as a consequence of the ribbon-gauze packing and they were therefore not included in the study. These specimens were held by fine forceps during separation from the underlying bone. The specimens were fixed in formalin for histological analysis. The entire specimen was assessed and the percentage of intact ciliated nasal mucosa calculated. Throughout the whole procedure, every attempt was made to avoid trauma to the lateral nasal wall mucosa and subsequent biopsy.

After 3 weeks of healing, the sheep were anaesthetised. The mucociliary clearance pattern and rate over the lateral nasal wall (care was taken not to injure this surface during the middle turbinectomy) was observed by placing carbon flakes above the turbinectomy stump and comparing this to the pre-operative rate. After this one side of the sheep's nose a 1 x 5 cm full thickness biopsy of nasal mucosa was removed and placed into formalin for histology, and assessment of percentage of intact ciliated nasal mucosa.

### 3.1.4 RESULTS

Prior to the middle turbinectomy, the carbon flakes were transported by mucociliary mechanism from the anterior end of the middle turbinate towards the posterior end of the medial surface and the superior surface of the middle turbinate. The average mucociliary transport rate was 1.19 mm per second (Standard deviation (SD)=1.36; 95% confidence interval (CI)=$\pm$0.97). At three weeks, after the turbinectomy, the turbinectomy stump was covered with crusts. The crust was removed carefully without touching the lateral nasal wall mucosa. Carbon flakes were placed and were noted to be transported over the lateral nasal wall directed posteriorly towards the nasopharynx. The carbon flakes were not transported across the turbinectomy stump. The average transport rate (0.84 mm per second; SD=0.63; 95% CI=$\pm$0.45) did not differ significantly following total middle turbinectomy (paired t test p=0.3974). Results are summarised in Table 1.

**Table 1**

Summary of mucociliary transport rates

| N=10 | Before middle turbinectomy | After middle turbinectomy |
|---|---|---|
| Mean speed (mm/s) | 1.19 | 0.84 |
| Standard deviation | 1.36 | 0.63 |
| Standard error | 0.43 | 0.20 |
| Minimum | 0.00 | 0.24 |
| Maximum | 4.50 | 2.16 |
| Median | 0.74 | 0.63 |
| 95% confidence interval | $\pm$0.97 | $\pm$0.45 |

paired t test p = 0.3974

mm/s = millimetre per second

Specimens (n = 6) from the lateral sides of the middle turbinate mucosa (unpacked surface) were examined as a control. The percentage of intact ciliated mucosa of the specimen varied from 54.1% to the 100% with a mean of 85% (SD=17.6; 95% CI=$\pm$14.7). The results of this control group are shown in Table 2. Specimens (n=10) from the lateral nasal wall mucosa were examined to assess the influence of turbinectomy on the respiratory epithelium. The percentage of intact ciliated mucosa of the specimen varied from 70% to the 100% with a mean of 89% (SD=10.75; 95% CI=$\pm$7.69). The results of this post-turbinectomy group are also shown in Table 2. There was no statistically significant difference between the means of the control group and the post-turbinectomy group (p=0.5571).

**Table 2**

Percentage of intact ciliated nasal mucosa following turbinectomy vs control

| Sheep No. | Control (unpacked mucosa) N=6 | Post-turbinectomy N=10 |
|---|---|---|
| | % intact ciliated mucosa | % intact ciliated mucosa |
| 1 | NA | 100 |
| 2 | NA | 80 |
| 3 | NA | 100 |
| 4 | NA | 100 |
| 5 | 71.7 | 80 |
| 6 | 75.7 | 80 |
| 7 | 78.3 | 90 |
| 8 | 100 | 95 |
| 9 | 54.1 | 70 |
| 10 | 100 | 95 |
| Mean | 79.9 | 89 |
| SD | 17.6 | 10.75 |
| 95% CI | ±14.7 | ±7.69 |

NA = not available (control specimens were contaminated by packing in the first 4 sheep)

### 3.1.5 DISCUSSION

ESS is practiced by many otolaryngologists worldwide for a variety of nasal and sinus conditions. There is a need for scientific evaluation into the effects of ESS in the nose. In order to achieve this a suitable animal model needs to be identified. The sheep is suitable in terms of size, anatomical configuration of the sinuses, physiology (similar nasal respiratory epithelium and mucociliary drainage) and pathology (Gardiner et al.,1996; Illum,1996, King, 1998). Many inflammatory conditions of the nasal cavity and sinuses of the sheep are similar to that found in humans. For example, sinusitis is very common in sheep as a response to larvae of Oestrus ovis (Jubb et al, 1988). The sheep model also offers an inexpensive and reliable model for nasal absorption of all types of drug formulations before testing in humans (Illum, 1996). However, for access to the nasal sinuses, the middle turbinate needs to be removed. This study has shown that removal of the sheep's middle turbinate does not significantly alter the mucociliary clearance or the histology of the nasal respiratory mucosa. However, there was a loss of ciliated nasal mucosa in both the control and post-turbinectomy groups. This loss can be explained in part due to mechanical damage occurring during removal. This was in spite of great care being taken by very gently holding the specimen with very fine pair of forceps during removal. In addition there may have been small areas of unintentional injury during the removal of the middle turbinate as the lateral surface (unpacked surface) of the turbinate and the lateral nasal wall were adjacent to where the turbinate was cut at its attachment. There seems to be an unavoidable degree of trauma to both sites despite the surgeon using an endoscope and ESS instruments. While the current

study failed to show the mucociliary transport rate after turbinectomy differs significantly from the baseline mucociliary transport rate, there was a trend for a slower mucociliary transport rate after turbinectomy. Turbinectomy results in crusts formation as shown in this study and this may play a role in reducing mucociliary clearance. The other aspect that requires comment is the lack of control specimens in the first four sheep. The first four sheep had the biopsies taken from the medial surface of the middle turbinate in contrast to the latter 6 sheep where the specimens were taken from the lateral aspect of the middle turbinate. In the first 4 sheep nasal packing was inserted between the middle turbinate and septum to decongest the nose. The ribbon gauze packing caused significant histological damage to the nasal mucosa and consequently these specimens were not included in this study. We have subsequently (this is the next study discussed) shown that nasal packing can significantly affect the integrity of the nasal mucosa (Shaw et al, 2000).

The results of this study using the sheep model have shown that total middle turbinectomy does not significantly alter either the mucociliary clearance rate or histology on the lateral nasal wall at 3 weeks following the operation.

## 3.2 EFFECT OF PACKING ON NASAL MUCOSA OF SHEEP

### 3.2.1 INTRODUCTION

Nasal packing is used as part of the pre-operative preparation for sinus surgery by many rhinologists (Greinwald and Holtel, 1996). Ribbon gauze has been used to pack the nose for many years (Kamer and Parkes, 1975; Fanous, 1980) and more recently neuropatties have been used because they have being thought to cause less mucosal trauma. Packs are soaked with various types of local anaesthetics and vasoconstrictor agents in order to facilitate vasoconstriction and analgesia. This is thought to improve the operative field by improving both visualisation and decreasing bleeding during surgery (Lips et al., 1987; Pfleiderer and Brockbank, 1988). Much has been written on the use of different materials for post-operative packing for both stenting and haemostasis (Kamer and Parkes, 1975; Fanous, 1980; Fairbanks, 1986) but little on the effects of pre-operative packing.

In order to study the effects of nasal packing, the sheep has been validated as a suitable animal model (Shaw et al, 2001). In addition to the similar placement of the sheep sinuses to the human sinuses (Gardiner et al., 1996) and the histological similarity of the nasal respiratory epithelium (Illum, 1996), the sheep model allows

the use of endoscopes and surgical techniques that are currently used in human sinus surgery.

### 3.2.2 AIMS

The aim of this study was to investigate the effects of two of the more commonly used nasal packing materials, ribbon gauze and neuropatties, on the nasal mucosa.

### 3.2.3 MATERIAL AND METHODS

This study was approved by the animal ethics committees of the Queen Elizabeth Hospital and the University of Adelaide. The sheep were given a general anaesthetic (GA) by intra-venous pentobarbitone, intubation and ventilation. A statistical analysis showed that for a power of 80% and an effect size of 25% (loss of ciliated mucosa between packed and unpacked mucosa greater than 25%), 14 sheep were required. Both nasal cavities were used. In each nasal cavity, packing was placed between the middle turbinate and septum under endoscopic control using a Weil's Blakesley forceps. The pack was soaked in 2 mls of 10% cocaine solution. The lateral side of the middle turbinate was not in contact with any packing. The pack was left in place for 10 minutes before removal. Initially the sheep were packed with ribbon gauze (6 sheep with 8 specimens). However, once the histology was

reviewed it was decided to try a less traumatic packing material (neuropatties). Neuropatties were chosen as they are the current material used in our department in the pre-operative preparation of the nose before endoscopic sinus surgery (ESS). Eight sheep with 16 specimens formed this group. A total of 2 mls of 2% lignocaine with 1:80,000 adrenaline was injected into the anterior end of the middle turbinate prior to the insertion of packing. The middle turbinate was removed under endoscopic control with an ESS scissors. The turbinate was removed by cutting the insertion of the turbinate with every attempt made not to damage the lateral or medial surfaces of the turbinate. Once the middle turbinate had been removed, a 1x1 cm full thickness strip of mucosa was taken from the middle turbinate from an area that had been visually confirmed to have been in contact with the nasal packs. A 1x1 cm full thickness strip of mucosa was also taken from the lateral aspect of the middle turbinate which was not in contact with any packing. This tissue was used as a control. These specimens were held by fine forceps during separation from the underlying bone. The specimens were fixed in formalin for histological analysis. Two blinded observers assessed the percentage of intact ciliated nasal mucosa in all specimens (packed and control). The entire specimen was assessed and the percentage of intact ciliated nasal mucosa determined.

