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Application of Amniotic Membrane for Covering Mastoid Cavity in Canal Wall Down Mastoidectomy

Mohammad Faramarzi, MD; Reza Kaboodkhani, MD; Sareh Roosta, MSc, MA; Negar Azarpira, MD; Mahmood Shishegar, MD; Hajar Bahranifard, MD

Objective: Prevention of granulation tissue formation and acceleration of epithelialization of the mastoid cavity in canal wall down (CWD) mastoidectomy by use of amniotic membrane (AM) as a biologic dressing.

Study Design: Prospective and randomized study.

Methods: During CWD mastoidectomy, an inferiorly base musculoperiosteal flap was rotated into the cavity. In order to coverage of this flap, the AM (75 ears) or the temporalis fascia (control group, 73 ears) was used. The times for mastoid cavity epithelialization were compared in both groups.

Results: In the AM group, duration of complete epithelialization of the cavity was $41.4 \pm 7.7$ days, whereas in the control group it was $59.2 \pm 9.1$ days. Duration of time for complete epithelialization in the AM group was shorter than in the control group, which was significant ($P < 0.0001$).

Conclusion: The use of AM in CWD mastoidectomy is beneficial in minimizing postoperative epithelialization time.

Key Words: Chronic otitis media, amniotic membrane, epithelialization, obliteration, canal wall down mastoidectomy.

Level of Evidence: 1b

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INTRODUCTION

Canal wall down (CWD) is one of the common procedures for chronic suppurative otitis media with cholesteatoma. However, in patients who undergo this procedure, cavity problem is a long-term health issue. In other words, the volume of mastoid cavity that becomes larger, it is slowly covered by epithelium. Therefore, the nonepithelialized denuded area develops granulation tissue, foul smelling discharge, and crusty debris. These are the most common reasons for postoperative visits and life-long referral to the otologist’s office. Three types of tissue may develop after CWD mastoidectomy: keratinized squamous epithelium, ciliated columnar respiratory epithelium, or granulation tissue. Due to probable bacterial infection, achieving a dry cavity is more difficult in the respiratory epithelium. Some researchers reported a cavity problem rate of 20% to 60%.

In order to eliminate open cavity problems, reducing the mastoid cavity volume and/or covering the denuded area is a critical factor to prevent granulation tissue and accelerate epithelialization. Clinical studies have shown that the most common free graft used to cover mastoid cavity is temporalis fascia. Despite its acknowledged application, fascia grafts are inadequate in size to cover all areas of the mastoid cavity. In addition, random flaps are the most common soft tissue flaps used for mastoid obliteration. Overall, the procedure of choice for mastoid obliteration is a controversial issue among otolaryngologists. Therefore, otolaryngologists need to acquaint themselves with various mastoid obliteration options to facilitate the healing process in a variety of clinical situations.

However, with respect to speeding up the healing process in reconstructive surgery, a growing body of literature recognizes the importance of human amniotic membrane (AM) in various fields such as plastic surgery, oropharyngeal surgery, ophthalmology, maxillofacial surgery, gynecology, dentistry, and neurosurgery.

The human AM has recently emerged as a potential acceleration tool in the healing process. These are the amniotic epithelial and mesenchymal cells mainly comprised of several growth factors and cytokines. The growth factors stimulate epithelialization through proliferation, migration, and differentiation of epithelial cells. Also, the AM has antibacterial, anti-inflammatory, immunomodulatory, plasticity, and analgesic characteristics. Furthermore, it acts as a biological wound dressing and biological scaffolds that regulates cell migration.

In otolaryngology, it is used to repair postlaryngectomy pharyngocutaneous fistula, for reconstruction of mandibular defects, for dorsal nasal and soft tissue augmentation in rhinoplasty, and for repair of oral mucosa and auricular cartilage.

