Preoperative Factors Affecting Tympanic Membrane Regeneration Therapy Using an Atelocollagen and Basic Fibroblast Growth Factor

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IMPORTANCE The use of growth factors to achieve closure of perforated tympanic membranes (TMs) has recently become popular. However, preoperative factors affecting treatment outcomes have seldom been discussed.

OBJECTIVE To evaluate preoperative factors contributing to the success or failure of healing of perforated TMs.

DESIGN, SETTING, AND PARTICIPANTS Retrospective cohort study of 153 patients (48 males and 105 females) in whom the duration of perforation was longer than 6 months prior to treatment and who were observed for at least 1 year after treatment between July 2009 and June 2012. Eight factors considered likely to affect the outcome of perforation closure were statistically evaluated using multivariate logistic regression analysis.

INTERVENTIONS Each perforated TM was filled with a synthetic graft material (atelocollagen sponge and silicone membrane) containing human basic fibroblast growth factor to promote wound healing after TM perforation closure.

MAIN OUTCOMES AND MEASURES Complete closure vs residual perforation.

RESULTS After 1 year of follow-up, 101 patients (66.0%) achieved complete closure, 30 patients (19.6%) had residual pinhole perforations (<1 mm diameter), and 22 patients (14.4%) had larger residual perforations. Multivariate logistic regression analysis adjusted for each explanatory variable identified a TM without calcification (odds ratio [OR], 2.68 [95% CI, 1.17-6.15]; P = .03) and a perforation not involving the tympanic annulus (odds ratio, 2.75 [95% CI, 1.09-6.94]; P = .04) as significant. Insignificant factors included perforation margin identified on microscopy (OR, 0.24 [95% CI, 0.99-6.27]; P < .001), perforation margin without epithelial migration (OR, 7.27 [95% CI, 0.66-80.49]; P = .11), absence of preoperative otorrhea (P = .38), no previous ear operations (P = .82), perforation size (P = .14), and patient age (P = .26).

CONCLUSIONS AND RELEVANCE Tympanic membrane regeneration therapy can be applied to all patients, except those with cholesteatoma or malignant neoplasm. However, patients with severe calcification of the TM and those with marginal perforations close to the fibrous annulus should be treated more prudently to achieve perforation closure.
Minimally invasive ambulatory surgical procedures, using growth factors such as autologous serum eardrops and basic fibroblast growth factor (BFGF), have recently been developed in efforts to achieve closure of perforated tympanic membranes (TMs). Such approaches are effective even when perforations are large because the regenerative activity of the TM is reduced at the perforation margins. However, preoperative factors affecting therapy outcomes remain controversial. Commencing in 2000, we have used an atelocollagen and BFGF to promote regeneration of the TM and close perforations. Herein, we provide the details of our treatment and discuss preoperative factors affecting closure outcomes.

Methods

Candidates for Tympanic Membrane Regeneration Therapy

The study was approved by the ethics committee of Ehime University Hospital and was applied only to patients who provided written informed consent. Among the 214 patients who presented to the outpatient clinic for TM regeneration at the otorhinolaryngology department of Ehime University Hospital between July 2009 and June 2012 and underwent TM regeneration therapy using an atelocollagen and silicone membrane and BFGF, 153 patients (48 males and 105 females) in whom the duration of perforation was longer than 6 months prior to treatment and who were observed for at least 1 year after treatment were included in the study. The patients’ ages ranged from 13 to 90 years, with a mean (SD) age of 64.9 (15.1) years. We believe that patients should be at least 10 years old at the time of operation and explained our methods, including local anesthesia, to the patient and their family. If the patient and their family wished and consented, we attempted the treatment. No patient was unable to tolerate the procedure.

Candidates for TM regeneration therapy were selected on the basis of their medical history and microscopic or fibroscopic findings of the TM. Fiberscopy allowed us to clearly identify marginal perforations, even when this was not possible microscopically. Patients with clear evidence of secondary cholesteatoma arising from the perforation margin (as evident fibrosically or microscopically), or malignant neoplasm of the middle ear, were excluded. However, patients with marginal perforations (evident microscopically) were not excluded.

Patients with otorrhea on initial examination were not excluded. If otorrhea was evident at the initial examination, we sought to treat the condition and temporarily dry the TM using appropriate treatment (eg, antibiotics). We usually irrigated the middle ear with saline 2 to 3 times per week and cleaned the ear canal using Burow or pyoktanin solution for at least 3 months before starting regenerative therapy. Such therapy was given even if otorrhea persisted, provided that computed tomography (CT) yielded no evidence of excessive formation of granulation tissue in the middle ear.