### 3.2.4 RESULTS

The two blinded observers histological analysis were found to have a high degree of correlation (correlation coefficient r = 0.93). In group 1, there were 8 specimens from nasal cavities packed with ribbon gauze. The percentage of intact ciliated nasal mucosa varied widely from complete absence of ciliated mucosa (cuboidal lining layer and the basement membrane only) in 3 to minimal epithelium loss (10% loss of ciliated epithelium). The mean loss of ciliated nasal mucosa after ribbon gauze nasal packing was 32.3% (standard deviation (SD)=35.4; standard error of mean (SEM)=12.5; 95% confidence interval (CI)=$\pm$29.6). The results of this group are shown in Table 3 and Figure 2. A typical loss of respiratory epithelium after ribbon gauze packing is illustrated in Figure 3.

**Table 3**

Percentage of intact ciliated nasal mucosa following ribbon gauze packing

| Sheep no. | | Percentage intact ciliated mucosa |
|---|---|---|
| 1 | Right | 10 |
| 2 | Left | 65 |
| 3 | Right | 0 |
| | Left | 90 |
| 4 | Right | 0 |
| 5 | Right | 0 |
| | Left | 60 |
| 6 | Right | 33 |
| Mean | | 32.3 |
| SD | | 35.4 |
| SEM | | 12.5 |
| 95% CI | | ±29.6 |

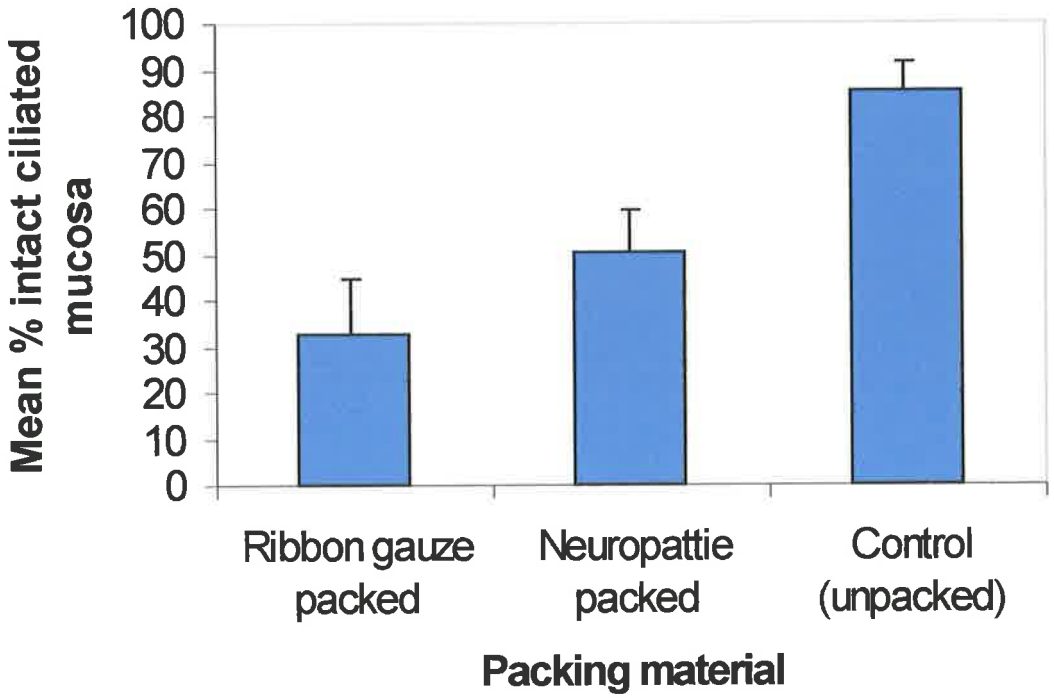


**Figure 2**

Effect of nasal packing with ribbon gauze, neuropatties vs control (unpacked), on the percentage of intact ciliated nasal mucosa. Both ribbon gauze packing and neuropattie packing resulted in significant loss of the ciliated nasal mucosa when compared with the control group (unpacked) ($p<0.005$). Values are presented as mean $\pm$SEM.

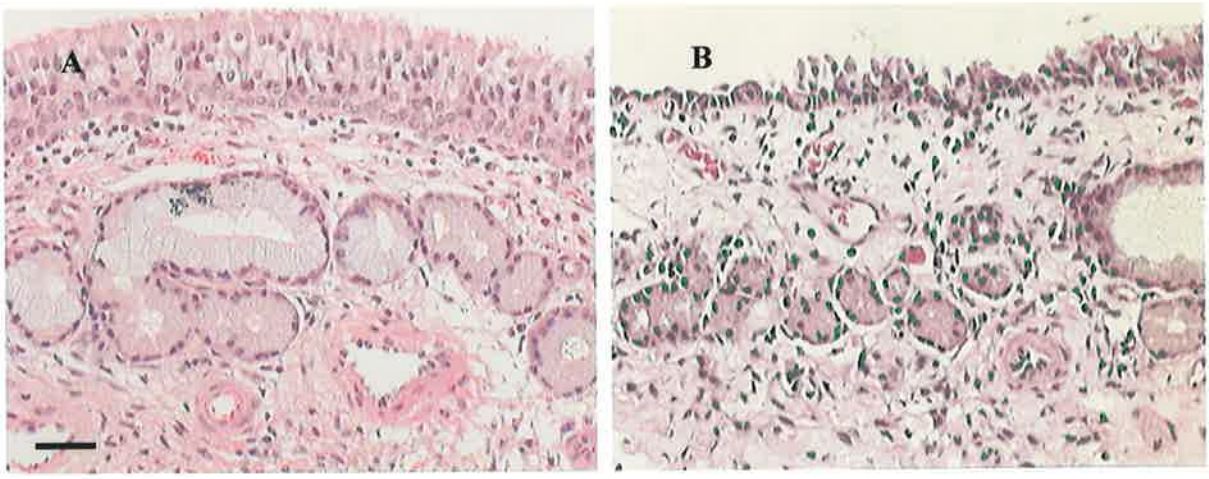


**Figure 3**

(a) Haematoxylin & Eosin (H & E) stained specimen from control (unpacked) mucosa showing 100% intact ciliated nasal mucosa. (b) H & E stained specimen from ribbon gauze packed mucosa showing approximately 15% intact ciliated mucosa. Magnification bar = 80μm.

In group 2, there were 16 specimens from sheep that had neuropatties inserted as packing. The percentage of intact ciliated mucosa varied from complete absence of ciliated respiratory epithelium (only cuboidal lining layer and the basement membrane visible) in 3 specimens to complete preservation (100%) of the ciliated mucosa. The mean of the percentage of intact ciliated mucosa after neuropattie nasal packing was 50.4% (SD=35.9; SEM=8.9; 95% CI=$\pm$19.1). The results of this group are shown in Table 4 and Figure 2. There was no statistically significant difference between the means of the ribbon gauze packing group and the neuropatties packing group (p=0.25). A typical loss of respiratory epithelium after neuropattie packing is illustrated in Figure 4.

**Table 4**

Percentage of intact ciliated nasal mucosa following neuropattie packing vs control

| Sheep no. | | Mucosa packed with neuropatties: Percentage intact ciliated mucosa | Control (unpacked mucosa): Percentage intact ciliated mucosa |
|---|---|---|---|
| 7 | right | 0 | 100 |
| | left | 0 | |
| 8 | right | 95 | 100 |
| | left | 67 | |
| 9 | right | 30 | 71.7 |
| | left | 100 | |
| 10 | right | 0 | 75.7 |
| | left | 70 | |
| 11 | right | 10 | 78.3 |
| | left | 100 | |
| 12 | right | 75 | 100 |
| | left | 50 | |
| 13 | right | 60 | 54.1 |
| | left | 70 | |
| 14 | right | 20 | 100 |
| | left | 60 | |
| Mean | | 50.4 | 85 |
| SD | | 35.9 | 17.6 |
| SEM | | 8.9 | 6.2 |
| 95% CI | | ± 19.1 | ± 14.7 |

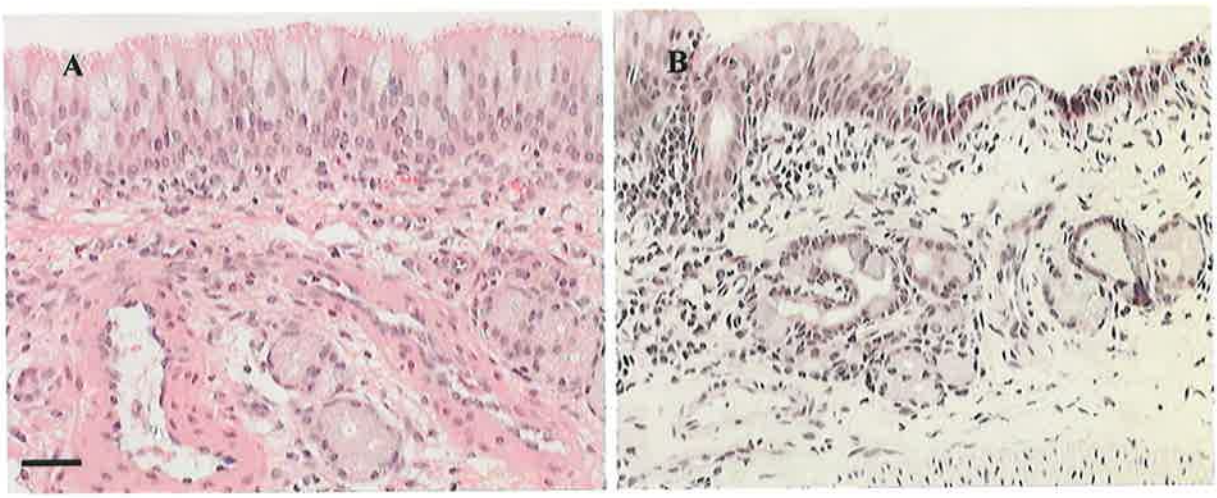


**Figure 4**

(a) H & E stained specimen from control (unpacked) mucosa showing 100% intact ciliated nasal mucosa. (b) H & E stained specimen from neuropattie packed mucosa showing approximately 57% intact ciliated mucosa.