In the otology domain, the first reference to this subject was made by Sugar in 1944. He used the dehydrated form of AM to cover the mastoid cavity. Sugar called the
AM aminoplasin and reported 24 cases of modified radical or radical mastoidectomy. During the first stage, the cavity was packed with iodoform paraffin gauze for 7 to 12 days. During the second stage, through a postauricular approach a flexible stent wrapped in aminoplasin was inserted into the cavity for 10 days and then was pulled out through the meatus. Sugar achieved a 75% favorable dry cavity, in a two-stage operation, albeit by applying iodine-boric powder. Overall, only a few studies have focused on the role of AM in sealing of the oval window, grafting of the tympanic membrane, and lining of the tympanic or mastoid cavity but without obliteration. 12,27–36

To best of our knowledge, no study has surveyed the effect of AM on the healing process in cases with mastoid obliteration. Therefore, the aim of this study was to determine the efficacy of AM as a biologic dressing on mastoid epithelialization following mastoid obliteration.

**MATERIALS AND METHODS**

This prospective and randomized clinical trial study was carried out from August 2012 to April 2017. The research protocol was approved by Shiraz University of Medical Sciences Local Ethics Committee. The design of this study was approved by the Iranian Registry of Clinical Trials (IRCT) (IRCT2015081915496N16). Written informed consent was obtained from all patients. Inclusion criteria were adult patients who had undergone primary CWD mastoidectomy due to chronic otitis media with cholesteatoma in the study period. Exclusion criteria were revision surgery; patients younger than 18 years of age; preoperative medical problems such as asthma, diabetes, coagulation disorders, cardiovascular disease, basic metabolic disorder, and chronic liver or renal disease; and canal wall down mastoidectomy due to other reasons such as tumors. Also, patients with follow-up less than 6 months were excluded from this study.

At first, 164 patients were randomly divided in two groups by blocked randomization method: AM group and temporalis fascia (control) group.

All procedures were performed by the first author (M.F.) at Dastgheib Hospital, which is affiliated with the Shiraz University of Medical Sciences (Shiraz, Iran). This hospital is a tertiary health care center in the field of otology in southern Iran. Generally, our surgical technique consisted of postauricular approach, CWD mastoidectomy, and tympanoplasty associated with mastoid obliteration and reconstruction of the external auditory canal (EAC). The incision is carried through the skin and into the subcutaneous tissue 1 cm posterior to the postauricular sulcus. Next, the auricle is retracted anteriorly and Korner’s flap is elevated. Then, a posterior canal wall skin incision is made 5 mm lateral to the tympanic annulus while we attempt to preserve as much of the posterior canal wall skin as laterally as possible. CWD mastoidectomy and eradication of cholesteatoma were performed. During removal of the posterior bony EAC, two small vertical ridges in the posterosuperior and posteroinferior portion were kept intact to provide a bony structural support for the newly reconstructed soft EAC. After insertion of silastic sheet in the middle ear and tympanoplasty, small pieces of cartilages were harvested from the concha through the postauricular incision and used for attic obliteration. Then, an inferiorly broad base musculoperiosteal flap was rotated to the mastoid cavity, posterior to the preserved supportive vertical ridges in the posterosuperior and posteroinferior sections of EAC, whereas anteriorly it was positioned on the facial ridge and superiorly was positioned over the lateral canal and tegmen (Fig. 1). In sclerotic mastoid cavities, usually this flap alone provides adequate bulk for obliteration. To complete obliteration in large mastoid cavities, cartilages were positioned in the posterior aspect of the flap in the cavity.

Amniotic membranes were supplied by the Nemazee Hospital, Transplant Research Center, which is affiliated with the Shiraz University of Medical Sciences (Shiraz, Iran). Human placenta was obtained after elective caesarean section in a woman who was seronegative for HIV, hepatitis B and C, and syphilis, with no history of premature rupture membranes, endometritis, or meconium ileus. Under a lamellar flow hood, the placenta was first washed of blood clots with sterile saline. The inner AM was separated from the chorion by blunt dissection, then washed three times with phosphate buffered saline (PBS) containing Antibiotic-Antimycotic (Gibco, Germany).

The membrane was flattened onto a nitrocellulose membrane (Protran Nitrocellulose membrane roll, Sigma Aldrich, Germany) with the epithelial surface up, and 10 x 10 cm pieces were prepared. Pieces were placed in 4%, 8%, and 12% dimethyl sulphoxide (DMSO) PBS for 5 minutes, and finally placed in a sterile vial containing 12% DMSO medium (Sigma-Aldrich, St. Louis, MO) and Antibiotic-Antimycotic (Gibco, Germany). Vials were frozen at −80°C. The membrane, previously defrosted in an operation room and rinsed in normal saline, was then used to cover the patient’s operation site.