Computed tomography scanning was performed in all patients with otorrhea, thickening of the middle ear mucosa, or migration of part of the perforation margin to the back of the TM. If CT revealed a suspected shadow around the ossicles, at the back of the TM, or in the tympanic attic, that patient was excluded. Also, those exhibiting poor aeration of the mastoid antrum, suggestive of excessive formation of granulation tissue in the middle ear or secondary cholesteatoma arising from the perforation margin, were excluded and were advised to undergo tympanoplasty. The preoperative factors were determined in each patient using TM photographic records reviewed for all patients for the purposes of the study.

Tympanic Membrane Regeneration Therapy Procedure

In this procedure, a perforated TM is filled with a synthetic graft material (atelocollagen sponge and silicone membrane; Terudermis, Olympus Terumo Biomaterials) to which human fibroblast growth factor is applied to promote wound healing (BFGF preparation; Fibrast Spray, Kaken Pharma Co). The details are as follows:

1. A small cotton ball soaked in anesthetic solution (lidocaine hydrochloride, 4%) is placed on the margin of the perforated TM for approximately 20 minutes to achieve surface anesthesia.

2. The perforation margin is circumferentially dissected with a sharp pick to expose fresh tissue.

3. The atelocollagen membrane is trimmed to an appropriate size with scissors and forceps and inserted into the perforation, making sure that the silicone membrane faces outward and no gap remains. During this step, the silicone membrane should be trimmed into a circle slightly larger than the perforation to allow the membrane to tightly fit the TM and so that the atelocollagen membrane can be immobilized.

4. Using a long thin needle, 0.1 to 0.2 mL of BFGF solution (100 µg/mL) is applied from a gap between the silicone membrane and the perforation onto the atelocollagen membrane to complete the procedure. The entire procedure is completed in approximately 30 minutes. The silicone membrane is removed at a follow-up visit 2 to 3 weeks after the procedure. If a perforation remains, steps 1 to 4 are repeated until the perforation is completely closed.

Evaluation of the Outcome of Tympanic Membrane Closure and Identification of Factors Affecting the Outcome

The outcome of TM closure was evaluated on the basis of the condition of the TM at least 1 year postoperative follow-up. The effects of 8 factors considered likely to affect the outcome of perforation closure were statistically evaluated by multivariate logistic regression analysis: (1) identifiable perforation margin, (2) perforation margin without epithelial migration, (3) TM calcification, (4) central perforation, (5) otorrhea, (6) prior ear operation, (7) perforation size, and (8) age. Logistic regression analysis was performed with residual perforation as the objective variable and the 8 factors as the explanatory variables. All variables were analyzed as Boolean variables of “Yes” (1) and “No” (0). Differences were deemed to be statistically significant for P values less than .05. The 8 factors that might produce a better outcome are detailed as follows:

1. Perforation margin identified on microscopy: patients without markedly curved ear canals such that the entire circum-
ference of the TM perforation margin could be viewed microscopically (Figure, A).

2. Perforation margin without epithelial migration: patients in whom the perforation margin had not partially migrated to
the back of the TM but with no CT evidence of secondary cholesteatoma.

3. Tympanic membrane without calcification: patients without marked calcification of the remaining TM involving 2 or more quadrants (Figure, B).

4. Central perforation: patients with central perforation not too close to the fibrous annulus (Figure, C).

5. No preoperative otorrhea: patients not found to have otorrhea at the initial examination or who were found to have otorrhea but who were effectively treated with antibiotics chosen via sensitivity testing, middle ear irrigation, and ear canal cleaning.

6. No prior ear operation: patients who had not undergone ear operations, such as myringoplasty (including the simplified method) and tympanoplasty.

7. Small or medium perforation: patients with a small or medium perforation of the TM involving only 1 or 2 quadrants (Figure, D).

8. Not old age: patients younger than 60 years.

Results

Outcome of Closure of Perforated Tympanic Membrane
The causes of the perforated TM in the 153 patients in whom the duration of perforation was longer than 6 months prior to treatment and who were observed for at least 1 year after treatment included chronic otitis media in 98 patients, traumatic perforation in 13 patients, perforation following myringotomy or tube placement in 19 patients, and reperforation following tympanoplasty in 14 patients. Nine of the 153 patients developed reperforation more than 1 year after TM perforation closure by regeneration therapy and underwent reoperation. The mean (range) duration of perforation prior to treatment was 32.0 years (6 months to 83 years).

