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Repair of the Tympanic Membrane with Urinary Bladder Matrix

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Abstract

Objectives/Hypothesis—To test urinary bladder matrix (UBM) as a potential treatment for tympanic membrane (TM) healing and regeneration.

Study Design—This prospective pilot study was designed to provide both qualitative and semiquantitative assessment of temporal and spatial healing events in the chinchilla model of chronic TM perforations with and without UBM patching.

Methods—Bilateral myringotomies were performed and repeated as necessary to create subtotal perforations over an 8-week period. Myringoplasty was then performed, with left TMs serving as controls and right TMs receiving UBM patches. TMs were excised at 4 weeks, 8 weeks, and 12 weeks. Fixed tissue samples were characterized for gross morphology, then processed for microscopic evaluation.

Results—Chronic perforations were maintained with one or more repeated myringotomies. Although both control and patched TMs were thicker than native tissue, patched TMs were transparent and uniform in thickness without any inclusions. UBM patches were readily degraded and replaced by newly deposited and organized host tissue that recapitulated the native TM layers.

Conclusions—UBM scaffolds were an effective biological scaffold for TM closure and tissue remodeling, leading to thicker than normal anatomy but otherwise normal morphology. Future studies are required to determine functional and temporal outcomes as well as alternative patch orientations. The results show particular promise as a superior alternative means of reconstructing not only chronic TM perforations but also dimeric TMs associated with retraction pockets and atelectasis.

Keywords

Tympanic membrane; perforation; chinchilla; extracellular matrix; urinary bladder matrix; wound healing

INTRODUCTION

Tympanic membrane (TM) perforations in the pediatric population are a consequence of trauma, diseases, including otitis media (OM), and clinical treatments for OM, such as tympanostomy tubes (TTs). The TM is a thin, ovoid-shaped, semitransparent structure

composed of three layers.¹ A keratinizing squamous epithelium outer layer overlies a collagenous connective tissue layer bounded internally by a cuboidal mucosal epithelium. Closure of a TM perforation is driven by epithelial migration.² Clinically, a perforated TM is generally considered healed once the epithelium has formed. However, the resulting neomembrane, or monomeric membrane, lacks the fibrous middle layer,³ which may affect long-term functional outcomes. However, this term clearly misrepresents the histologic composition, as both the outer and inner layers are present; therefore, our group favors the term dimeric membrane. Current techniques most often result in a dimeric neomembrane that is acoustically less efficient, that is more vulnerable to forming a retraction pocket with the associated risk of cholesteatoma formation, and that is more susceptible to reperforation by even minor barotraumatic insults. Therefore, reconstructive approaches that reconstitute the integrity and resilience of the trilaminar structure of the native TM are still needed.

Rates of spontaneous TM closure are reported as high as 90% for trauma and middle ear disease^{4,5}; however, the development of chronic perforations as a consequence of TTs has been reported to be significant as well.^{6,7} Observation of TMs for spontaneous closure can average many months before surgery is considered.⁸ Perforations can lead to pain, increased susceptibility to infection, cholesteatoma formation, nonphysiologic gas exchange, and tympanosclerosis, and contribute to conductive hearing loss. Small central TM perforations that do not heal may be reconstructed with a paper patch myringoplasty; however, the efficacy of this technique is questionable.⁹ After myringoplasty failure, autologous graft material is often utilized to surgically reconstruct the TM. About 90% of tympanoplasties are successful¹⁰; however, reperforations can still occur.^{8,11} Reperforations can require multiple surgeries to reconstruct, which, in turn, may limit donor fascia in the operative field and can increase donor site morbidity.¹² Failure rates increase with a history of recurrent infections and multiple tympanoplasties.⁸ Graft failure and improper healing can compromise structure and thus normal function and lead to severe pathologic complications.¹⁰