AM graft was extended anteriorly medial to the tympanic membrane graft, which covers the facial ridge. Posteriorly, it was positioned to fully cover the flap. The Korner’s flap was repositioned over the AM-covered flap. The lateral pedicle of the Korner’s flap was sutured to the inferior pedicle muscle flap using Vicryl suture 3-0 (Vicryl Ethicon, Bridgewater, NJ); thus, in the newly reconstructed EAC anteriorly, preserved lateral posterior EAC skin could be returned to the newly modified canal wall between the AM graft and the EAC dressing. In the control group, the procedure was similar to that used for the AM group, with the exception that we did not use AM for muscle flap coverage.

The meatus was then packed with Gelfoam (Pfizer, New York, NY) and a gauze pack impregnated with ophthalmic tetracycline ointment. In most cases, nearly normal contour EAC was achievable; therefore, minimal mastoplasty was required. The postauricular incision was sutured in two-layer Vicryl 3-0 (Ethicon, Bridgewater, NJ) and nylon 4-0. The tetracycline gauze was
removed 7 days postoperatively. Then, the patients were given antibiotic ear drops for 1 week simultaneous to oral antibiotics.

Follow-up was done by the independent assessor otolaryngologist. Patients were evaluated by microscopic otoscopy on postoperative week 3. Then, follow-up interval depended on the granulation tissue formation. If it did exist, patients were observed every couple of weeks, but if did not exist, they were observed within the second month, third month, fourth month, sixth month, and 12th month in the first year, and then once a year. If granulation tissue was observed, chemical cautery was performed with trichloroacetic acid 5%. The assessor investigated the average time to complete mastoid cavity epithelialization as a primary outcome and also evaluated the granulation tissue formation, graft success rate, recurrence of cholesteatoma, and pre-postoperative hearing results as secondary outcomes.

In order to assess hearing results, pure tone audiometry at frequencies of 0.5, 1, 2, and 4 kHz were analyzed. Because we do not measure the 3-kHz frequency in our center, we use the mean of 2- and 4-kHz frequencies instead of the 3 kHz. Also, preoperative and postoperative air conduction (AC), bone conduction (BC), air–bone gaps (ABG), speech discrimination score (SDS), and speech reception thresholds (SRT) were measured. Preoperative audiogram was performed 1 week prior to the surgery, and audiogram of 6 months after the surgery was selected as postoperative audiogram.

Data were analyzed using SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY). Fisher exact test and chi-square tests were used to compare categorical variables between groups. Normality of quantitative variables was assessed by the Kolmogorov–Smirnov test. Normal (non-normal) variables were analyzed using t test (Mann-Whitney test) to compare between groups. P values less than 0.05 were considered significant.

RESULTS
In this study, at first 170 ears were enrolled. As the consort flow diagram in Figure 2 shows, five ears were excluded due to revision CWD mastoidectomy, and one 14-year-old patient was excluded due to age. In addition, 16 cases were excluded due to inadequate follow-up. Minimum follow-up period was 6 months, and the maximum was 55 months (mean 25 months). In the AM group, 75 ears and the control group 73 ears were analyzed.

There were 40 (53.3%) men and 35 (46.7%) women in the AM group. A total of 35 (47.9%) men and 38 (52.1%) women were in the control group. This difference was not statistically significant (P = 0.622). The age range in the AM group was 20 to 65 years of age (mean age of 35 years) and in the control group was 24 to 73 years of age (mean age of 33.6 years). There was no significant difference between age in both groups (P = 0.280).

In the AM group, duration of complete epithelialization of the cavity was 41.4 ± 7.7 days, whereas in the control group it was 59.2 ± 9.1 days. Duration of time for complete epithelialization in the AM group was shorter than the control group, which was statistically significant (P < 0.0001).

Within 2 months, in the AM group four ears (5.3%) had suffered from granulation tissue. In the control group, 12 ears (16.4%) had developed granulation tissue on the muscular flap. Incidence of granulation tissue in the AM group was less than in the control group, which was statistically significant (P = 0.035). In addition, according to otomicroscopic examinations in the study period we did not find cases with recurrence of cholesteatoma.

