Sprayable Chitosan/Starch-Based Sealant Reduces Adhesion Formation in a Sheep Model for Chronic Sinusitis

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Objectives/Hypothesis: Postoperative adhesion formation after endoscopic sinus surgery (ESS) remains a complication associated with high revision rates. This study determines the efficacy of a sprayable chitosan/starch-based sealant for reducing adhesions in an ESS sheep model for chronic sinusitis.

Study Design: Prospective, blinded, randomized controlled trial.

Methods: Sheep (n = 14) with eosinophilic rhinitis (determined by the presence of eosinophilia in nasal secretions) underwent ESS with middle turbinectomies, standardized mucosal injuries created on the lateral nasal wall, and partial thickness wounds created around the ethmoid cell region. Surgery was performed bilaterally (28 nasal cavities). Animals were randomized into treatment with sprayable chitosan/starch-based sealant (n = 7, 14 nasal cavities) or no treatment (n = 7, 14 nasal cavities). Two animals in the treatment group expired due to anesthetic complications associated with the turbinecomies, leaving five animals (10 sites) that completed the study. Presence of adhesions was assessed by endoscopic evaluation at days 14 and 28 after initial surgery. Adhesion formation was confirmed via necropsy of sinus cavities at day 28 after initial surgery.

Results: Adhesions were observed in all seven control animals, resulting in an 86% (95% confidence interval [CI], 65–100) adhesion rate (12 of 14 sites). The five surviving treatment animals had a 10% (95% CI, 0–33) adhesion rate (one of 10 sites). Treatment with the sprayable chitosan/starch-based sealant resulted in a 76% reduction (95% CI, 32–100) of adhesions (P < .002).

Conclusions: In this sheep model for chronic sinusitis, treatment with sprayable chitosan/starch-based sealant reduced adhesion formation by 76% after ESS (P < .002).

Key Words: Animal model, adhesion, chitin/chitosan, ear/nose/throat surgery, wound healing.

Level of Evidence: N/A.

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INTRODUCTION

Endoscopic sinus surgery is performed >250,000 times annually in the United States, in most cases for the management of chronic rhinosinusitis.1–3 Although surgical interventions for the management of chronic rhinosinusitis are often successful, >15% of patients require a repeat operation.4 Wound healing in the perioperative period following endoscopic sinus surgery is an important determinant for surgical success, with one of the primary causes of short-term complication being postoperative adhesion or synechiae formation.4,5 Multiple bioabsorbable and nonabsorbable packing materials have been utilized throughout the history of endoscopic sinus surgery in an attempt to reduce postoperative bleeding and adhesion formation. However, there are no current therapies commonly accepted by otolaryngologists that can reliably reduce postoperative adhesion formation in the perioperative period following human endoscopic sinus surgery.6

Chitosan is a linear polysaccharide commercially produced by the deacetylation of chitin, a structural molecule found in crustaceans, insects, and the cell walls of fungi.7 Chitosan has been used in numerous biomedical applications, in part due to its hypoallergenicity,8 its ability to rapidly clot blood,9 and its antimicrobial effects.10 In addition, chitosan maintains solubility in the acidic environment of epithelial wound healing.11 As a result, there has been significant interest in using chitosan-based compounds intraoperatively during endoscopic sinus surgery.12–14 Chitosan-in-gel formulations have demonstrated improved sinus wound healing following endoscopic sinus surgery in sheep,15 and in an Australian human study.16 Ideally, a chitosan formulation would also exist in a sprayable form to allow for uniform application to sinus epithelium and would be visible during intraoperative delivery. The formulation would act as a sealant, that is, would remain adherent to freshly operated sinus surfaces for the duration necessary for re-epithelization to occur and allow for airway patency.
We recently reported on our initial endeavors to evaluate the efficacy of such a sprayable, chitosan-based sinus sealant.\textsuperscript{17} We demonstrated dose-dependent inhibition of migration and proliferation of cultured fibroblasts obtained from human surgical polyp explants using this sinus sealant, demonstrated reduction in adhesion scores by 94\% in a well-established rabbit cecal-sidewall model commonly utilized for adhesion testing, and demonstrated a reduction of adhesion formation by 70\% in healthy sheep undergoing endoscopic sinus surgery and wounding to induce adhesion formation.