New treatment methods for enhancing TM repair and healing include application of extracellular matrix components,¹³ acellular dermal grafts,¹⁴ and growth factors.¹⁵ Alternatively, scaffolds derived from selected xenogeneic extracellular matrix (ECM) provide a biocompatible material with inductive tissue remodeling properties for tissue engineering and regenerative medicine applications.¹⁶ ECM scaffolds act as templates for constructive and tissue-specific remodeling due to their composition of well-recognized structural and functional proteins. The aim of this pilot study was to test the efficacy of a specific ECM, urinary bladder matrix (UBM), that contains both stroma and a basement membrane, as a grafting alternative for TM reconstruction to achieve healing and regeneration of all three TM layers. The challenge in testing any material for TM repair is the use of an appropriate animal model since the vast majority of models have proven inadequate.¹⁷ The chinchilla animal model has been used successfully due to favorable anatomy.¹⁸ We utilized a modification of the original method for creating chronic TM perforations in chinchillas¹⁸ by using only the infolding technique without thermal injury to mimic the damage seen clinically as a result of trauma or TTs. Therefore, this pilot study was designed to assess the creation of chronic TM perforations in the chinchilla animal model in addition to exploring the utility of UBM as a potential material for TM repair.

MATERIALS AND METHODS

Preparation of UBM

The UBM was prepared as previously described.¹⁹ Briefly, porcine urinary bladders were harvested from market weight pigs (approximately 110 kg–130 kg). Residual external connective tissues were trimmed and all residual urine was removed by repeated tap water

washes. The urothelial layer was removed by soaking in 1 N saline. The tunica serosa, tunica muscularis externa, tunica submucosa, and most of the muscularis mucosa were mechanically delaminated. The remaining basement membrane of the tunica epithelialis mucosa and the subjacent tunica propria constitute the biological scaffold called UBM. The UBM was decellularized and disinfected by immersion in 0.1% peracetic acid, 4% ethanol, and 96% deionized water for 2 hours. The material was then washed twice and terminally sterilized with ethylene oxide. UBM patches, 50–100 μ m thick, were cut with dermal punches (4 mm–6 mm in diameter) large enough to cover the entire perforation. In some cases, slightly larger rectangular pieces were needed for the largest perforations. The UBM patches were hydrated with 0.9% sterile phosphate buffered saline (PBS) prior to use.

Perforation Model

Fourteen chinchillas (~500 g, Jim Ritterspack Chinchilla Ranch, Genoa, OH) were used in this study. All procedures complied with and were approved by the Children's Hospital of Pittsburgh Animal Research and Care Committee. Animals were anesthetized with ketamine (5.7 mg/kg), xylazine (0.14 mg/kg), and acepromazine (0.02 mg/kg) intramuscularly for all procedures (Webster Veterinary, Middletown, PA). Myringotomy was performed as previously described,¹⁸ but without thermal injury, on 11 animals (three additional animals were used for native TM tissue). The ear canal was sterilized with an iodine solution and then flushed several times with sterile 0.9% PBS. In order to otoscopically visualize the entire TM with a surgical microscope, a speculum was used to depress the bony ridge within the ear canal that partially blocks anterior exposure. A myringotomy knife was used to make an incision in both TMs, and an otologic hook was inserted and used to disrupt the mucosal layer on the medial aspect of the perforation edge. Flaps were then created by incising radially along the perforation edge and folded back to create a subtotal perforation of more than 50% of the pars tensa in the inferior region. These flaps were folded medially to appose the disrupted mucosal layer of the medial surface in order to establish the epithelial ridge found in chronically perforated TMs.

Over the course of the following 8 weeks the perforations were evaluated clinically, twice the 1st week then once a week using a surgical microscope to ensure that a stable hole was present without re-epithelialization, closure, or infection. If perforations were observed to be closing (i.e., either closed or small), the myringotomy procedure was repeated. In addition to otoscopic confirmation that the perforations remained patent, tympanometry was performed adjunctively (American Electro-medics, Amherst, NH) to corroborate our observations. Perforations were scored based on their size: none = 0, small = 1 (less than 25%), medium = 2 (25%–50%), and large = 3 (greater than 50%).