Small, medium, and large perforations, defined as those involving 1, 2, and 3 or more quadrants of the TM, respectively, were found in 73 (47.7%), 55 (35.9%), and 25 (16.3%) patients before treatment.

Seventeen patients were recognized as having otorrhea at the first visit. We applied ear treatments, as mentioned, for at least 8 weeks. In 8 patients, the otorrhea stopped, whereas 9 patients underwent regenerative therapy despite the presence of otorrhea. The mean time it took for the ear treatment to heal the otorrhea was 3.9 weeks.

At 3 months after treatment, 129 patients (84.3%) achieved complete closure, 9 patients (5.9%) were found to have perforations smaller than 1 mm in diameter (residual pinhole perforation), and 15 patients (9.8%) were found to have perforations of 1 mm or larger in diameter (larger residual perforation). In the latest evaluation performed 1 year after operation, 101 patients (66.0%) achieved complete closure, 30 patients (19.6%) had residual pinhole perforations, and 22 patients (14.4%) had larger residual perforations.

Of the 101 patients who achieved complete closure, 52, 37, and 12 patients had small, medium, and large perforations before operation, with a closure rate of 71% (52 of 73), 67% (37 of 55), and 48% (12 of 25), respectively. When stratified by pre-treatment perforation size, the mean number of operations to complete closure was 1.3, 1.4, and 1.8 in patients with small, medium, and large perforations, respectively.

Of the 30 patients with residual pinhole perforations, the mean (SD) improvement in hearing was 8.9 (7.9) dB, which was not significantly different from that in the complete closure group (t test). During 24 months of postoperative follow-up, pinhole perforations developed a mean (SD) of 7.5 (8.5) months after the regeneration therapy. Patients with residual pinhole perforations were observed every 3 to 4 months on an outpatient basis and were advised to undergo a second operation if enlargement of the pinhole perforations and worsening of hearing were observed. At last follow-up, 4 patients with pinhole perforations were found to have progressed to small perforations and had undergone a second session of regeneration therapy. Of the 22 patients with residual perforations, during 21 months of posttreatment follow-up, reperforations developed a mean (SD) of 2.3 (4.2) months after the regeneration therapy.

Postoperative epithelial pearl formation in the TM was observed in 6 patients (3.9%) at the dissected perforation margin in all cases.

Relationship Between Preoperative Factors Likely to Affect Closure Outcome and the Rate of Complete Closure or Residual Perforation
The Table summarizes the 8 factors likely to affect the outcome of perforation closure and the rate of complete closure or residual perforation after at least 1 year of follow-up. Logistic regression analysis adjusted for each explanatory variable identified a TM without calcification (odds ratio, 2.68 [95% CI, 1.17-6.15]; P = .03) and a perforation not involving the tympanic annulus (odds ratio, 2.75 [95% CI,1.09-6.94]; P = .04) as significant (Table).