Herein, we tested the hypothesis that this sprayable, chitosan-based sealant formulation would be effective in reducing intraoperative adhesions during endoscopic sinus surgery in a cohort of sheep with eosinophilic rhinitis (ER). We attempted to replicate this ER sheep adhesion model previously reported in the literature solely in Australia,\textsuperscript{15} and developed a reproducible scoring system and photography protocol for sinus adhesions formed in sheep following endoscopic sinus surgery.

**MATERIALS AND METHODS**

**ER Sheep Model**

Sheep were maintained and procedures were performed at the Ibex Preclinical Research, Inc. animal facility in Logan, Utah, a facility registered by the US Department of Agriculture that operates in accordance with the Guide for the Care and Use of Laboratory Animals. The animal study protocol was approved by the Ibex Institutional Animal Care and Use Committee prior to commencement of the studies. Adult female domestic sheep (\textit{Ovis aries}) with obvious nasal discharge were screened from a pool of 99 animals for the presence of eosinophils in their nasal discharge. Sample size estimation was performed a priori using Mead’s resource equation method. Fifteen animals (14 for use in the study and one reserve animal) identified as in good health, eosinophil positive, and weighing between 50 and 80 kg were selected and acclimated for 11 days in a combination of indoor and outdoor housing. Animals were fed alfalfa hay and/or alfalfa pellets and potable water ad libitum.

**Anesthesia and Surgical Procedures**

At 2 to 5 days prior to surgery, the animals were weighed, and a physical exam was conducted including an examination of the nares and muzzle. Approximately 24 hours prior to surgery, food was removed from the animals to minimize regurgitation/aspiration. On the evening prior to surgery, water was withheld from the animals. Cefazolin (1 g, intravenous) was given perioperatively. Prior to induction of anesthesia, animals were premedicated with atropine (0.04–0.4 mg/kg intramuscular) to reduce intraoperative airway secretions and butorphanol (0.3–0.5 mg/kg, intramuscular) to provide preemptive analgesia. Anesthesia was induced using ketamine (6–11 mg/kg) and diazepam (0.3–0.6 mg/kg) intravenously or midazolam (0.2–0.5 mg/kg) intramuscularly. After induction of anesthesia, a cuffed endotracheal tube was placed, and anesthesia was maintained using isoflurane (% to effect) in oxygen. An orogastric tube was passed into the stomach to control eructation. Ophthalmic ointment was applied to both eyes to prevent corneal drying and reduce the risk of corneal abrasions. The vital signs (temperature, heart rate, respiration rate, oxygen saturation) of the animal were monitored during the procedure. Lactated Ringer solution was administered at a rate of approximately 10 mL/kg/hr during the procedure through an intravenous catheter. The animal was placed on an operating table in sternal recumbency with the head positioned to allow for best access to the nasal airway. Operations required approximately 45 minutes to 1 hour per animal of general anesthetic.

**Experimental Sinus Surgical Adhesion Formation**

An endoscope was inserted in one of the nares, and anatomic landmarks were identified. The middle turbinate was resected with endoscopic scissors along its lateral ridge of attachment. The stump of the middle turbinate was cauterized as necessary to control any arterial bleeding. Following resection of the middle turbinate, full thickness lateral nasal wall mucosal wounds were created above and below the ridge of bone that remains after middle turbinectomy (two areas of wounding per side). To create the wounds, a 3-mm cutting bur attached to a microdebrider (Medtronic Surgical Technologies, Jacksonville, FL) was placed either just superior or inferior to the middle turbinectomy bone ridge, starting at the stump of the resected middle turbinate, and then powered for approximately 30 seconds to create a full thickness wound penetrating to the underlying lateral nasal bone. The size of the wounds was 4 cm × 2 cm (± 0.5 cm) made by advancing anteriorly via a circular motion and measured via a demarcated suction tube. Additionally, the sheep have an ethmoid turbinal that is exposed following resection of the middle turbinate that approximates a human middle turbinate and a human middle meatus. A full thickness wound was made around the exposed anterior perimeter of this ethmoid turbinal via a circular motion timed for 10 seconds. The same procedure was conducted on the contralateral side. Hemostasis was obtained via monopolar suction cautery, and the use of epinephrine-soaked gauze as needed. A bur was preferred to a microdebrider blade due to our opinion that this instrument produces a more reliable and controllable full thickness epithelial injury with less mucosal stripping.