Initially, a prophylactic antibiotic (ampicillin) was given intramuscularly for the first 2 days after the first myringotomy; however, some animals experienced gastrointestinal distress. Therefore, repeated myringotomies were performed without prophylactic antibiotics. Animals were observed three to four times per week during the entire study for neurological signs of infection.

Myringoplasty

After the 8-week observation period, all the chinchillas used in the myringoplasty experiment had perforations between 25% and 75% of the pars tensa. The epithelial rim of each TM perforation was ablated to expose fresh tissue as would normally be performed clinically prior to patching. The left ears served as controls for normal wound healing. The right TM perforations were treated with the UBM patches placed with the basement membrane surface apposed to the superficial surface of the TM. The middle ear was not packed with Gelfoam, as described in other animal studies and performed clinically, in order

to avoid any interactions with the UBM patches. TMs were clinically evaluated weekly (twice the 1st week) under anesthesia for up to 12 weeks to document the healing process. Animals were euthanized at 4 weeks, 8 weeks, and 12 weeks; animals with patent left perforations were kept in the study until 12 weeks to allow for the maximum time for potential healing, which was a conservative choice with respect to UBM-treated right TM comparisons.

Tissue and Histological Analyses

Three animals were euthanized by intracardial injection of Euthasol (195 mg, Webster Veterinary) at 4 weeks, while four animals each were euthanized at 8 weeks and 12 weeks. In addition, three normal, untreated animals were used and their TMs recovered to serve as samples of normal tissue structure. Immediately after euthanasia, each bulla was excised and extraneous tissue trimmed with scissors. Each bulla was filled with and soaked in 10% formalin overnight, then the surrounding bone was trimmed away leaving the intact TM on the bony annulus. In order to document their gross morphology, the medial surface of the fixed TMs was imaged using a dissecting microscope (Olympus, Melville, NY), a digital camera (Diagnostic Instruments, Sterling Heights, MI), and MetaMorph morphometric imaging software (Universal Imaging, Downing-town, PA). The samples were then decalcified, marked with colored dye to label the anterior edge, and cut flat along the inferior edge for orientation purposes. After paraffin embedding, serial sections (7 μm) were cut at 500 μm intervals starting at the inferior edge of the TM. Sections were stained with hematoxylin and eosin and with Masson's trichrome for examination. Entire TM cross-sections were imaged using a dissecting microscope, and magnified views were obtained using a 40 $\times$ oil immersion objective with a light microscope (Zeiss, Thornwood, NY), a digital camera (QImaging, Surrey, Canada), and the imaging software. Samples chosen for analysis came from approximately the same transverse plane of the pars tensa. Area measurements, indicative of TM thickness, were performed by thresholding three to four successive images of the TMs and quantifying the thresholded area using a 4 $\times$ objective with a light microscope (Nikon, Melville, NY), digital camera, and the imaging software. These area measurements were then normalized to the mean values for native TMs.

STATISTICS

Since this was a pilot study, the n-values were low and statistical analyses were not employed. However, data are presented in both a qualitative and semiquantitative manner in order to present a timeline of events associated with perforation healing. Quantitative information is given as mean values.

RESULTS

Perforation Creation and Outcome

Perforations were initially created in both TMs of 11 animals following the modified infolding technique. As described for the original technique,¹⁸ we attempted to create four flaps to fold back onto the roughened mucosa. However, this proved technically challenging, and we typically created fewer than four flaps. After the initial procedure, only 32% of TMs (7 of 22) remained patent throughout the 8-week observation period (Fig. 1A). Thirty-six percent (8 of 22), 23% (5 of 22), and 9% (2 of 22) of the TMs required one, two, and three reperforations, respectively. A total of 14 and 10 reperforations were performed on the left and right TMs, respectively. Reperforations were performed when the holes were categorized as small (86% or 12 of 14 and 80% or 8 of 10 for the left and right TMs, respectively) or closed (14% or 2 of 14 and 20% or 2 of 10 for the left and right TMs, respectively). The average time until the first, second, and third reperforations were created

was approximately 4.4 weeks, 5.8 weeks, and 6 weeks for the left TMs and 4.1 weeks, 6 weeks, and 7 weeks for the right TMs (Fig. 1B). However, the need for subsequent reperforations decreased directly with the number of perforations (Fig. 1C).

