

# Myringoplasty: Comparing Fresh Porcine Submucosa with Rice Paper Patch

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## Abstract

**Objective:** To evaluate and compare the efficacy of freshly prepared porcine small intestine submucosa (SIS) with rice paper patch in the repair of large chronic tympanic membrane perforations.

**Materials and methods:** Chronic tympanic membrane perforations were bilaterally produced in five adult chinchillas using a 3mm aural speculum and a myringoplasty knife for a total of 10 perforations. Each chinchilla was post-operatively monitored for five weeks to assess whether perforations spontaneously closed or became infected prior to surgical repair with myringoplasty. The left and right ear of each chinchilla was repaired with a rice paper patch onlay and freshly prepared porcine SIS graft, respectively.

**Results:** One chinchilla was excluded from the study due to an ear infection, leaving four chinchillas for a total of 8 perforations. Four out of four (100%) myringoplasties performed with the fresh SIS xenograft remained healed after 5 weeks. Harvest of rice paper patch grafts demonstrated little healing and only one out of four (25%) ears showed TM epithelium under microscopic examination.

**Conclusion:** The results and histological analysis indicate fresh SIS xenografts a practical alternative to rice paper patch for the repair of chronic tympanic membrane perforations.

## Introduction

Given that the tympanic membrane is thin and exposed to the environment, it is vulnerable to infections, physical trauma, and barometric changes in pressure. The mechanism of damage and the size of the perforation influence the ability for the TM to heal itself. While acute TM perforations are likely to spontaneously heal and regenerate, some can become chronic requiring surgical repair through myringoplasty.

An ideal animal model for graft placement must have a TM that is similar to humans and a perforation that is free from infection. Chinchillas have a large TM and wide ear canal, which allow easy access in positioning a graft. Moreover, the TM resembles that of humans in that it consists of three layers including a squamous epithelium, middle fibrous layer, and mucosal layer. Not only is the range of hearing similar to that of man, but also the TM is of similar proportion with a diameter ranging from 6 to 9mm in size.

A graft that is biocompatible, easy to handle, and readily available in a range of sizes would be most practical for tissue repair via myringoplasty. SIS has been successfully used as a scaffold for tissue repair. This study will examine a cost-effective option by using grafts made from fresh, native SIS prepared on location as opposed to relying on commercially available sources. Porcine SIS is a xenograft that enhances wound healing by inducing the host tissue to proliferate, regenerate, and remodel post-implantation. This natural biomaterial is able to stimulate cell division, migration, and differentiation as a result of growth factors found within the submucosa including vascular endothelial growth factor (VEGF), fibroblast growth factor (b-FGF), and transforming growth factor  $\beta$  (TGF $\beta$ ).

Myringoplasty has limitations including the need for general anesthesia, costly equipment, surgical expertise, and donor site morbidity. With this in mind, rice paper patch was developed and successfully used on small perforations in order to reduce operating time and cost with a simple nonsurgical procedure. Rice paper patch acts as a scaffold that guides migrating epithelium from the borders of the perforation in order to close the opening without the limitations seen in myringoplasty. The economic benefits are substantial, especially for patients with time constraints that wish to avoid a surgical procedure.

## Material and Methods

**Animal model:** The study was approved and monitored by the Philadelphia College of Osteopathic Medicine Institutional Animal Care and Utilization Committee. Five healthy adult female chinchillas each weighing 490 to 626g were purchased from Rauscher's Chinchilla Ranch (LaRue, Ohio). Only one gender was used, because the sex of the animal was not clinically considered to be a factor.

**Production of SIS graft:** Fresh porcine small intestine was obtained from a USDA approved vendor. The jejunum was sectioned and sliced longitudinally to produce a sheet, which was positioned serosal side up. The serosa and muscularis layers were removed from the submucosa and discarded. The sheet was inverted and the mucosal surface denuded. The fresh SIS was appropriately sized and stored into a sterilizing, chilled 10% gentamicin: 0.9% saline solution until placement.