Magnification bar = 80µm.

Specimens (n = 8) from the lateral sides of the middle turbinate mucosa (unpacked surface) were examined as a control. The percentage of intact ciliated mucosa of the specimen varied from 54.1% to the 100% with a mean of 85% (SD=17.6; SEM=6.2; 95% CI=±14.7). The results of this control group are shown in Table 4 and Figure 2. When analysed with unpaired students t-test the control group had significantly higher percentage of intact ciliated respiratory epithelium than the ribbon gauze packed group ($p<0.005$) or the neuropatties packed group ($p<0.005$).

### 3.2.5 DISCUSSION

Both ribbon gauze and the neuropatties are very common nasal packing materials for a wide variety of nasal surgery and ESS. The results from this study showed that both types of packing material resulted in significant loss of nasal mucosa with mean percentage loss of more than 50% of mucosa. The control (unpacked) group had significantly less loss (15%) of ciliated mucosa. There was no statistically significant difference between the nasal packing materials in the amount of nasal mucosa lost on histology. The study was performed by investigators who are experienced with nasal packing techniques and every attempt was made to place the packing material in the nose without damaging the mucosa. It was thought that there might well have been a difference between the packing materials as the ribbon gauze has a much more abrasive feel than the neuropatties. However, while blinded pathology assessments failed to confirm this, there was a trend showing less

mucosal loss with the neuropatties than with the ribbon gauze (Figure 2). It remains to be seen if other packing materials such as cotton wool would cause a similar traumatic mucosal injury. From the results of this study cotton wool would be likely to cause some mucosal trauma. It would also be of interest to evaluate the effect of cocaine alone on the nasal respiratory epithelium. It was interesting to note that the control group also had 15% mucosal loss. This can probably be explained in part due to the need to very gently hold the specimen with a very fine pair of forceps during removal. In addition there may have been small areas of unintentional injury during the removal of the middle turbinate as the lateral surface (unpacked surface) was adjacent to where the turbinate was cut at its attachment.

During the placement of the nasal packing the surgeons (C-KLS, PJW) used ESS instruments under endoscopic control. Care was taken to avoid unnecessary mucosal contact between the packing and the mucosa as the packing was placed. However, there seems to be an unavoidable trauma (with these packs) that results in a varying degree of mucosal injury. This loss of cilia may result in impaired mucociliary clearance and subsequent crusting which may increase the overall mucosal injury sustained during the nasal surgery and prolongs the healing period.

Surgeons who wish to continue to pack the nose should do so with gentleness under endoscopic control in an attempt to limit the nasal mucosal injury as much as possible. An alternative to packing is to decongest the nose with oxymetazoline or xylometazoline nasal spray in the anaesthetic holding area (Riegle et al., 1992; Latorre and Klimek 1999). This should shrink the nasal mucosa and allow an

improved endoscopic view. Once the patient is under general anaesthetic, local anaesthetic with adrenaline can be used to infiltrate the operative areas and in doing so minimise the possibility of bleeding intra-operatively (Pfleiderer and Brockbank, 1988). This would also allow the use of topically applied cocaine to be minimised and decrease the potential for toxicity (Pfleiderer and Brockbank, 1988; Bromley and Hayward, 1988). If bleeding becomes problematic during ESS, patties soaked in cocaine could be placed in the operative field for haemorrhage control (Pfleiderer and Brockbank, 1988). By using packing only in the surgical area, the trauma to the non-operated areas would be minimised.

In conclusion, ribbon gauze and neuropatties cause a significant mucosal injury in sheep when these packs are placed under endoscopic control with endoscopic instruments. Alternatives to packing the nose pre-operatively should be considered.

## 3.3 TEMPORAL HEALING PATTERN AND RETURN OF CILIAL FUNCTION IN THE NASAL CAVITY AFTER ENDOSCOPIC RESECTION OF FULL THICKNESS AND PARTIAL THICKNESS MUCOSAL SECTIONS

### 3.3.1 INTRODUCTION

Rhinosinusitis is a common condition that affects up to 18% of the general population (Jones, 1998). Rhinosinusitis is usually accompanied by severe mucosal changes which impair mucociliary clearance by affecting either ciliary function or the rheological properties of mucus. Endoscopic sinus surgery (ESS) is considered by many otolaryngologists to be the most exciting development for the treatment of chronic rhinosinusitis and many other various nasal and sinus pathology. This minimally invasive technique is aimed at restoring the natural mucociliary clearance mechanism, drainage and aeration of sinuses while maintaining as much of the normal anatomy as possible (Mackay, 1992). During ESS, it is extremely important to preserve as much ciliated respiratory epithelium as possible since an intact ciliated respiratory epithelium is the key to maintain the health of nose and sinuses. However, ESS involves removal of varying amount of ciliated respiratory epithelium. Surgical removal of this nasal and sinus mucosa raises the questions concerning mucosal regeneration and subsequent mucociliary function. Currently little is known about the healing of the mucosa in the nose and the effect of the healing process on the regeneration of normal mucociliary function (Ikeda et al,

1997). In order to study this healing process and the influence of surgery on the mucociliary function, a suitable animal model has been established (Gardiner et al, 1996; Illum, 1996; Shaw et al, 2001).

### 3.3.2 AIMS

The standardized sheep model was used to evaluate the temporal healing pattern of nasal mucosa and the return of cilial function after endoscopic resection of full-thickness and partial thickness mucosa.

### 3.3.3 MATERIAL AND METHODS

The study was approved by the animal ethics committees of the Queen Elizabeth Hospital and the University of Adelaide. The average healing time for the nose in rabbits is 21 days with a range from 13 to 29 days (Forsgren et al, 1993). A power study was done using a power 90% and with an effect size of 25% (> 25% change in mucociliary clearance rates or histological re-epithelialisation) and a significance level of 5%. A sample size of 14 sheep was required in a one-tailed test. Using the standardised sheep model under general anaesthetic (Shaw et al, 2001), a baseline mucociliary transport rate was measured using time elapse for carbon flakes to move from A to B. Thereafter two strips of mucosa of 5 cm by 1 cm (full-thickness

including the periosteum) were removed from the lateral nasal wall. In the opposite nasal cavity (computer randomised) a similar area of partial thickness mucosa was removed with the microdebrider (Xomed Straightshot Microdebrider). On day 7, 14, 21, 28, 42 (6 weeks), 56 (8 weeks) and 84 (12 weeks), a repeat endoscopy was performed under sedation and local anaesthetic and a 5 mm by 5 mm biopsy was taken from the previously wounded area. Seven different areas of the original wound were biopsied and these were noted and excluded from the final examination of the original wounds. The biopsies were examined histologically for the regeneration of epithelium and for the presence of cilia. On day 84, the mucociliary transport rate was measured by carbon flakes and the sheep were then sacrificed by overdose of Phenobarbitone.

### 3.3.4 RESULTS

During the initial study period 3 sheep died as a result of incorrect amount of sedation given and their incomplete results were excluded from the analysis. The results of the mucociliary transport rates are summarized in Table 5. The baseline mucociliary transport rate was 0.84 mm per second (standard error of mean (SEM)=0.20; 95% confidence interval (95% CI)=$\pm$0.45). On day 84 the mean mucociliary transport rate of the full-thickness wounded side was 0.90 mm per second (SEM=0.37; 95% CI=$\pm$0.86) while the partial thickness wounded side was 2.49 mm per second (SEM=1.02; 95% CI=$\pm$2.34). The mucociliary transport rate of the full-thickness wounded side did not differ significantly from that of the partial

thickness wounded side (paired t test p=0.22). In addition, the mucociliary transport rate of either full-thickness or partial thickness wounded side did not differ significantly from the baseline mucociliary transport rate (paired t test p=0.88; p=0.14 respectively).

**Table 5**

Summary of mucociliary transport rates

| | Baseline | Full thickness injury (n=9) | Partial thickness injury (n=9) | P value |
|---|---|---|---|---|
| Mean speed (mm/s) | 0.84 | 0.90 | 2.49 | P>0.05 |
| SEM | 0.20 | 0.37 | 1.02 | |
| 95% CI | ±0.45 | ±0.86 | ±2.34 | |

Baseline compared to day 84 (12 weeks) post mucosal injury

The full thickness wounded mucosa healed with significant crusting covering the raw area. Crusting was carefully removed before the biopsy was taken from the healing area. The partial thickness wounded mucosa healed with minimal crusting. Histologically the mean percentage of epithelium regeneration was much higher in the partial thickness wounded side (80.7%; SEM =10.3) when compared with the full thickness wounded side (64.9%; SEM = 19.2) after 12 weeks of healing. However, this was not statistically significant (paired t test $p > 0.05$). The results of the epithelium regeneration post mucosal injury are shown in Figure 5. A typical day 84 post full thickness wounded epithelialised mucosa and a typical day 84 post partial thickness wounded epithelialised mucosa are illustrated in Figure 6.