After the first week, perforation size stabilized at between 25% and 50% of pars tensa area (Fig. 2A). Beginning in the 3rd week, many of the TMs were covered with a crust (e.g., eschar) indicating some degree of healing (Fig. 2B). If crusts were present, they were removed so the TMs could be visualized. When compared to the reperforation timeline, crust formation for both left and right TMs was delayed the most after the initial procedure (3 weeks) but occurred more frequently after successive reperforations peaking at 5 weeks and 8 weeks. However, crust formation did not directly correlate with complete healing (i.e., perforation closure). No signs of infection were noted.

Repair With UBM Patches

At 8 weeks, all TMs had perforations that varied across the size range (from small to large) that were patched. In two animals, the myringoplasties were repeated the next day because the UBM patches detached from the TMs and were found in the external auditory canal. One week after the myringoplasty, the TMs appeared yellowish-white and were either dry or moist. In some cases, the UBM patches medialized into the perforations and resembled a pie crust, with a central depression. A ridge or edge was also noticeable in the adjacent external auditory canal of a few animals due to a slight shift in the position of the UBM patches. After the 2nd week, crusts were observed on all patched TMs (Fig. 3). The majority of control TMs also developed crusts by 3 weeks to 4 weeks. The crust of one patched TM at the 4-week time point was removed prior to tissue excision. Unfortunately, the crust was attached to the TM and led to injury and creation of a perforation. Therefore, crusts were not removed subsequently to avoid further injury to the TMs during the healing process, but they were gently manipulated when appropriate to assess TM closure and healing. The persistence of crusts in the following weeks varied, but more crusts were evident on patched TMs over the entire 12-week period. None of the patched TMs had perforations after the patches were applied. Seven of the 11 control TMs were closed by 4 weeks. The four other control TMs retained perforations (one small, two medium, and one large) throughout the 12-week period.

Gross Morphology

Animals were sacrificed at 4 weeks, 8 weeks, and 12 weeks based on the degree of closure of the control TM perforations. Of the seven control TMs that healed at 4 weeks, three and four animals were euthanized at 4 weeks and 8 weeks, respectively; the remaining four animals were euthanized at 12 weeks. Gross morphologic examination of the intact TMs supported the clinical observations of TM integrity, with one exception in which a very small hole was found in one of the TMs at 12 weeks that had originally had a large perforation. Upon inspection of the entire excised TMs, the area of damage from the myringotomy procedures and location of perforations and/or healing was found mainly in the inferior region of the pars tensa with some anterior quadrant bias (Fig. 4). Qualitatively, patched TMs were transparent and resembled native tissue. Controls appeared much less clear with areas that looked thickened with white patches suggestive of myringosclerosis. In two cases (4 weeks and 12 weeks), the patched TMs had perforations in the pars flaccida adjacent to the malleus, a region that was not injured or treated.

Thickness and Histological Analyses

Histological examination of the TM cross-sections revealed profound differences in tissue morphology (Fig. 5). Overall, both control and patched TMs were thicker than native tissue. However, patched TMs appeared more uniform in thickness throughout the section, while

control TMs were more variable. In many cases, thickening was more prominent in the inferior/anterior region. In order to quantify changes in TM thickness, the entire area of the TM cross-section was measured at the same bullar depth (i.e., similar TM lengths measured to be $7.91 \text{ mm} \pm 0.41 \text{ mm}$). When normalized to native tissue, the area of the TMs was larger for the patched group indicating thicker TMs (Fig. 6).