**Operative technique: I myringotomy:** Animals were fasted for no more than two hours prior to anesthesia with 1% to 3% isoflurane (per 1L oxygen) as not to disrupt the intestinal microflora. Body temperature was monitored closely with a rectal thermometer as adverse reactions to anesthetics such as hypothermia can develop in small mammals. The trans-canal approach to visualization of the TM through a 3mm otologic speculum was used in creating bilateral TM perforations in each chinchilla. An incision was made with a myringoplasty knife, severing at least 70% of the pars tensa. Postoperatively, pain was controlled by buprenorphine (0.05 mg/kg) 30 minutes prior to emergence from anesthesia and enrofloxacin (20 mg/kg) once a day for five days. Each chinchilla was post-operatively monitored for five weeks. Changes in behavior such as reduced food and water intake, restlessness, vocalizations, and excessive rubbing or biting indicative of pain were managed with 2 mg/mL acetaminophen added to the drinking water and buprenorphine as needed. Furthermore, signs of infection were treated with antibiotic drops containing 10,000 U/mL polymyxin B sulfate, 10 mg/mL hydrocortisone, and 3.5 mg/mL neomycin. Despite these precautions, one chinchilla succumbed to an ear infection and was excluded from the study. Non-infected TM perforations that lacked signs of epithelial regeneration were deemed chronic.

**Operative technique: II myringoplasty:** The remaining 4 chinchillas totaling 8 TM perforations underwent bilateral myringoplasty. After induction of anesthesia, the overlay technique was used to place grafts over the edges of the perforation and annulus. Each chinchilla served as its own control with an SIS graft and rice paper patch being placed in the right and left ears respectively. SIS graft placement was facilitated by aseptically blotting the excess with 10% gentamicin/normal saline solution. Rice paper patch was placed in the opposite ear by following the same general procedure. Gelfoam (The Upjohn Co., Kalamazoo, MI) saturated with antibiotic drops was then inserted into the external auditory meatus (EAM) of both ears.

**Post-surgical procedure:** Post-surgical monitoring was conducted as previously stated. Each TM repair was evaluated under anesthesia 3 weeks' post-surgery. All 4 animals were euthanized with a lethal dose of Euthasol (pentobarbital sodium and phenytoin sodium) solution (Virbac Corp., Fort Worth, Texas); 2 mL/first 10 lbs and 1mL each additional 10 lbs, IV.

**Graft harvest and preparation:** Eight TM's were excised by dissection from the annular groove and placed in 10% buffered formalin. Prior to paraffin embedment and sectioning, each specimen underwent a decalcification procedure. A rotary microtome was used to cut sections at 6 $\mu$ m, which were then deparaffinized and stained using Torren's method. Sections were imaged using a Nikon Eclipse E800<sup>TM</sup> microscope at 10x, 20x, 40x, 60x, and 100x and photographed using a Phase 3 Imaging<sup>TM</sup> system.

## Results

**Gross observations:** Subtotal perforations that involved the pars tensa, but did not include annulus were produced in 8 TMs of four chinchillas. All (100%) remained perforated over the course of 5 weeks. They were of uniform size, extending over 70% of the pars tensa. After 35 days of observation, none of the TMs demonstrated complete healing or showed any signs of infection. However, some perforations did reduce in size to approximately 50% of the pars tensa.