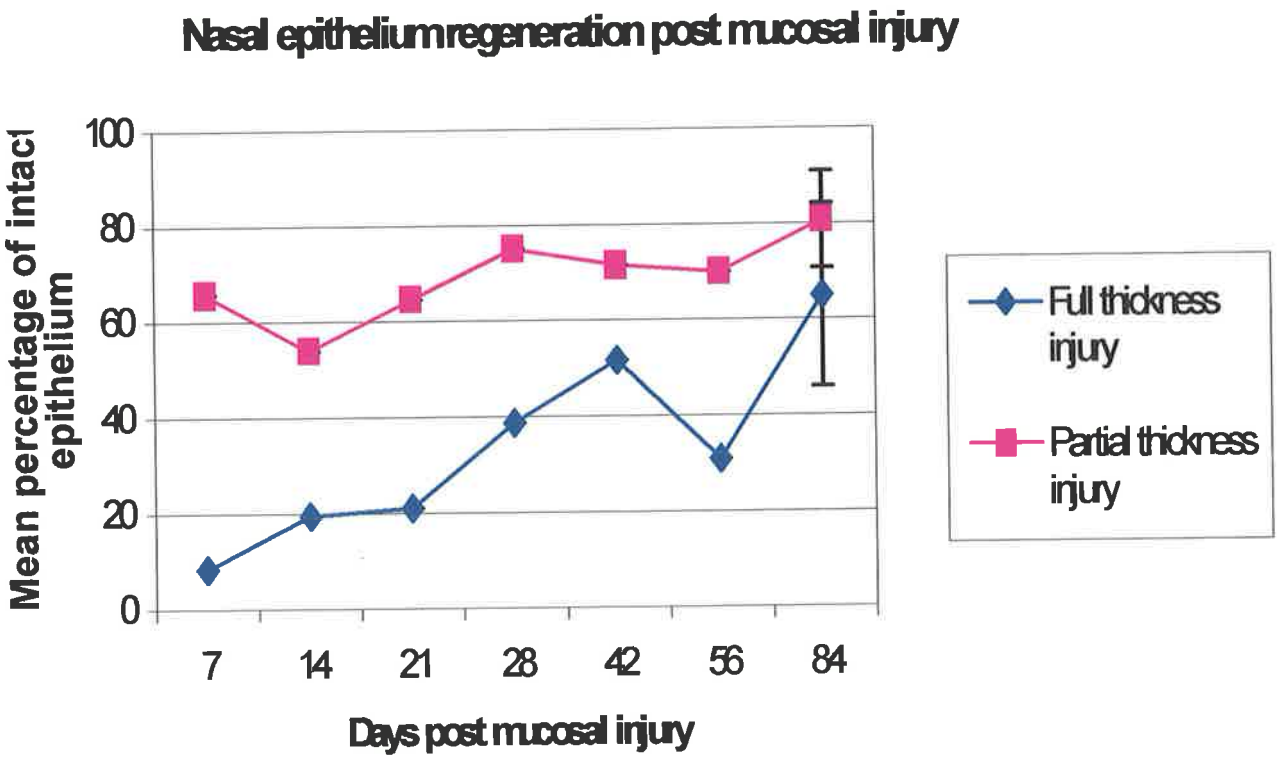


**Figure 5** Graph showing the healing of nasal respiratory epithelium after full thickness and partial thickness mucosal injury.

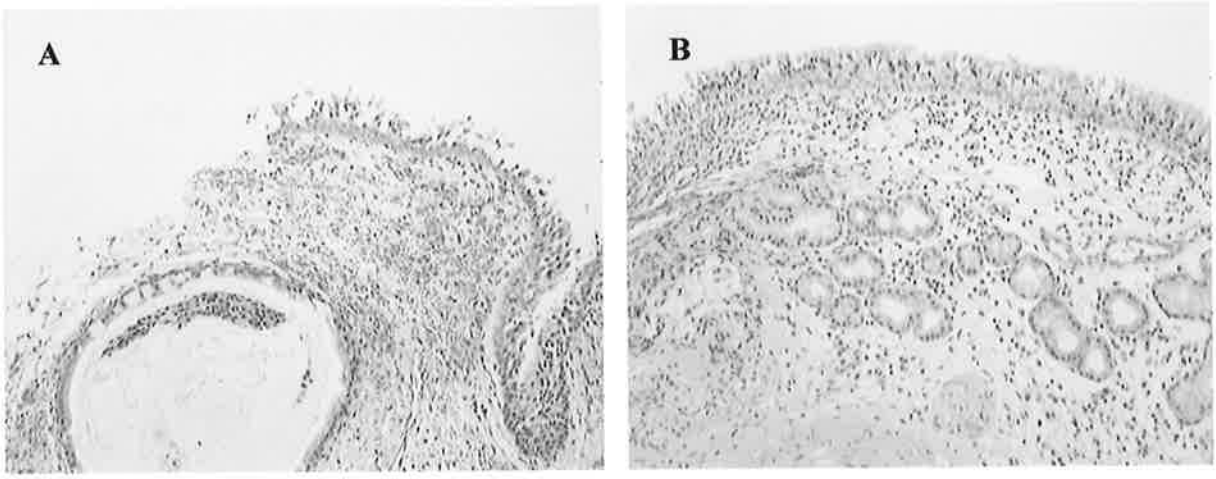


**Figure 6**

(a) Haematoxylin & Eosin (H & E) stained specimen from day 84 post full thickness wounding showing typical incomplete regeneration of epithelialised mucosa. (b) H & E stained specimen from day 84 post partial thickness wounding mucosa showing typical more complete regeneration of epithelialised mucosa. Magnification bar in (b) refers to both (a) and (b) and is equal to 100μm.

On histology the partial thickness wounded side showed gradual cilia regeneration in the first 4 weeks post injury. This was followed by a rapid regeneration of cilia with the mean percentage of cilia present being 68.35% (SEM =10.2; 95% CI = ±26.2) on day 84 post injury. In contrast the full thickness wounded side showed poor cilial regeneration with a mean 32.96% (SEM =19.17; 95% CI = ±14) of cilia present on day 84 post injury. This was statistically significant (paired t test $p < 0.05$). The results of the cilia regeneration post mucosal injury are summarized in Figure 7. A typical day 84 post full thickness wounded ciliated mucosa and a typical day 84 post partial thickness wounded ciliated mucosa is illustrated in Figure 8.