At higher magnification, these differences in thickness resulted from changes in collagen content of the middle connective tissue layer (Fig. 7). At 4 weeks, the control TMs had less collagen that appeared disorganized. At 8 and 12 weeks, the collagen in the control TMs appeared thicker and more organized than the 4-week time point. In contrast, the patched TMs had a fibrous, uniform layer of well-organized collagen starting at 4 weeks that appeared to thicken over time. In addition, a thicker keratinized layer was also noted in the patched TMs starting at 4 weeks. In both experimental groups, hypercellularity was seen as evidenced by the influx of round, mononuclear cells at 4 weeks, but the amount of cellularity was greater in the patched TMs. By 8 weeks, the cellularity had decreased but still remained higher than that of native tissue. The cells had a spindle-shaped morphology consistent with fibroblasts in both groups. Hyperplasia of the epithelial layers was seen for the control and patched TMs. Epithelial cells were seen on both sides of the newly deposited collagenous middle layer, and in the case of the 12-week controls, the epithelial cells grew around the edge of the perforation. In the patched TMs, the epithelial cells on the external auditory canal side were flattened, whereas those on the mucosal side were more cuboidal in shape and showed structures resembling cilia on their surface. Residual UBM was not evident at any of the time points, and attachment sites between the implanted ECM and adjacent native tissue were not recognizable. The control TMs had irregular regions containing small inclusions and fibrous nodules which contributed to their variable thicknesses (Fig. 8). Both groups lacked evidence of inflammation, increased vascularity, or neutrophils and macrophages.

DISCUSSION

The infolding technique used to create chronic perforations is thought to only work in chinchillas¹⁷ and is most successful with the use of a thermal technique.¹⁸ Although thermal injury to the TM does create a chronic perforation, the histology of the perforated edge differs from that seen clinically.¹⁷ Given that we did not utilize the thermal technique, but a modification used successfully by others,²⁰ we were only able to create two or three flaps instead of four because the TMs quickly lost tensile strength after the first incision and consequently became difficult to further manipulate. These variations may have contributed to our decreased incidence of chronic perforation but reflect the clinical situation since the majority of chronic perforations occur in the inferior/anterior quadrant of the pars tensa.

Our results indicated that only 32% of TMs remained perforated during the 8-week observation period. While many other studies have used shorter time frames, 8 weeks has been suggested as the minimum duration for a perforation to be characterized as chronic.¹⁷ Although we were not able to create a chronic perforation using a single myringotomy in the majority of TMs, and reperforation was required to maintain a patent hole, the frequency at which successive procedures were required decreased substantially over time. Clinically, repeated injuries to the TM and successive failures associated with surgical procedures can lead to chronic cases.⁸ Multiple sets of TTs are commonly associated with an increased incidence of nonhealing TM perforations. Therefore, repetitive myringotomy utilizing this modified procedure may produce a chronic perforation over a longer time period if reperforation is routinely performed.

In an attempt to characterize the natural healing process, we evaluated the TMs clinically every week, and on average, were able to maintain medium-sized perforations. The presence of crusts was highly variable, but did occur more rapidly after each successive reperforation (approximately 1 week after each additional procedure), which is consistent with a reset of the wound healing response. However, since the frequency of reperforations diminished over time, this suggests that the healing response was being modified each time the TM was injured.

We evaluated the efficacy of the UBM scaffold for its ability to stimulate healing and regeneration of the TM layers. The UBM scaffold is a resorbable, temporary, inductive template for tissue remodeling, providing both the structural support and signaling molecules crucial for inducing tissue repair.¹⁶ These characteristics make ECMs distinct from other sources used for TM repair. To our knowledge, only one other study has utilized an ECM, small intestinal submucosa (SIS), for TM perforation repair.²⁰ Although SIS and UBM share many characteristics in the process of tissue remodeling, UBM is thought to provide an advantage in terms of epithelial cell repopulation due to the presence of an intact basement membrane.²¹

Clinical evaluation of the TMs after UBM application revealed that the majority of patches remained in place and perforations were no longer evident. As a simple approximation of healing, crust presence on the TMs was noted and found to be similar between patched and control TMs, although they did persist longer on the patched TMs. These slight differences could be a result of variations in individual animal healing or simply be a result of the crust detaching from the TM and falling out of the external auditory canal.