Analyzing the hearing outcome revealed that there was no significant difference between pre- and
### TABLE I.
Pre- and Postoperative Hearing Variables in Two Operative Groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AM Group (N = 75)</th>
<th>Control Group (N = 75)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative AC (dB)</td>
<td>42.5 ± 12.2†</td>
<td>45.4 ± 14.3</td>
<td>0.186</td>
</tr>
<tr>
<td>Postoperative AC (dB)</td>
<td>39.9 ± 13.5</td>
<td>43.1 ± 17.4</td>
<td>0.213</td>
</tr>
<tr>
<td>Gain (dB)</td>
<td>2.6 ± 13.1</td>
<td>2.3 ± 15.7</td>
<td>0.900</td>
</tr>
<tr>
<td>Preoperative BC (dB)</td>
<td>7.8 ± 10.2</td>
<td>10.1 ± 7.5</td>
<td>0.121</td>
</tr>
<tr>
<td>Postoperative BC (dB)</td>
<td>11.3 ± 8.9</td>
<td>12.5 ± 10.7</td>
<td>0.459</td>
</tr>
<tr>
<td>Gain (dB)</td>
<td>3.5 ± 9.0</td>
<td>2.4 ± 9.2</td>
<td>0.463</td>
</tr>
<tr>
<td>Preoperative ABG (dB)</td>
<td>34.7 ± 12.2</td>
<td>35.3 ± 12.7</td>
<td>0.770</td>
</tr>
<tr>
<td>Postoperative ABG (dB)</td>
<td>28.6 ± 14.2</td>
<td>30.7 ± 13.1</td>
<td>0.352</td>
</tr>
<tr>
<td>Gain (dB)</td>
<td>6.1 ± 14.5</td>
<td>4.6 ± 12.4</td>
<td>0.500</td>
</tr>
<tr>
<td>Preoperative SRT (dB)</td>
<td>41.7 ± 14.3</td>
<td>44.7 ± 16.1</td>
<td>0.232</td>
</tr>
<tr>
<td>Postoperative SRT (dB)</td>
<td>38.6 ± 17.8</td>
<td>43.7 ± 18.2</td>
<td>0.087</td>
</tr>
<tr>
<td>Gain (dB)</td>
<td>3.1 ± 17.1</td>
<td>1.0 ± 17.9</td>
<td>0.467</td>
</tr>
<tr>
<td>Preoperative SDS (%)</td>
<td>93. ± 12.2</td>
<td>91.9 ± 17.2</td>
<td>0.654</td>
</tr>
<tr>
<td>Postoperative SDS (%)</td>
<td>95.1 ± 16.4</td>
<td>91.5 ± 13.6</td>
<td>0.149</td>
</tr>
<tr>
<td>Gain (%)</td>
<td>2.1 ± 13.5</td>
<td>0.4 ± 14.1</td>
<td>0.455</td>
</tr>
</tbody>
</table>

†Frequency of 0.5–3 kHz.

Values are Mean ± SD.

ABG = air–bone gap; AC = air conduction; BC = bone conduction;
AM = amniotic membrane; SD = standard deviation; SDS = Speech Discrimination Score; SRT = speech reception threshold.

postoperative mean AC, mean BC, mean ABG (0.5–3 kHz), SRT, and SDS in both groups and among them (P > 0.05) (Table I). In the present study, we found that graft success rate was 93.3% (n = 70) and 91.8% (n = 67) in the AM group and the control group, respectively. There was no significant difference between the two groups (P = 0.763).

**DISCUSSION**

For over a decade, inferior base musculoperiosteal flap has been used to obliterate the mastoid cavity at our center. In some cases, we found that the flap was not sufficient in size and bulk. Because patients developed granulation tissue over the bare areas of musculoperiosteal flap and mastoid bone, we decided to change our technique by combining method with AM.