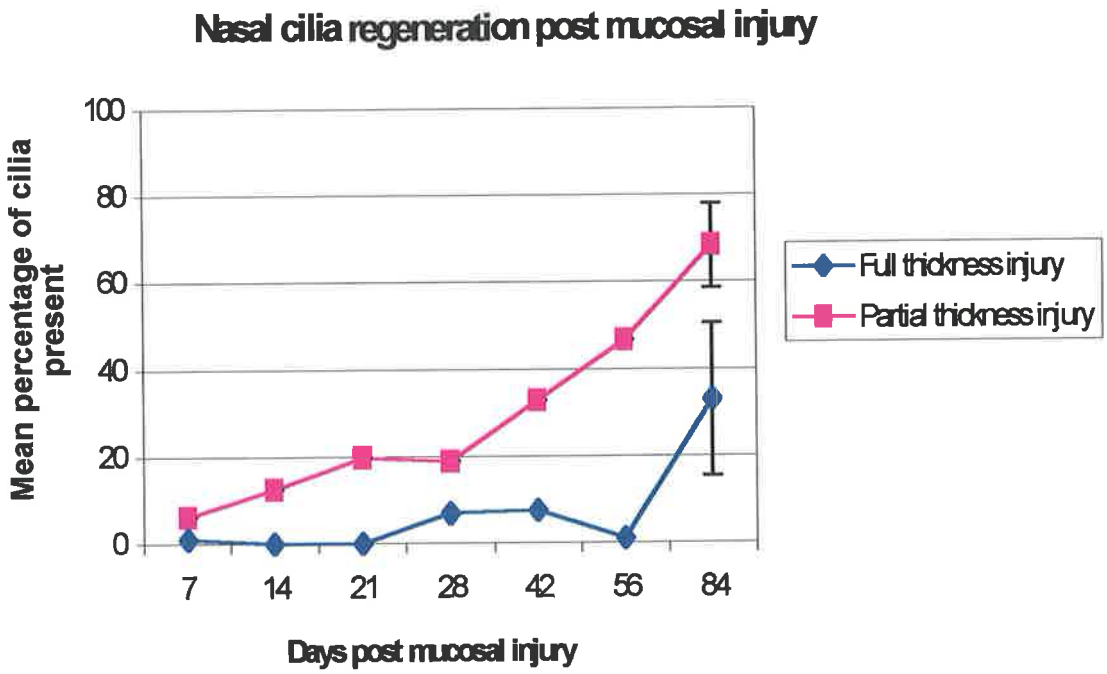


**Figure 7** Graph showing the regeneration of cilia after full thickness and partial thickness wounding.

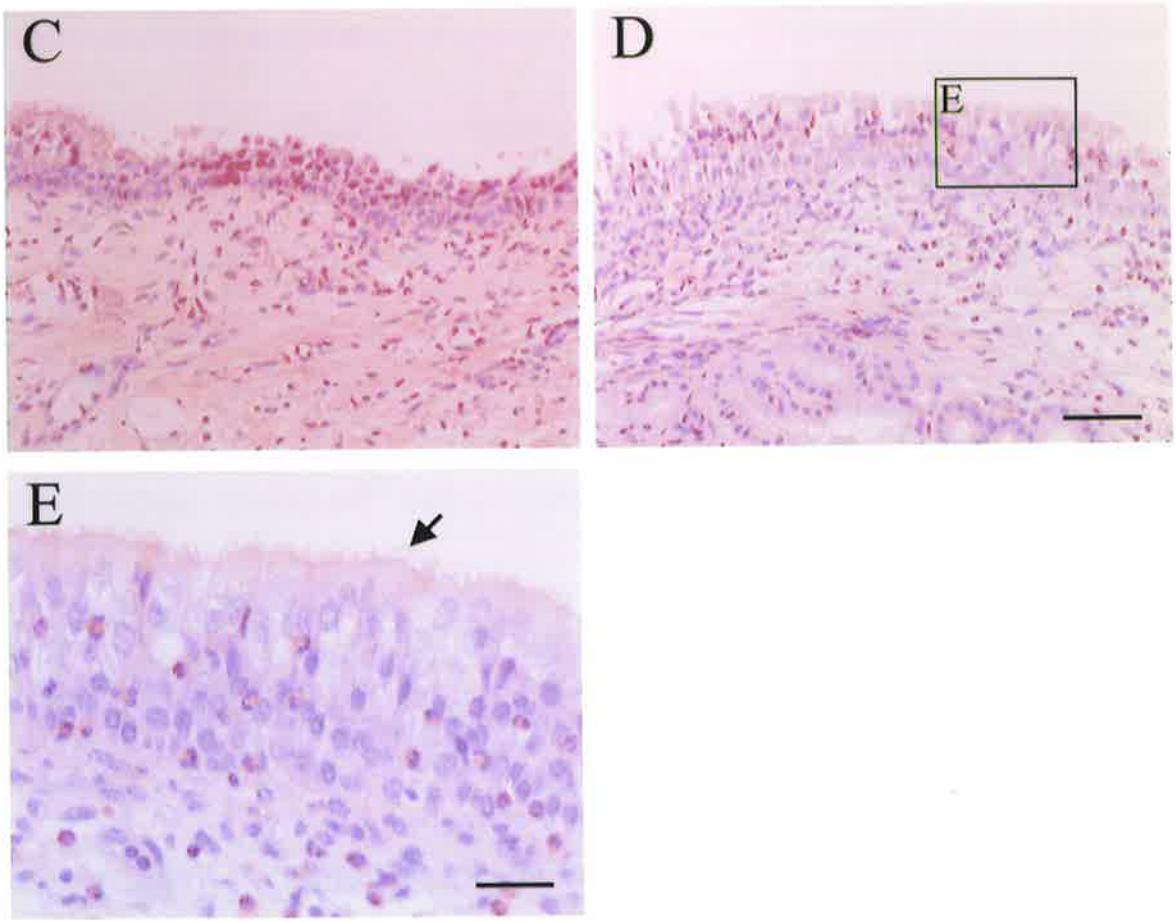


**Figure 8**

(C) H & E stained specimen from day 84 post full thickness wounding showing typical poorly ciliated nasal respiratory mucosa. (D) H & E stained specimen from day 84 post partial thickness wounded mucosa showing typical well ciliated nasal respiratory mucosa. Magnification bar in (D) refers to (C) and (D) and is equal to 50μm. (E) A magnified image of part of the ciliated nasal mucosa of (D) showing clearly the presence of cilia. The arrow points to cilia. Magnification bar = 10μm.

### 3.3.5 DISCUSSION

Normal nasal mucociliary clearance is the key to maintaining the health and defense of the nose and the sinuses. Effective mucociliary clearance is only possible when both the appropriate ciliary movement and an adequate mucous blanket are present. Therefore, the quantity and rheology (viscous and elastic properties) of mucous are intimately related with the mucociliary transport. These properties of nasal mucus facilitate effective movement of the cilia. Insoluble tracers such as carbon flakes are transported in the gel layer and provide a convenient visual measurement of the mucociliary transport rate and pattern. As can be seen from Figure and Figure , cilia appear to need a reasonable base of epithelium before regeneration occurs. In partial thickness injury, re-ciliation started relatively early and progressed at a steady rate. After full-thickness injury, there was a considerable lag time while the epithelium regenerated. This is partly due to the lack of basement membrane and the need for the epithelium to migrate from the edges of the wound to fill the defect. It is also apparent from Figure that re-ciliation was not complete at the time of sacrifice as the graph does not appear to have reached a plateau at this point. The time point for sacrifice had been selected on the basis of previous work (average healing time 21 days) (Forsgren et al, 1993; Weber et al, 1995), and it was expected that the healing process would have been complete at this time. Although there was a significant difference between the re-ciliation in full and partial thickness wounds at day 84, it would have been interesting to evaluate this 1 or 2 months later to see if this difference remained significant. As far as the epithelium is concerned, the difference in full and partial thickness epithelial regeneration closed with time and

was not significant at day 84. This healing process was considerably slower than expected and this would account for the symptoms seen in patients in the post-operative period (Weber et al, 1995). Currently additional studies are under way to assess whether re-ciliation will continue after day 84 and whether there is any significant difference between full and partial thickness injuries in the long term. This is an important question for nasal surgeons as full-thickness removal of mucosa around sinus ostia may predispose towards poor muco-ciliary movement over full-thickness scars. Surgeons would need to take more care to ensure that mucosa is spared where possible and limit the removal of full-thickness mucosa to an absolute minimum.

It is interesting to note that although this study showed a difference in the speed of epithelial regeneration and re-ciliation after full and partial thickness wounding, there was no significant difference in the muco-ciliary transport rates on day 84 ($p > 0.05$). The possible reasons for this are as follows: the carbon flake measurements are done by dropping a series of carbon flakes on the nasal mucosa and measuring the transit of the most distal flake. Even though care was taken with flake placement, some flakes may have landed on relatively normal adjacent nasal mucosa. In addition normal adjacent moving cilia can exert traction on the viscous mucous layer over the wounded epithelium and help the mucociliary transport rate (Deitmer, 1989). Giordano et al. also observed this bridging effect on incompletely re-epithelialised tracheal anastomosis (Giordano and Holsclaw, 1976). The mucociliary transport system can also bypass the healing non-ciliated epithelium by changing the direction of the transit by changing the viscosity of the mucus

(Deitmer, 1989). Other animal studies have shown changes in cilia following sinus surgery. Benninger et al. studied the effect of inferior meatal antrostomy (with mucosa stripping on one side) on the respiratory mucosa in rabbits. Histology showed that 10% of the cilia in the stripped side were abnormal and that the proportion of ciliated cells was 54% of that on the unoperated (control) side (Benninger et al, 1991). Another animal study showed that 7 of 10 sinuses subjected to Caldwell-Luc procedure reestablished ciliated respiratory epithelium but with abnormal histology (Benninger et al, 1989). However, mucociliary clearance was not examined in these studies so it is unclear what effect these ciliary abnormalities had on the mucociliary drainage.

This study has shown that regeneration of the epithelium and cilia occurred at a more rapid rate in partial-thickness wounding than after full-thickness wounding. Nasal surgeons should attempt to limit mucosal removal as the regeneration of epithelium and cilia take a considerable length of time. This may lead to post-operative symptoms as mucous stasis within the sinuses may predispose the sinuses to secondary bacterial colonization and infections. Such infections may also result in long-term consequences for the health of the sinuses and nose.

## 3.4 EVALUATION OF CILIAL REGENERATION BY SCANNING ELECTRON MICROSCOPY

### 3.4.1 INTRODUCTION

Mucociliary transport is the final result of the mucus rheology, and the functional and ultrastructural organization of the cilia at different levels. It is necessary that the cilia coordinate and have a metachronal waveform before mucociliary pathways are formed (Sleigh et al, 1988). Transmission electron microscopy (TEM) plays an important role in identification of ciliary dysmorphology whereas ciliary orientation and coordination can be studied using scanning electron microscopy (SEM) and TEM. The metachronal wave that is formed by the cilia is organized into different streams that can be measured by mucociliary transport. This metachronal wave can be visualized to some degree using SEM (Jorissen, 1998). Although SEM gives less information at the single cilium level, it can provide an overview of ciliary coordination (Jorissen, 1998).

Traditionally, the extent of ciliated nasal mucosa is assessed histologically. The true extent of cilial regeneration cannot be accurately reflected histologically as only a cross section of the specimen is assessed. With the advent of electron microscopy technology, the scanning electron microscopy (utilizing Soft Imaging System Analysis ®) provides excellent magnified images of cilia. Theoretically, the amount

of cilia present across the entire surface of specimen should be more accurately estimated when compared with the conventional histological examination. Currently, little is known about the role of SEM in healing of nasal epithelium and in particularly with respect to cilial regeneration.

### 3.4.2 AIM

The aim of this study was to evaluate the role of scanning electron microscopy in assessment of cilial regeneration post nasal mucosal injury.

### 3.4.3 MATERIAL AND METHODS

In study 3.3 biopsies for histological assessment were taken on day 56 and day 84 post injury. At the same time additional specimens were taken for assessment by scanning electron microscopy using a special computer software program (Soft Imaging System Analysis ®). These specimens were prospectively assessed by two blinded observers (LS, DM). Before the observations were started, both observers went through a number of specimens together to confirm the technique to be used in the experiment. This would have the effect of lessening any learning trend. After the biopsies of the nasal mucosa were taken from the lateral nasal wall, the specimens were immediately rinsed gently in normal (0.9%) saline in an attempt to remove any

mucus or blood clot from the mucosal surface. The specimens were then fixed in buffered glutaraldehyde. All the specimens were examined at 500x magnification by 2 blinded observers (LS and DM) separately using Soft Imaging System Analysis ®. The entire surface of each specimen was assessed. Each blinded observer assessed the specimens on 2 different occasions one month apart. For each observer's assessment, the intra-observer difference in assessing the same specimens on 2 occasions was calculated. The inter-observer difference between the two observers for each specimen was calculated. The results of SEM assessment was also compared with the results of histological assessment.

### 3.4.4 RESULTS

Eight specimens were covered with mucus or blood clot and therefore, not suitable for assessment. Eighteen specimens were suitable for scanning electron microscopy. The results of each individual observer's ($1^{st}$ or $2^{nd}$ or average) assessments are shown in Table 6 and Table 7. Typical SEM appearances on day 56 post full thickness wounded ciliated mucosa and day 56 post partial thickness wounded ciliated mucosa are illustrated in Figure 9 and Figure 10 respectively. Typical SEM appearances of day 84 post full thickness wounded ciliated mucosa and day 84 post partial thickness wounded ciliated mucosa are illustrated in Figure 11 and Figure 12 respectively.

