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 3 mm otologic spear and a myringoplasty knife. Each ear was postoperatively monitored for five weeks to assess whether perforations spontaneously closed or became infected prior to surgical repair with myringoplasty. The left ear of each chinchilla was repaired with a rice paper patch onlay and freshly preserved 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 allows for comparison with 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 healing similar to that of man, but also the TM is of similar proportion with a diameter ranging from 6 to 10mm 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, naïve 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 produce a sheet, which was positioned serosal side up. The serosa and muscular 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 anesthesia such as hypothermia can develop in small mammals. The trans-annular approach to visualization of the TM through a 3mm otologic spearg was used in creating bilateral TM perforations in each chinchilla. An incision was made with a myringoplasty knife, securing 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 morphine (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 monitored 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 tolerated bilateral TM perforations under bilateral myringoplasty repair. After induction of anesthesia, the overlying tissue was used to place grafts over the edges of the perforation and annulus. Each chinchilla served as its own control with a 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% povidone-iodine normal saline solution. Rice paper patch was placed in the opposite ear following the same general procedure. Gel foam (The Upjohn Co., Kalamazoo, MI) saturated with antibiotics 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 Euthatal pentobarbital sodium and an intravenous (IV) solution (Virbac Corp., Fort Worth, Texas): 2 mL/first 10 lbs and 1 mL/ additional 10 lbs with 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 embedding and sectioning, each specimen underwent a decalcification procedure. A rotary microtome was used to cut sections at 6µm, which were then deparaffinized and stained using Toren’s method. Sections were imaged using a Nikon Eclipse E600 microscope at 10x, 20x, 40x, 60x, and 100x and photographed using a Phase 3 Imaging system.

Histological observations: Torens’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 fixed with the TM (Fig. 2). As compared to their intact state, TMs are disorganized and appear to be undergoing modification. At 10µs 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.

Results

Gross observations: Subtotal perforations that involved the pars tensa, but did not include annulus were produced in 8 TMs of four chinchillas. AR (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 (100%) of the TM perforations repaired with SIS graft showed partial or complete healing after 21 days (Fig. 1). 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.

Conclusion

In this study, we examined the efficacy of freshly prepared porcine SIS grafts for 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 harvestable, 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.

References


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