We attempted to confirm that using our fast-and-simple method, a dry ear, small-size mastoid cavity, and normal contour EAC is achievable in CWD mastoidectomy by using a combination of cartilage, inferior base musculoperiosteal flap, AM, and canal wall skin. Occasionally, in large mastoid cavities, it is necessary to place autogenous cartilage pieces posterior to the musculoperiosteal flap as a sustainable filler. Another advantage of this technique is the small cosmetic meato-plasty. We believe that a normal EAC contour is achievable using AM. The surface of the obliterating flap would be smoother without any dead space and retraction pocket that eventually facilitate epithelialization. In addition, we preserved much of the posterior EAC skin and sutured the lateral pedicle of the Korner’s flap to AM-covered muscle flap. This would create less skin defect over AM, which eventually leads to early epithelialization. Inferior base musculoperiosteal flap is a random flap that occipital and postauricular arteries supply. Although flap might be prone to atrophy, after mean 25 months of follow-up we did not observe a significant shrink-back. This might be due to two forces of posterior cartilaginous support and fixed anterior canal skin as an anterior support. These are particularly important stabilizing factors. Also, it is clear that potential biological properties of extracellular matrix proteins in the basement membrane of the AM make it an important scaffolding material. Another significant aspect of AM is that it prevents partial or complete flap necrosis. It has established that AM application as a biologic dressing in random skin flaps yields significant capillary proliferation and decreases neutrophil infiltration, which facilitates flap survival.

Mastoid cavity obliteration using soft tissue flaps is considered highly effective (78%–100%) in solving a cavity problem; however, it is very difficult to compare them due to the variety of obliteration techniques. Also, a few authors have considered the effects of various soft tissue flaps on duration of epithelialization in CWD mastoidectomy. Overall, they found the duration of epithelialization was 25 to 90 days.

Our work is a modified version of Lee et al.’s method. They used pieces of cartilage from the concha for EAC reconstruction, inferiorly based musculoperiosteal flap for mastoid obliteration, and posteriorly transposed Palva flap to cover the obliterated attic area. Duration of time for complete epithelialization in our control group with inferior base musculoperiosteal flap without AM was approximately 10 weeks, which is in line with the Kim et al. study. They used anterior-based musculoperiosteal flap, and the mean duration of epithelialization was about 11 weeks.

Review of the literature showed that a few researchers have addressed the issue of lining the middle ear or mastoid cavity by AM but failed to address its usage in mastoid obliteration techniques. In 1959, Liu et al. used amnioplastin to cover the mastoid cavity, but this was unsuccessful. In 1990, Turan et al. found that lyophilized AM as a biologic dressing was a useful material for lining the tympanic cavi ties. In a retrospective chart review by Shojaku et al. (2011), they compared 11 ears for which hyperdry AM was used with 11 ears in the other group for which temporalis fascia graft was used. Also, they used fibrin glue to attach the grafts in both groups. They found that the time of epithelialization in the AM graft was considerably shorter than in the fascia graft (32 days vs. 45 days). In another retrospective chart review by Kanazawa et al. (2012), they compared times for mastoid cavity epithelialization in three groups: the hyperdry AM without fibrin glue (7 ears), AM (11 ears), or temporal fascia (11 ears) attached with fibrin glue. The mean time to complete epithelialization of the cavity in the AM with and without fibrin glue groups was considerably shorter than that of the temporal fascia group (31.9 days and 32.4 days vs. 44.5 days). However, direct comparison of studies is difficult due to differences in techniques, but it seems that duration of time for complete epithelialization in our AM group was 10 days longer than Sojakuetal et al. and Kanazawa et al. studies.

One of the strengths of this study was its prospective and randomized nature. The second strength of...
This study was that all procedures were performed by the senior author; hence, differences in the level of expertise can be excluded as a confounding factor. However, the only major drawback was its relatively short follow-up period. It is plausible that a mean follow-up period of 25 months might influence the results. Also, cholesteatoma recurrence rate needs to be checked, but a follow-up at 25 months post-surgery may be too soon. Albeit due to obliteration of mastoid cavity, observation of potential recurrence of cholesteatoma by otomicroscopic controls is not practical. Therefore, diffusion-weighted MRI is indicated with a longer follow-up period.

CONCLUSION

This initial result is optimistic and suggests that the clinical application of AM in mastoid obliteration is effective in minimizing postoperative epithelialization time.

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BIBLIOGRAPHY