**Table 6**

Summary of SEM assessment of cilial regeneration evaluated by observer, LS

| Specimen Number | 1st assessment (% cilia present) | 2nd assessment (% cilia present) | Difference between trials (% cilia present) | Average of 2 trials |
|---|---|---|---|---|
| 1 | 69.31 | 68.82 | 0.49 | 69.07 |
| 2 | 66.99 | 30.85 | 36.14 | 48.92 |
| 3 | 76.61 | 76.44 | 0.17 | 76.53 |
| 4 | 78.22 | 78.95 | 0.73 | 78.59 |
| 5 | 81.83 | 75.24 | 6.59 | 78.54 |
| 6 | 48.38 | 71.15 | 22.77 | 59.76 |
| 7 | 64.28 | 63.13 | 1.15 | 63.71 |
| 8 | 80.65 | 79.13 | 2.67 | 79.89 |
| 9 | 70.56 | 66.67 | 3.89 | 68.62 |
| 10 | 88.75 | 90.14 | 1.39 | 89.45 |
| 11 | 82.9 | 86.61 | 3.71 | 84.75 |
| 12 | 78.63 | 78.41 | 0.22 | 78.52 |
| 13 | 80.54 | 79.48 | 1.14 | 80.01 |
| 14 | 72.11 | 77.76 | 5.65 | 74.94 |
| 15 | 71.12 | 75.99 | 4.87 | 73.55 |
| 16 | 70.77 | 78.23 | 7.46 | 74.50 |
| 17 | 81.10 | 83.88 | 2.78 | 82.49 |
| 18 | 65.75 | 72.29 | 6.54 | 69.02 |
| Mean of difference between 2 assessments | | | 6.335 % | |
| SD | | | 9.191 | |
| Median of difference between 2 assessments | | | 3.245 % | |

**Table 7**

Summary of SEM assessment of cilial regeneration evaluated by observer, DM

| Specimen Number | 1st assessment (% cilia present) | 2nd assessment (% cilia present) | Difference between trials (% cilia present) | Average of 2 trials |
|---|---|---|---|---|
| 1 | 64.67 | 69.50 | 4.83 | 67.08 |
| 2 | 48.6 | 60.06 | 11.39 | 54.37 |
| 3 | 67.30 | 68.30 | 1 | 67.80 |
| 4 | 69.67 | 61.30 | 8.37 | 65.50 |
| 5 | 66.25 | 67.50 | 1.25 | 66.88 |
| 6 | 72.50 | 56 | 16.50 | 64.25 |
| 7 | 67.60 | 65.60 | 1 | 66.60 |
| 8 | 64.33 | 59 | 5.33 | 61.67 |
| 9 | 59 | 69.5 | 10.5 | 64.25 |
| 10 | 64 | 74.5 | 10.5 | 69.25 |
| 11 | 72.73 | 69.30 | 3.43 | 71.02 |
| 12 | 85.64 | 85.52 | 0.12 | 85.58 |
| 13 | 78.73 | 76.10 | 2.63 | 77.42 |
| 14 | 70.69 | 84.05 | 13.36 | 77.37 |
| 15 | 79.65 | 82.07 | 2.42 | 80.86 |
| 16 | 74.62 | 71.39 | 3.23 | 73.01 |
| 17 | 81.81 | 73.57 | 8.24 | 77.69 |
| 18 | 67.33 | 78.58 | 11.25 | 72.95 |

| | |
|---|---|
| Mean of difference between 2 assessments | 6.08 % |
| SD | 5.02 |
| Median of difference between 2 assessments | 4.13 % |

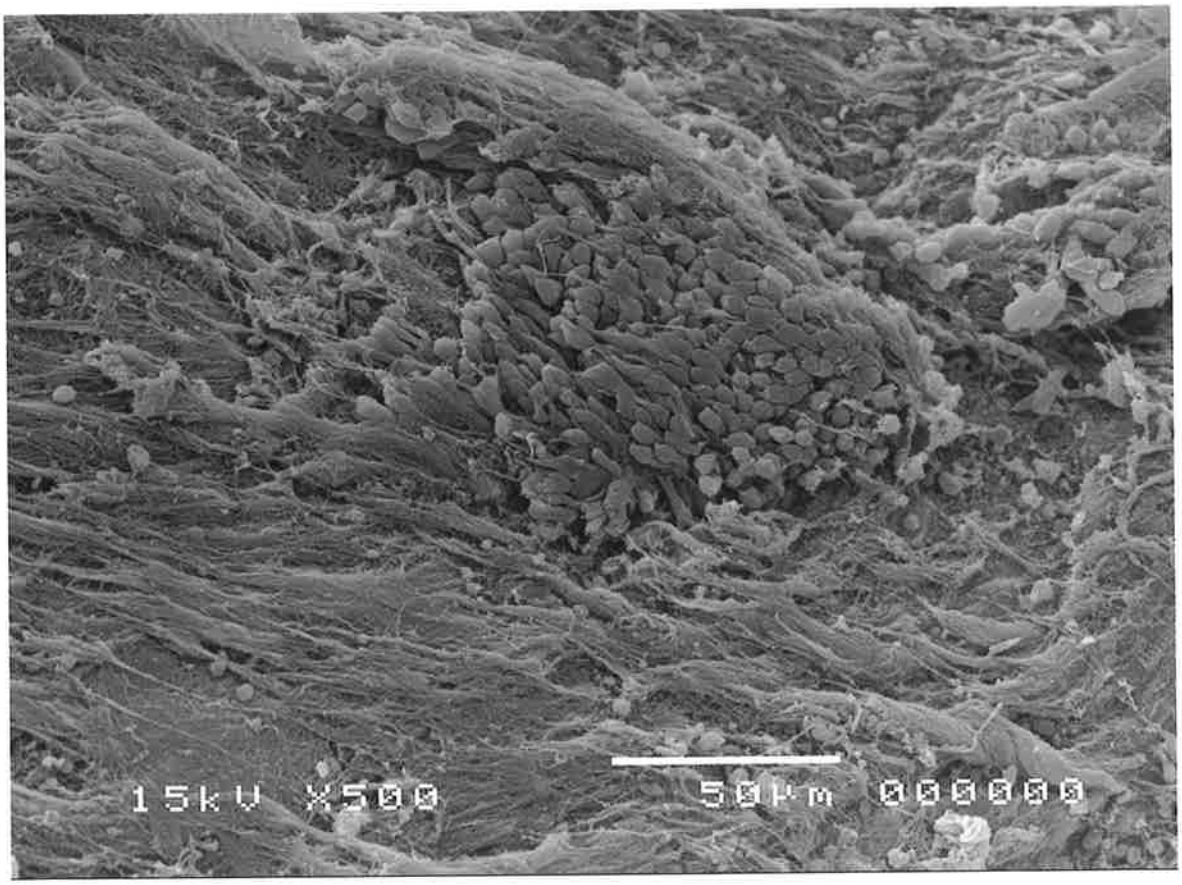


**Figure 9**

SEM specimen from day 56 post full thickness wounding showing typical poorly ciliated nasal respiratory mucosa.

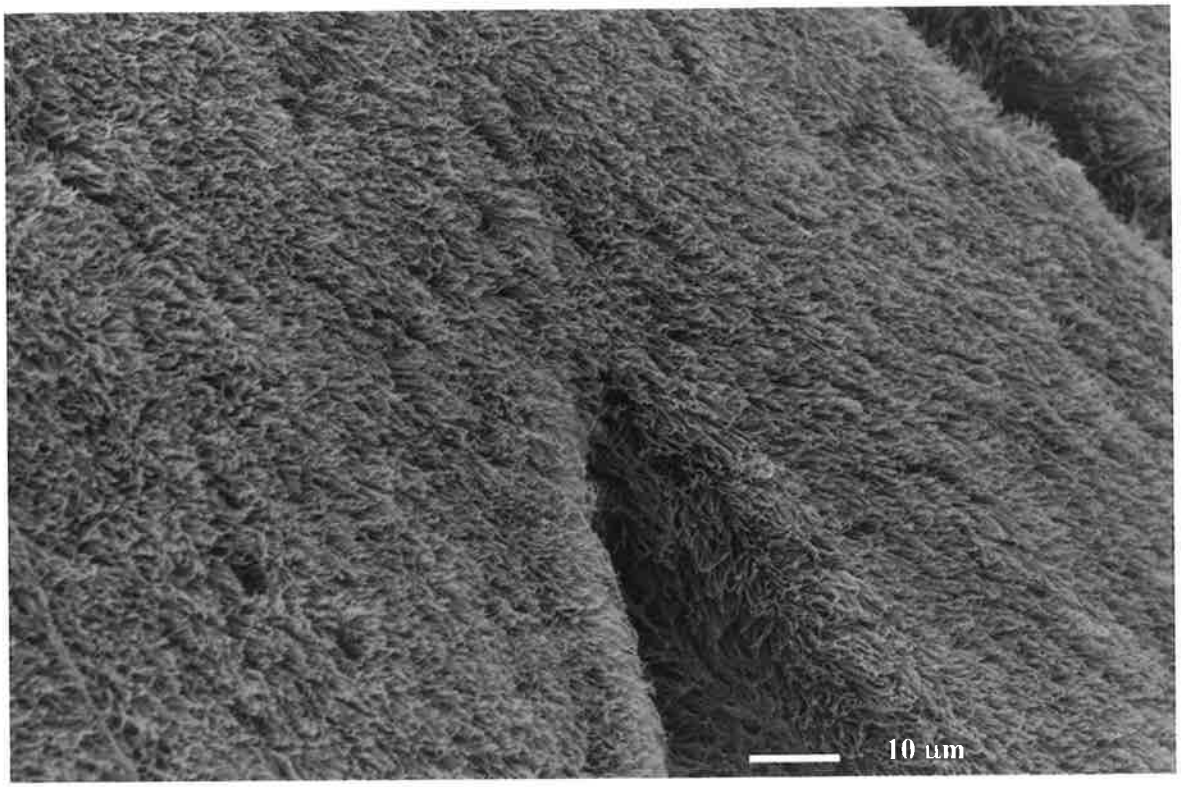


**Figure 10**

SEM specimen from day 56 post partial thickness wounded mucosa showing typical well ciliated nasal respiratory mucosa.