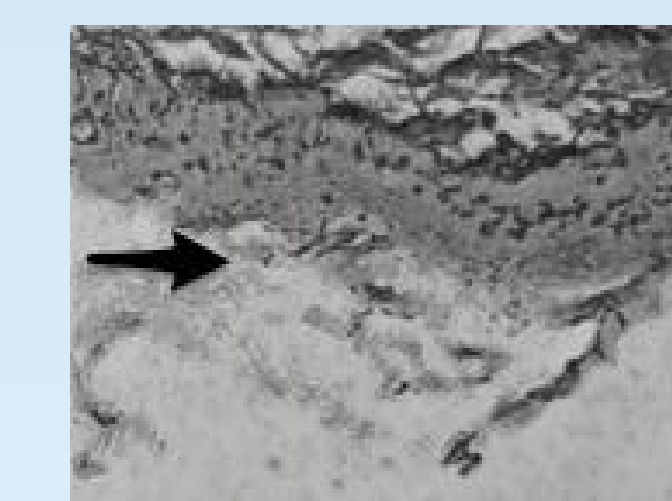
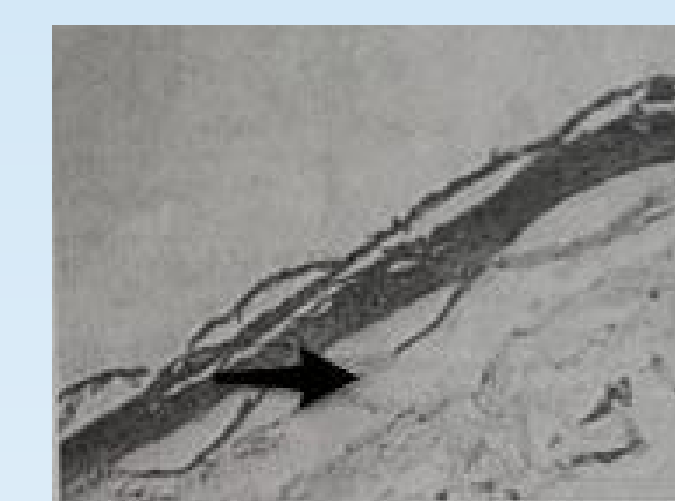
All four (100%) of the TM perforations repaired with SIS graft showed partial or complete healing after 21 days (Fig. 1). If SIS was not completely absorbed, its location was indicated by a translucent appearance on the repaired surface. Perforations repaired with rice paper patch on the other hand, appeared thin and semitransparent.



Fig. 1. Posterior view of an SIS repaired TM in the bulla.

**Histological observations:** Toren's method of staining was used in which SIS appears pink, collagen blue-green, and mast cell granules deep blue. In two SIS repaired TMs, SIS fused with the TM (Fig. 2). As compared to their intact state, TMs are disorganized and appear to be undergoing modification. At 100x magnification, SIS appears to be forming attachments to the TM. However, in one repair there seems to be slight inflammation between the SIS and collagen. In the rest of the repairs, no inflammation is indicated at the site of infiltration of SIS and collagen. Lastly, one SIS-repaired TM demonstrated neovascularization in a collagen-dense region.

A



B

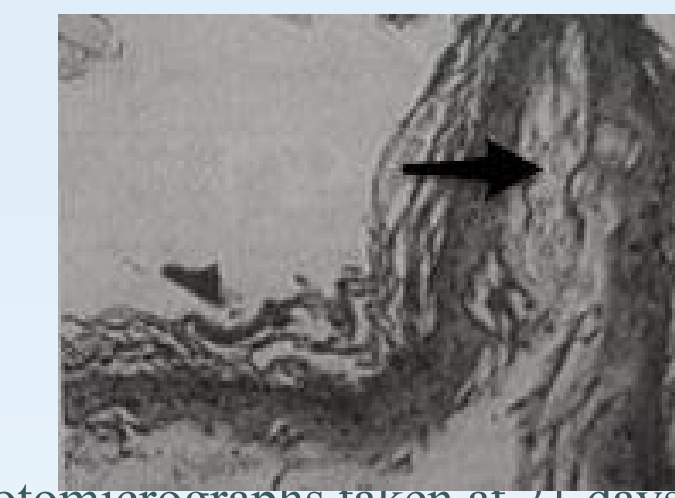


Fig. 2. Photomicrographs taken at 21 days demonstrate ingrowth of SIS on two different TMs (A and B) (Toren's method; left, 40x; right, 100x).

Repairs with rice paper patch were fragile and proved difficult to harvest. On histological examination, only one of four ears (25%) showed epithelial tissue of a healed TM.

## Conclusion

In this study, we examined the efficacy of freshly prepared porcine SIS xenograft in comparison to rice paper patch in the chinchilla animal model. Although rice paper patch can be effective in healing small TM perforations it isn't without its downsides. As the size of the perforation increases, the efficacy of rice paper patch decreases clearly demonstrating an inverse relationship between size and success rate. The results of this study demonstrate freshly harvested porcine SIS as an effective graft for myringoplasty. SIS offers several advantages for TM repair, as it is readily obtainable, affordable, and easy to handle. Multiple applications for SIS in various animal models both in our laboratory and those of other investigators have previously demonstrated a high success rate using freshly prepared SIS grafts and the present study further extends its application to TM perforations. In under-resourced areas where other alternatives are not feasible, freshly prepared SIS could be a viable option in treating patients with chronic TM perforations.

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