Discussion
Regeneration therapy based on the novel concept of tissue engineering—creating new tissue in situ—is referred to as in situ tissue engineering.7 Based on this concept, 3 factors, including growth factors, scaffolds, and new cells, are required to produce new tissue. Basic fibroblast growth factor, a growth factor that was identified following the identification of epidermal growth factor, is known to directly promote the proliferation of vascular endothelial cells and fibroblasts via its receptor and formation of well-vascularized granulation tissue in vivo.8 Basic fibroblast growth factor has been used by dermatologists for the treatment of pressure sores,9 ulcers,10 and burns.11 Recent advances in the development of scaffold materials for in situ tissue engineering include the development of synthetic graft materials with lower antigenicity and high biocompatibility. Collagen is highly compatible with surrounding tissue, can serve as a scaffold for newborn cells and tissues, and is eventually absorbed12; consequently, it is considered an excellent synthetic graft material for perforation closure.13 The addition of BFGF,7 a growth factor known to help wound healing by acting on fibroblasts and vascular endothelial cells to promote epithelial pearl formation in the TM was observed in 6 patients (3.9%) at the dissected perforation margin in all cases.
promote neovascularization, proliferation of granulation tissue, and reepithelialization\(^a\) to atelocollagen, is expected to improve the rate of successful closure of perforated TMs and extend the indications of the treatment.\(^{1,4,15-17}\) Lou et al.\(^2\) and Zhang and Lou\(^4\) reported that direct application of BFGF improved healing and shortened the closure time of acute traumatic perforations. We performed regenerative therapy on patients with chronic cases in which the duration of perforation prior to treatment was longer than 6 months. We suggest that mechanical disruption of the edge of the perforation, and the scaffold for newly formed cells and tissues, is required to stimulate the activity of TM stem cells.\(^3\) This is because the outer squamous epithelium around the edge of a chronic perforation prevents TM closure.\(^18\) In our previous study reported in 2003,\(^5\) we compared the outcome of 9 patients treated with atelocollagen and silicone membrane plus BFGF therapy with the outcome of 5 patients treated with saline for chronic otitis media and achieved a 100% closure rate with the addition of BFGF, whereas the closure rate was only 40% with the saline-only treatment. We also reported the outcome of 87 patients treated using the same procedure and found that the proportions of patients with complete closure, residual pinhole perforation, and larger residual perforation were 92%, 6%, and 2%, respectively.\(^6\) In the present study, the rate of complete closure, residual pinhole perforation, and larger residual perforation was 66.0%, 19.6%, and 14.4% after at least 1 year of postoperative follow-up, indicating a poorer outcome compared with the previous study. Two possible reasons for the poorer treatment outcome in the present study are suggested. One is that patients who would have been considered inappropriate candidates for TM regenerative therapy in the previous study were allowed to undergo the therapy if they wished and consented to do so after being informed of the challenging situation. Another reason may depend on the difference in the follow-up period after treatment. In the initial study, not all patients were observed for longer than 1 year. In this study, complete closure was achieved in 101 patients (66.0%) after at least 1 year of postoperative follow-up, although 129 (84.3%) had achieved complete closure 3 months after the operation. Twenty-eight complete closure cases worsened to pinhole perforation or larger residual perforation with 1 year of postoperative follow-up. If we defined the observation period after treatment to be longer, the success rate would be worse. Of the 214 patients who presented to the outpatient clinic for TM regeneration, 61 were not enrolled because they were lost to follow-up for at least 1 year after treatment or clinical information was missing. Furthermore, the final decision on the choice of TM regeneration therapy or other primary treatments depended on the patients’ wishes after they were informed about each method. Thus, there was likely to have been selection bias in this study.

Logistic regression analysis of factors likely to affect the outcomes of perforated TM closure revealed significant differences between patients with and without calcification of the TM and marginal perforations, which reduce blood supply to the site of TM regeneration\(^{19}\) and thus to the residual TM.
turn, the supply of new cells, essential for in situ tissue engineering, is inadequate, increasing the incidence of residual perforation. The odds ratio showed that patients with marginal perforations were at approximately a 4.1-fold higher risk of residual perforation than those with central perforations. The 105 patients who achieved complete closure after at least 1 year of postoperative follow-up consisted of 71%, 67%, and 48% of the patients who had small, medium, and large perforations before operation, respectively, demonstrating that patients with a smaller perforation achieved a higher closure rate. The closure rate for regenerative therapy is certainly higher than that for a paper patch in large perforations (48% vs 12%) but was not significantly better for small perforations (71% vs 63%). Certainly, obtaining closure in half of large perforations without surgery is worthwhile.

However, the statistical results suggested a limited effect of preoperative perforation size on the outcome of regenerative therapy and instead identified the location of perforation (ie, marginal or central perforation) as a more important predictor of therapy outcome. Kim et al found no significant effect of preoperative perforation size on the closure rate after fat-graft myringoplasty. Perforation closure is more difficult to achieve in patients with a marginal perforation, even when the perforation itself is small or medium sized, than in patients with a large perforation whose margin can be completely identified. It is thus likely that the outcome of TM regeneration therapy can be predicted by comprehensive examination of the perforation, including location of the perforation, condition of the perforation margin, and the presence or absence of calcification of the remaining TM. However, additional studies with a larger number of patients are needed to more precisely identify relevant factors.

Postoperative epithelial pearl formation was reported in our previous study. In the present study, epithelial pearl formation in the TM was observed in 6 patients (9%), in each case at the dissected perforation margin, suggesting that the lesion arose from the residual epithelium. Given that the incidence of postoperative epithelial pearl formation after overlay myringoplasty is 2.5% to 7.0%, it seems unlikely that the regeneration therapy induced epithelial pearl formation.

### Conclusions

Tympanic membrane regeneration therapy can be applied to all patients except those with cholesteatoma or cancer. However, in patients with severe calcification of the TM and those with marginal perforations close to the fibrous annulus, regenerative therapy should be given prudently to achieve perforation closure, with patient consent being given only after detailed explanation of the difficulties that might be encountered.