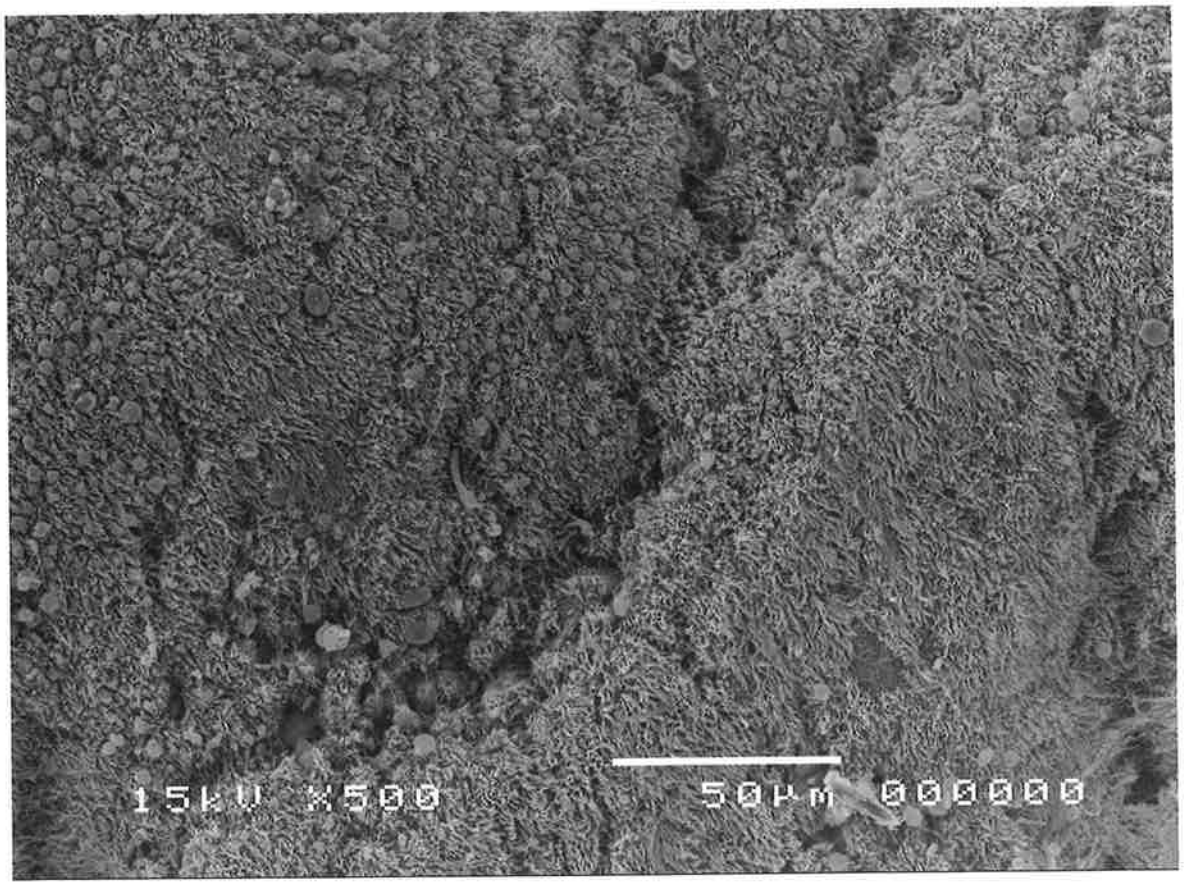


**Figure 11**

SEM specimen from day 84 post full thickness wounding showing typical incomplete ciliated nasal respiratory mucosa.

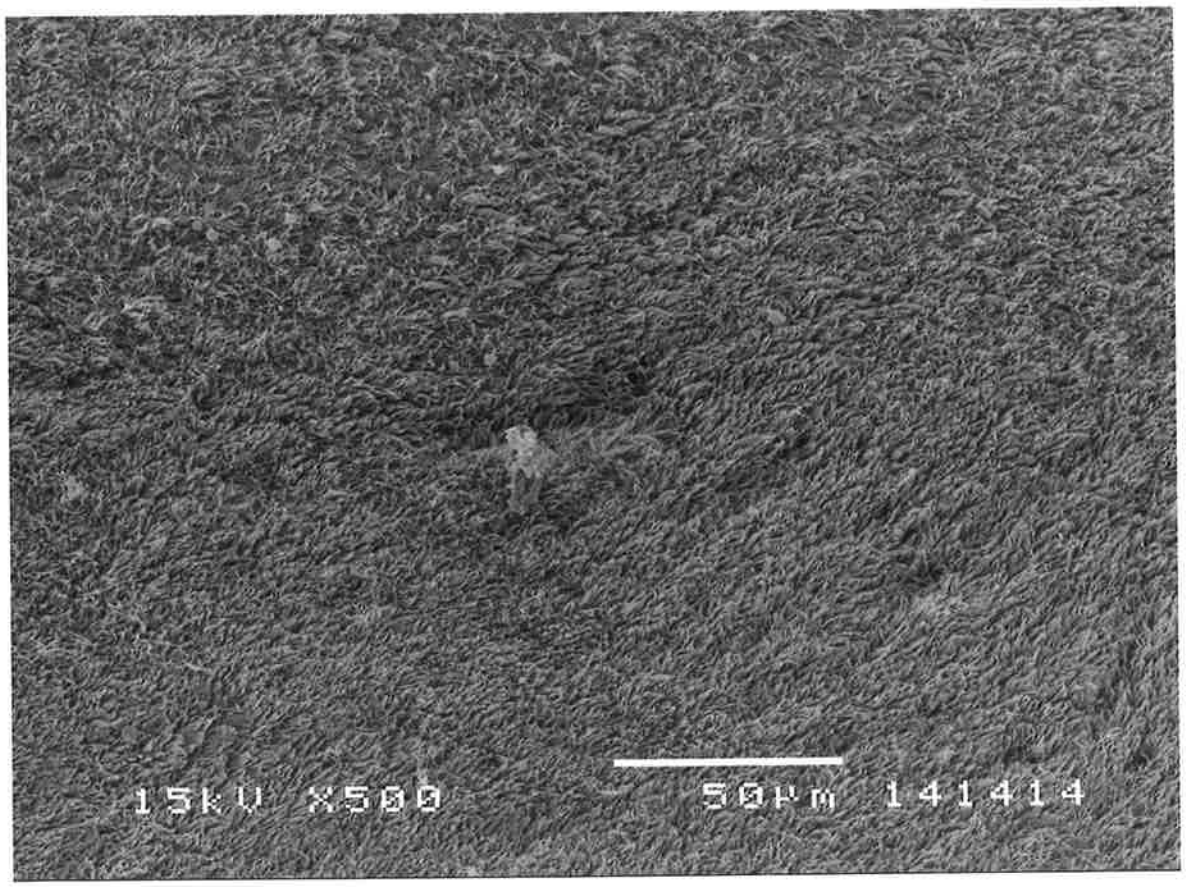


**Figure 12**

SEM specimen from day 84 post partial thickness wounded mucosa showing typical well ciliated nasal respiratory mucosa.

The difference between the 1st and 2nd assessments for each observer (LS, DM) was very small (LS: mean of the difference between the 1st and 2nd assessments 6.335 %, Standard deviation (SD)= 9.191, median 3.245%; DM: mean of the difference between the 1st and 2nd assessments 6.08 %, SD=5.02, median 4.13%). In addition, the correlation between the 1st and 2nd assessments for each observer was high and the correlation was significant at the 0.05 level (LS: Pearson correlation coefficient r = 0.533, p = 0.023; DM: r = 0.549, p = 0.018). Therefore, there was little intra-individual variability for each blinded observer's assessment.

When comparing the results between the observers, the difference between the observer's assessments was small (mean of the differences between the observer's assessments 7.47 %, SD= 5.67; median 5.13 %). The two blinded observers assessments were found to have a high degree of correlation which was significant (Pearson correlation coefficient r = 0.508, $p < 0.05$). The results of comparison of inter-observer's assessments are shown in Table 8.

**Table 8**

Summary of the results of comparison between 2 observers' SEM assessments of cilial regeneration

| Specimen Number | Observer, LS (mean % cilia present) after 2 assessements | Observer, DM (mean % cilia present) after 2 assessments | Difference between 2 observers |
|---|---|---|---|
| 1 | 69.07 | 67.08 | 1.99 |
| 2 | 48.92 | 54.37 | 5.45 |
| 3 | 76.53 | 67.80 | 8.73 |
| 4 | 78.59 | 65.50 | 13.09 |
| 5 | 78.54 | 66.88 | 11.67 |
| 6 | 59.76 | 64.25 | 4.49 |
| 7 | 63.71 | 66.60 | 2.89 |
| 8 | 79.89 | 61.67 | 18.22 |
| 9 | 68.62 | 64.25 | 4.37 |
| 10 | 89.45 | 69.25 | 20.2 |
| 11 | 84.75 | 71.02 | 13.73 |
| 12 | 78.52 | 85.58 | 7.06 |
| 13 | 80.01 | 77.42 | 2.59 |
| 14 | 74.94 | 77.37 | 2.43 |
| 15 | 73.55 | 80.86 | 7.31 |
| 16 | 74.50 | 73.01 | 1.49 |
| 17 | 82.49 | 77.69 | 4.80 |
| 18 | 69.02 | 72.95 | 3.93 |

| | |
|---|---|
| Mean of difference between 2 observers' assessments | 7.47 % |
| SD | 5.67 |
| Median of difference between 2 observers' assessments | 5.13 % |

When comparing the results between SEM and histological assessment of cilial regeneration, the results have shown that the true extent of cilial regeneration cannot be accurately reflected histologically as no cilia was identified histologically in three specimens. There was a large difference between SEM and histological assessments of cilial regeneration (mean of difference between SEM and histological assessment 30.61%, SD 30.95, median 15.12%). In addition the correlation between SEM and histological assessments was poor and the correlation was not significant (Pearson correlation coefficient $r = -0.239$, $p > 0.05$). Overall histological assessment identified less cilia (mean percentage cilia present 60.19%, SD 38.50) when compared with SEM assessment (mean percentage cilia present 73.94%, SD 9.65). The results of SEM and histological assessments of cilial regeneration are shown in Table 9.