**Application of Chitosan/Starch-Based Sealant**

Animals were selected to be control animals (n = 7) or receive the test agent (n = 7) a priori via randomization. Randomization was performed by an independent veterinarian with concealed instructions for the surgeon. The surgeon was blinded to the randomization until all surgical procedures were completed in the animal. For animals in the test group, the chitosan/starch-based sealant was delivered by a sprayer (Medtronic Surgical Technologies) that delivered the sealant in a 180° (half-spherical) zone. All areas of surgical wounding bilaterally were treated until visibly covered completely (6 mL per nasal cavity) with the purple sealant. Control animals were left untreated.

**Postoperative Evaluation**

The animals were moved to a recovery area and monitored for recovery from anesthesia. Once each animal had a strong swallowing reflex, the endotracheal tube was removed. Once sternal recumbency was achieved, each animal was returned to its individual pen. After full recovery from the anesthetic, food and water were returned to each animal. Animals were returned to group housing at the discretion of the attending veterinarian. Animals underwent clinical observations for general health and weight daily. Animals had a fentanyl transdermal patch (100 µg/hr) placed on the skin, just prior to surgery, to provide additional postoperative analgesia for up to 3 days. Additional analgesia was given at the discretion of the veterinarian. Enrofloxacin (2.5–5.0 mg/kg, subcutaneous) was
given postoperatively daily for up to 7 days. Seven days postoperatively, each animal received ivermectin treatment via intramuscular injection. Animals underwent endoscopic evaluation on day 14 and day 28 after the surgical procedure to examine for the development of adhesions and monitor for the need of early termination of the study if any visible signs of material-mediated inflammation, infection, or necrotic tissue were observed. Day 14 was also selected as the first endoscopic evaluation point to allow adequate time for the sealant to be fully cleared from the nasal cavity and allow for blinded evaluation of adhesions, which occurs within postoperative days 4 to 10 as documented in our previous study. Day 28, all surviving

Fig. 1. Sheep sinus surgical adhesion grading system: (A) 0, no adhesions; (B) 1, a single thin adhesion; (C) 2, >1 thin adhesions; (D) 3, a single thick/large adhesion; (E) 4, >1 thick/large adhesions; (F) 5, complete obliteration of a meatus.
animals were euthanized using an injectable concentrated barbiturate euthanasia solution at an approximate dose of 1.2 mL/10 lb. Day 28 was selected for necropsy based on previous work that showed adhesions were most severe at day 28 in a sheep model that examined adhesions for 112 days postoperatively. Day 28 was felt to represent the endpoint that would be most cost-effective and humane to our animals. Necropsies were performed, and the wound sites were exposed using an oscillating bone saw, rongeurs, and any other necessary instruments. The nasal airway was visualized to determine whether any additional adhesions were present. The number of adhesions and location of adhesions were documented. Standardized high-resolution digital photographs of each of the sites (a mid-sagittal image examining the lateral ridge injury and coronal images examining the ethmoid turbinals) were taken after the necropsy on day 28.

**Adhesion Scoring and Statistical Analysis**

The presence or absence of adhesions was noted by a surgeon, veterinarian, and veterinary technician at the time of necropsy. Although nonblinded, these assessments were performed 28 days after surgery, with no record of which nasal cavities received the sealant and no evidence of any sealant present in the animals. Adhesions were also additionally scored by 3 blinded scorers not affiliated with the surgery by reviewing photographs of the necropsies and utilizing the adhesion grading system described in Figure 1. Adhesion grading was scored collectively for the entire nasal cavity. Wilcoxon rank sum tests were performed to evaluate for statistical significance at $P < .05$. Pearson product–moment correlation coefficient was used to assess inter-rater reliability for adhesion grading.