Our clinical observations were supported by gross and histological analyses. The one exception was the presence of a very small hole in a treated TM at 12 weeks that may not have been covered by the UBM patch due to its location in the center of the TM. Upon gross morphological analysis of the intact TMs, patched TMs appeared more transparent than the healed control TMs and resembled native tissue in overall appearance. TMs patched with SIS have also been shown to be translucent, but in contrast to our findings, showed evidence of mild calcifications.²⁰ The control TMs were opaque in some areas and contained white patches similar to tympanosclerotic plaques. Although these perforations were not deemed truly chronic, the presence of plaque-like inclusions suggests that the TMs underwent substantial trauma leading to a more pathological outcome from our perforation procedure.

Histological analysis showed that patched TMs were thicker and contained better organized collagen than the control TMs despite the fact that they appeared more translucent. In addition, fibrous nodules were found in the control TMs, which is consistent with plaque formation. Histology did not reveal any identifiable remnants of the UBM patches at any of the reported time points. Nodules were not observed in the histological sections of patched TMs despite greater cellularity than the control TMs. The presence of mononuclear cells in response to the UBM is typical of tissue healing responses to ECMs.¹⁶ A thicker keratinized layer and hyperplasia were also found much earlier and were more pronounced in the patched TMs. The formation of excess keratin is a normal product of TM wound healing and acts as a scaffold for epithelial migration.¹⁷ Ciliated epithelial cells, which are found on the mucosal side of normal chinchilla TMs,²² were noted on the mucosal side of the patched TMs but not observed on the control TMs. This response in the patched TMs to the UBM is similar to the response observed when UBM scaffolds were used to reconstruct the trachea, another tissue that normally is populated by ciliated epithelial cells.²³ Although patching with SIS utilizing an underlay technique has also resulted in healed TMs with a trilaminar structure,²⁰ noticeable inflammation was present as well as residual SIS which is in contrast to our findings with UBM. Therefore, UBM may represent a better ECM scaffold for TM

repair, and the structure and composition of the basement membrane may play a role in this favorable healing response.

One concern of the results of the present study is the thickness of the organized connective tissue layer that resulted from the use of the UBM patches. Although the gross morphology of the treated TMs appeared similar to the native tissue, and is consistent with the typical response to ECM scaffolds that are not chemically cross-linked, the thick, organized connective tissue layer that resulted may impact hearing function. Acoustic evaluation using tympanometry was not performed on the ears after UBM application since the time course of healing was unknown. Cross-sectional areas of ECM-treated medial collateral ligament defects have been shown to be significantly greater at 12 weeks, but subsequently decrease in size approximating sham treatments at 26 weeks.²⁴ Therefore, longer time points may be required to fully evaluate the efficacy of the UBM scaffold as a material for repairing and regenerating a functional TM similar to native tissue. On the other hand, there may be distinct advantages to a reconstructive outcome that results in a thicker TM. A more pronounced connective tissue framework is less likely to form a retraction pocket or cholesteatoma and may maintain a more functional vibratory middle ear resulting in improved hearing despite a thicker TM with reduced admittance. Therefore, this alternative method may be a viable means of reconstructing the TM and a treatment option for a healed TM composed only of a neomembrane that has been complicated by these conditions. Other future considerations include the orientation of the UBM patches relative to the basement membrane as well as surgical techniques for patch placement.

CONCLUSION

Although this study was limited by the number of animals utilized and evaluated at each time point, the results do suggest several important conclusions. The study was designed to evaluate the use of the chinchilla model for chronic TM perforations and the efficacy of UBM as a patch material for repair. In order to fully evaluate the perforation and healing process, we employed both qualitative and semiquantitative analyses to characterize our outcomes. The use of an ECM scaffold clearly altered the cellular repair response and appears to be an effective template for TM reconstruction. UBM patches were readily integrated into the tissue and degraded completely, which led to accelerated tissue remodeling and reconstruction of the three layers of the TM, including the dense connective tissue stroma. Further studies are required in order to assess long term changes in TM structure as well as functional outcomes relative to the mechanics of the TM and proper hearing. The outcome of this study shows particular promise as a superior alternative means of reconstructing not only chronic perforations of the TM but also dimeric TMs associated with retraction pockets and atelectasis.