**Table 9**

Summary of the results of comparison between SEM (observer LS) and histological assessments of cilial regeneration

| Specimen Number | SEM (LS) (mean % cilia present) after 2 assessements | Histology (% cilia present) | Difference between SEM & Histological assessments |
|---|---|---|---|
| 1 | 69.07 | 1.40 | 67.67 |
| 2 | 48.92 | 100 | 52.08 |
| 3 | 76.53 | 99.7 | 23.17 |
| 4 | 78.59 | 85.90 | 7.31 |
| 5 | 78.54 | 74.00 | 4.54 |
| 6 | 59.76 | 93.40 | 32.64 |
| 7 | 63.71 | 64.50 | 0.79 |
| 8 | 79.89 | 93.50 | 13.61 |
| 9 | 68.62 | 52.00 | 16.62 |
| 10 | 89.45 | 0 | 89.45 |
| 11 | 84.75 | 0 | 84.75 |
| 12 | 78.52 | 79.00 | 0.48 |
| 13 | 80.01 | 92.00 | 11.99 |
| 14 | 74.94 | 84.00 | 9.06 |
| 15 | 73.55 | 66.00 | 7.55 |
| 16 | 74.50 | 14.60 | 59.9 |
| 17 | 82.49 | 83.36 | 0.87 |
| 18 | 69.02 | 0 | 69.02 |

| | |
|---|---|
| Mean of the difference between SEM & histological assessments | 30.61% |
| SD | 30.95 |
| Median | 15.12% |

### 3.4.5 DISCUSSION

Traditionally, the extent of ciliated nasal mucosa has been assessed histologically. While this may be representative of the specimen it may also reflect only a small cross section of the specimen and thus may be prone to selection bias. The results of this experiment has shown that histological assessment failed to identify cilia in three specimens and thus is not a reliable tool for assessing cilial return. In contrast scanning electron microscopy using the Soft Imaging System Analysis® software provides excellent magnified images of cilia across the entire specimen. Thus the amount of cilia present across the entire surface of specimen can be estimated and is less likely to be influenced by selection bias. The results of the study have shown that SEM was able to detect more cilial return when compared with histological analysis. Therefore, SEM is a more reliable tool for assessing cilial return.

Before the observations were started, both observers went through a number of specimens together to confirm the technique to be used in the experiment. This would have the effect of lessening any learning trend. The skill of assessing the cilia accurately with the software was easily acquired with training as indicated by the small intra- and inter-individual difference in assessing the specimens. SEM as a tool for assessing cilial return after mucosal injury is thus validated by the low intra- and inter-observer variation and can be recommended for further studies where accurate and reliable analysis of cilial return is required.

As far as specimen preparation is concerned, it is crucial to wash off any mucus or blood clot from the mucosal surface during the preparation of the specimens for electron microscopy. The specimens should be handled with gentleness as cilia can be easily damaged during the preparation process.

Return of cilial function after surgery is crucial in restoring the natural mucociliary clearance mechanism, and the health of nose and sinuses. Therefore, there is a need to be able to scientifically assess this cilial return as different surgical interventions and postoperative treatments are compared. However, the role of scanning electron microscopy in assessing cilial regeneration requires further study to confirm its usefulness and accuracy as the present study involved a relatively small sample size.

# CHAPTER4

# SUMMARY

In the first experimental study, ten sheep were used to establish a standardized model for the study of endoscopic sinus surgery. The sheep was selected as the most suitable animal after extensive investigation of all possible models. The sheep's nasal cavity and sinuses are orientated in a similar fashion to man's. However, to access the maxillary, ethmoid and frontal sinus, the sheep's middle turbinate needs to be removed. Consequently the effects of middle turbinectomy on the nasal respiratory epithelium and cilial function were studied. Baseline mucociliary transport rates and patterns over the middle turbinates were observed using carbon powder as an insoluble tracer medium. The obstructing middle turbinates were then resected endoscopically. Mucosa on the lateral aspect of the middle turbinate which was not in contact with any nasal packing was taken as a control for histological examination. The average mucociliary transport rate was 1.19 mm per second (Standard deviation (SD)=1.36; 95% confidence interval (CI)=$\pm$0.97). Three weeks after the turbinectomy, the cilial function of the lateral nasal wall was compared to the baseline mucociliary function. The average transport rate (0.84 mm per second; SD=0.63; 95% CI=$\pm$0.45) did not differ significantly following total middle turbinectomy (paired t test p=0.3974). The percentage of intact ciliated mucosa of lateral nasal wall after turbinectomy was compared to the control. The percentage of intact ciliated mucosa of the control group varied from 54.1% to the 100% with a mean of 85% (SD=17.6; 95% CI=$\pm$14.7). The percentage of intact ciliated mucosa of the post-turbinectomy group varied from 70% to the 100% with a mean of 89% (SD=10.75; 95% CI=$\pm$7.69). There was no statistically significant difference between the means of the control group and the post-turbinectomy group (p=0.5571). The turbinectomy did not significantly alter cilial function or respiratory

epithelium. We conclude that the sheep is a suitable model for studying the healing process after endoscopic surgery.

After the standardized sheep model had been established, the effects of packing with ribbon gauze and neuropatties on the nasal mucosa was assessed using this standardized sheep model. Fourteen sheep either underwent ribbon gauze or neuropattie nasal packing. Trauma to nasal mucosa caused by ribbon gauze and neuropatties was compared to mucosa on the lateral aspect of the middle turbinate which was not in contact with any packing. This tissue was used as a control. Ribbon gauze packing resulted in significant loss of 68% of the ciliated surface of the mucosa when compared with the control group with a 15% loss of ciliated surface ($p<0.005$). Neuropattie packing also resulted in significant loss of 50% of the ciliated surface of the mucosa when compared with the control group ($p<0.005$). There was no significant difference in loss of ciliated mucosa in the specimens packed with ribbon gauze or neuropatties ($p=0.25$). Nasal packing results in a significant mucosal injury with loss of cilia. This may influence the mucociliary clearance of the nose in the post-operative healing phase. Pre-operative nasal packing should be used circumspectly and if possible avoided.

The aim of the third experimental study was to assess the temporal healing process of nasal epithelium after full-thickness and partial thickness mucosal removal in sheep. Healing was assessed by histologically examining serial biopsies of the healing wounds. The histology assessed the regeneration of epithelium and return of cilia. Muco-ciliary clearance was measured before and after injury. On day 84 post

injury partial thickness injuries had 80.7% (standard error of the mean (SEM) =10.25) normal epithelium and 68.35% (SEM =19.2) re-ciliation. Full-thickness wounds had 64.98% (SEM =19.17) normal epithelium and 32.96% (SEM = 17.46) re-ciliation. On day 84 the difference for epithelium regeneration was not significant ($p > 0.05$) but re-ciliation was significant ($p < 0.05$). The baseline mucociliary clearance was 0.84 mm per second (SEM=0.2) and did not differ significantly from either the partial thickness wound transport rate (2.49 mm per second; SEM=1.02) or the full-thickness transport rate (0.9 mm per second; SEM = 0.37) (paired t test $p> 0.05$). The time period (84 days) for evaluation of re-ciliation was insufficient, as re-ciliation appeared to be continuing. The healing process took place over a longer period than what had been previously reported in literature and this may account for symptoms seen in the post-operative period in patients after sinus surgery.

Finally, the role of scanning electron microscopy in assessment of cilial regeneration was assessed. Cilial regeneration after mucosal injury was assessed on day 56 and day 84 post injury by scanning electron microscopy (using Soft Imaging System Analysis ®) by 2 blinded observers on two different occasions one month apart. Eighteen specimens were suitable for scanning electron microscopy. The difference between the 1st and 2nd assessments for each observer was very small (For LS: mean of the difference between the 1st and 2nd assessments 6.335 %, Standard deviation (SD)= 9.191, median 3.245%; For DM: mean of the difference between the 1st and 2nd assessments 6.08 %, SD=5.02, median 4.13 %). In addition, the correlation between the 1st and 2nd assessments for each observer was high and the correlation was significant ($p < 0.05$). The Pearson correlation coefficient for intra-observer

error for observer LS was $r = 0.533$, $p < 0.05$, and for observer DM was $r = 0.549$, $p < 0.05$. Therefore, there was little intra-observer variability for each blinded observer's assessment. When comparing the results between the observers, the difference between the observer's assessment was small (mean of the differences between the observer's assessments 7.47 %, SD= 5.67; median 5.13 %). The two blinded observers assessments were found to have a high degree of correlation (Pearson correlation coefficient $r = 0.508$) and was statistically significant ($p < 0.05$). Traditionally, the extent of ciliated nasal mucosa is assessed histologically but may be open to selection bias. The scanning electron microscopy using Soft Imaging System Analysis ® provides excellent images of cilia and the amount of cilia present across the surface of specimen can be easily and more accurately estimated. Return of cilial function after surgery is crucial in restoring the natural mucociliary clearance mechanism, and the health of nose and sinuses. Therefore, there is a need to be able to scientifically assess this cilial return as different surgical interventions and postoperative treatments are compared to one another. However, the role of scanning electron microscopy in assessing cilial regeneration requires further studies to confirm its usefulness and accuracy as the present study only involved small sample size. Specimen handling specifically regarding washing mucus or blood clot from the mucosal surface during the preparation of the specimens for electron microscopy is crucial. The specimens should be handled with gentleness as cilia can be easily damaged during the preparation process.

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