**RESULTS**

Surgical procedures were successfully completed on control sheep ($n = 7$) and sheep receiving the chitosan/starch-based sealant ($n = 7$). One animal exhibited cardiac arrest shortly after the commencement of anesthesia and was replaced with the reserve animal. Cause of death after necropsy was determined to be related to an adverse reaction to anesthesia. One animal in the test group died on the day of the surgical procedure. Necropsy revealed significant aspiration of blood and clots in bilateral bronchi. Cause of death after necropsy was determined to be due to aspiration of blood intraoperatively and subsequent respiratory failure. One animal in the test group died on postoperative day 6. Necropsy revealed evidence of blood in the trachea, resolving blood clots in the secondary bronchi, and wet heavy lung appearance. Cause of death was determined to be aspiration of blood. No treatment-related adverse effects were observed, and no additional morbidity or mortality occurred. Re-epithelialization of all wounds was complete in all animals at necropsy. Adhesions were observed in all seven control animals, resulting in an 86% (95% confidence interval [CI], 65–100) adhesion rate (12 of 14 sites). The five surviving treatment animals had a 10% (95% CI, 0%, 33%) adhesion rate (one of 10 sites). Treatment with the sprayable chitosan/starch-based sprayable sealant resulted in a 76% reduction (95% CI, 32–100) of adhesions ($P < .002$; see Fig. 2). Mean total adhesion score for the control animals was $3.1 \pm 0.2$ (95% CI, 2.7–3.5). Mean total adhesion score for the chitosan/starch-based sealant treated animals was $0.4 \pm 0.2$ (95% CI, 0.0, 0.8). Chitosan/starch-based treatment resulted in a reduction of $2.7 \pm 0.4$ (95% CI, 1.9–3.5) in total adhesion score ($P < .00001$). Inter-rater reliability was strong, with a Pearson $r$ between .81 and .94. Representative images of adhesions from control and treatment animals are seen in Figure 3.

**DISCUSSION**

The prevention of perioperative adhesion or synechiae formation is an important goal for endoscopic sinus surgeons. Although multiple types of therapies have been developed and tested for the prevention of adhesion formation, few have demonstrated conclusive proof of the ability to do so. For example, a recent prospective, randomized, double-blinded, placebo-controlled trial using a triamcinolone-impregnated commonly used bioresorbable dressing demonstrated improved short-term Kennedy–Lund endoscopy scores and a trend toward reduced adhesion formation. However, earlier reports have suggested that the same bioresorbable dressing may impair wound healing and possibly promote adhesion formation. Similar conflicting findings have been reported with many other middle meatal spacers and bioabsorbable packing materials. As a result, no single biomaterial for use during sinus surgery has gained widespread acceptance. The difficulty lies in the fact that the ideal biomaterial would be hemostatic, promote wound healing, reduce adhesion formation, visibly and uniformly coat all healing epithelial surfaces, and reduce the need for future debridements. To date, no biomaterial commonly used appears to fulfill all of these ideal requirements.

However, there has been significant interest in the potential for chitosan, a linear polysaccharide derived from chitin, to serve as the active component for this ideal biomaterial. Chitosan is biodegradable, nontoxic, nonimmunogenic, antimicrobial, and dissolves in the acidic environments of wound healing. Furthermore, it is available as medical grade material and has been proven to be an outstanding hemostatic agent for trauma patients and for use on the battlefield. Wormald and colleagues have utilized a chitosan-dextran gel and demonstrated reduced adhesion formation in a sheep model.
of chronic sinusitis\textsuperscript{15} and demonstrated significantly reduced adhesion formation in a trial of 40 Australian patients undergoing sinus surgery.\textsuperscript{16}

Medtronic Surgical Technologies has endeavored to develop a unique, sprayable, and visible formulation of a chitosan-based sinus sealant suitable for use perioperatively. It is hypothesized that a sprayable formulation would provide more uniform coverage of healing epithelium, be easier and more reliable for the surgeon to use intraoperatively, and best fulfill the goals of the ideal biomaterial for use during endoscopic sinus surgery. We recently reported on our initial efficacy studies\textsuperscript{17} with such a sprayable, chitosan/starch-based sinus sealant, demonstrating its ability to inhibit migration and proliferation in vitro of human fibroblasts cultured from surgical polyp explants, its ability to significantly inhibit adhesion formation in a well-established rabbit cecal sidewall model, and its ability to significantly inhibit adhesion formation in an experimental sinus surgical adhesion model in healthy sheep.