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Fig. 1.

(A) Number of TMs that required re-perforations, (B) the average time until each re-perforation, and (C) frequency for successive re-perforations for left and right TMs through the 8 week perforation period. As time progressed, the number of TMs requiring re-perforations decreased. Values represent means based on $n = 11$ for left and right TMs.

Fig. 2.

(A) Average perforation size and (B) crust removal as an indicator of healing for left and right TMs through the 8 week perforation period. On average, perforations were maintained at a medium size despite indicators of healing. Perforations were scored based on their size: none = 0, small = 1 (less than 25%), medium = 2 (25% to 50%), and large = 3 (greater than 50%). Values represent means based on n = 11 for left and right TMs.

Fig. 3. Crust presence as an indicator of healing through the 12-week healing period for controls (left) and tympanic membranes (TMs) treated with urinary bladder matrix (UBM) patches (right). Crusts persisted on more of the right TMs after treatment with UBM patches with no perforations present. Values represent means based on $n = 11$ for left and right TMs.

Fig. 4.

Examples of gross morphology of intact excised tympanic membranes (TMs) at 4 weeks, 8 weeks, and 12 weeks for controls (left) and TMs treated with urinary bladder matrix (UBM) patches (right). TMs treated with UBM patches appeared more uniform and transparent. TMs were visualized medially with a dissecting microscope. S = superior, I = inferior, P = posterior, and A = anterior regions of the TMs. Scale bar represents 5 mm, and dotted circles represent perforation areas without (left) and with (right) UBM patches.

Fig. 5.

Representative histological sections (hematoxylin and eosin stained) of control (left) and tympanic membranes TMs treated with urinary bladder matrix (UBM) patches (right) at 4 weeks, 8 weeks, and 12 weeks. TMs treated with UBM patches exhibit larger but more uniform thicknesses across the tissue. Entire TM cross-sections were imaged with a dissecting microscope. M = mucosal side, E = squamous epithelial side, P = posterior region, and A = anterior region of the TMs. Scale bar represents 5 mm.

Fig. 6.

Thickness of control tympanic membranes (TMs) (left) are less than TMs treated with urinary bladder matrix patches (right) at 4 weeks, 8 weeks, and 12 weeks. TM cross-sections were visualized with a 4× objective with light microscopy, and successive images were quantified based on thresholding the entire TM areas at a constant length ($7.91 \text{ mm} \pm 0.41 \text{ mm}$) in successive images. Values were normalized to left and right native TMs of $n = 2$ and $n = 3$, respectively; for $n = 2$ and $n = 3$ left and right TMs at 4 weeks, respectively; for $n = 4$ for both left and right TMs at 8 weeks; and $n = 2$ for right TMs at 12 weeks (left TMs were not evaluated since all of them had perforations at 12 weeks).

Fig. 7.

Representative histological sections stained with Masson's trichrome of control tympanic membranes (TMs) (left) and TMs treated with urinary bladder matrix (UBM) patches (right) at 4 weeks, 8 weeks, and 12 weeks. TMs treated with UBM patches were thicker and exhibited more organized collagen and showed a layer of ciliated cells at 12 weeks (arrow). The epithelium of the control TMs at 12 weeks had grown around the edge of the perforation instead of closing. TMs were visualized with a 40× oil immersion objective with light microscopy. M = mucosal side, E = squamous epithelial side, P = posterior region, and A = anterior region of the TMs. Scale bar represents 40 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 8. Control tympanic membranes (TMs) exhibited (A) small inclusions and (B) fibrous nodules (arrows) that were not evident in TMs treated with urinary bladder matrix patches. TMs were visualized with a 40× oil immersion objective with light microscopy. M = mucosal side, E = squamous epithelial side, P = posterior region, and A = anterior region of the TMs. Scale bar represents 40 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]