In this study, we attempted to build on our work by replicating the chronic sinusitis model from Australia.\textsuperscript{15} Eosinophilic rhinitis in sheep is induced by a parasitic larval infection in the sinuses. Although not normally parasitically induced, a large subset of chronic sinusitis sufferers has eosinophilic disease, so we concur that this sheep model might also be clinically relevant to a large subset of human patients. Treatment with the chitosan-starch based sealant resulted in statistical improvement over control in the ER sheep model. The necropsy scores showed a 76% reduction in presence of adhesions; this result is consistent with the 70% reduction in adhesions we reported in the healthy sheep surgical model. Thus, the efficacy of the chitosan-starch based spray was not hindered in the presence of ER.

In efforts to limit bias of data, we developed a new scoring protocol for sheep adhesions. Adhesions were evaluated using a 0 to 5 scale from high-resolution macro photographs taken of each sinus cavity at necropsy so we could acquire scores from independent, blinded reviewers. Scorers were first trained on the scale using images of sheep sinus cavities taken during previous sheep trials. After training, scorers were allowed to view the images for this study and recorded their scores on a provided score sheet. The use of high-resolution photographs will allow for independent comparisons between individual experiments and across institutions, improving the usefulness of this valuable animal model.

During our study, three sheep died from presumed anesthetic complications determined by necropsy evaluation by licensed veterinarians; one animal suffered cardiac arrest under anesthesia, and two animals suffered from respiratory failure. At necropsy both respiratory failure animals had significant volumes of blood in their lungs. We believe these deaths are probably due to poorly fitting cuffs on the endotracheal tubes used during these experiments. Due to the long snouts, relatively separate nasal and oral anatomy, and position in neck extension during the surgery, all nasal bleeding that flows posteriorly has direct access to the upper respiratory tree. For this study compared to our previous work with sheep, we used an endotracheal tube from a different vendor that did not form as tight a seal in the sheep trachea. For our future studies and for future users of this animal model, we feel it is imperative to aggressively protect the upper airway from aspiration of blood. We will consider modification of our technique, including the possibility of posterior nasal packing and/or other techniques to minimize aspiration of blood.

This prospective, randomized, blinded, controlled animal trial has important limitations. In this study, we chose to randomize the treatment to each animal, as opposed to each nasal cavity in our previous study on healthy sheep. Although this minimizes the confounding bias from potential paracrine effects of treatment on the contralateral nasal cavity, it does allow for an allocation bias due to possible differences in surgical technique, differing amounts of bleeding, differing use of monopolar

Fig. 3. (A) Example of adhesions (arrows) formed in ethmoid turbinal (analogous to middle meatus) in right nasal cavity of control sheep 28 days postsurgery. (B) Example of chitosan/starch-based sealant-treated nasal cavity in sheep 28 days postsurgery.
 cautery, differences in anesthetic parameters, nutritional and environmental differences, and genetic differences in adhesion formation between cohorts. In our previous study, we utilized a methodology where each animal received the sealant on one side and had one side serve as a control. Collectively, both studies had similar rates of reduction in adhesion formation (70% and 76%), suggesting that these potential biases, if present, were minimal. Also, the adhesion scoring system we have developed would ideally be clinically validated from outcome studies in human endoscopic sinus surgery. Although it is obvious that obliteration of the frontal sinus outflow tract is a harmful complication from sinus surgery, the clinical significance of small, thin adhesions, for example those seen between the middle turbinate and the lateral nasal wall, are unknown and are possibly of little clinical significance. To our knowledge, there are no studies examining the significance of various, well-defined types of adhesions or synechiae that develop after human endoscopic sinus surgery. Without such a study as a guide, this scoring system is our best estimation of clinical severity. Despite these limitations, this study, to our knowledge, is the first to show statistically significant reductions of adhesion formation compared to control in an ER model of sheep. This study and our previous report collectively provide strong evidence to support human studies of this sprayable, chitosan-based sinus sealant and its ability to reduce adhesion formation following endoscopic sinus surgery.

CONCLUSION

In a sheep model of chronic sinusitis, a sprayable formulation of chitosan/starch-based sealant reduced experimental adhesion formation following sinus surgery by 76% \( (P < .002) \). Mean total adhesion scores were reduced from 3.1 ± 0.2 (95% CI, 2.7–3.5) in control animals to 0.4 ± 0.2 (95% CI, 0.0–0.8) in treated animals \( (P < .00001) \). This study supports the further investigation of this sealant for potential use during human endoscopic sinus surgery.

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BIBLIOGRAPHY