Chitosan patch scaffold for repair of chronic safe tympanic membrane perforation

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Objective
This study was designed to evaluate the performances of chitosan patch myringoplasty in patients with small and medium sized chronic safe tympanic membrane (T.M) perforations.

Patients and methods
After preparation of the chitosan patch scaffold (CPS), 60 consecutive dry small and medium sized(from <3 mm up to 5 mm) central tympanic membrane perforations were included in this study.

Results
Healing of T.M perforations was achieved in 14 out of 19 in small perforations (73.68%) and 25 out of 41 in medium sized perforations (60.97%) also significant improvement of hearing and tinnitus were achieved post-operatively.

Conclusion
Myringoplasty by water insoluble chitosan patch scaffold with 3% chitosan and 3% glycerol is easy, safe and quick method. It can be done in outpatient clinic without risk of general anesthesia and with noticeable reduction in the time of operation.

Keywords:
tympanic membrane perforations, chitosan patch scaffold, healing

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Introduction

Tympanic membrane (TM) perforations are a commonly encountered disorder by otolaryngologist head and neck surgeons [1]. Closure of TM perforations was initially attempted with a segment of pig's bladder by Marcus Banzer in 1640. Berthold [2] introduced the first surgical cure of TM perforations in 1878 using full-thickness skin autografts from the forearm. Heermann in 1961 [3] performed successful autografts for closure of TM perforations using temporalis fascia.

Currently, an autologous autograft including muscle fascia or perichondrium is generally used for treatment of TM perforations.

However, surgical treatments have shortcomings, affecting both patients and doctors, such as high cost, defective donor sites, the need for general anesthesia in some patients, the complex microsurgical skills of surgeons, and aseptic procedures [4]. Several agents or factors such as hyaluronic acid, epidermal growth factor, and basic fibroblast growth factor have been tried experimentally to promote the healing of TM perforations [5]. Blake in 1887 [6] introduced the simple technique of a paper patch graft that guides the migrating epithelium to a scaffold from the borders of perforation.

Several scaffolds that could stimulate tympanic regeneration, guideear drum growing, or replace the perforated defects have been introduced until recently, such as hydrogels [7], collagen [8], Seprafilm [9], or calcium alginate [10], and tested for closure of TM perforations [11].

Chitosan (poly-b(1-4)-d-glucosamine) is an aminopolysaccharide obtained by deacetylation of chitin and has characteristics such as biocompatibility, tensile strength and flexibility, good cell adhesion, antifungal and antibacterial functions, and properties that stimulate wound cure [12].

Chitosan was first tested experimentally as a patch scaffold by Kim *et al.* [11] to repair traumatic TM perforations.

The study aimed to evaluate water-insoluble chitosan patch scaffold (CPS) for repair of chronic dry safe TM perforations.

Materials and methods

First, Institutional Review Board approval of the study design and the obtaining of informed consent were performed.

After exclusion of traumatic perforations, large perforation greater than 5 mm, bilateral perforations, previous operated ear, middle ear cholesteatoma, A-B

gap greater than 30 dB, eustachian tube dysfunction, and middle ear or other ENT infections, 60 patients were selected randomly from outpatient clinic with small (<3 mm), medium-sized (3–5 mm), safe, and dry perforations.

Preparation of chitosan patch scaffold

Water-insoluble chitosan powder with molecular weight 190 000–310 000, as shown in Figure 1, acetic acid, and glycerol were purchased from Egyptian International Center for Import and all were used to prepare the CPS.

Acetic acid was used as a solvent for the water-insoluble chitosan and glycerol was used as a plasticizer. Chitosan solution was prepared by adding 3 or 5 g of chitosan powder to 100 ml of 2% acetic acid. Thereafter, chitosan solution was mixed with 1–5 ml of glycerol. This chitosan mixture was filtered through a pore size of 1 μl to remove large and impure particles. The prepared chitosan mixture was poured into Petri dishes and dried at 45°C for 12 h to obtain different preparations of CPSs as shown in Figure 2.

Measurement of mechanical properties of chitosan patch scaffold

The tensile strength, elongation at break, and Young's modulus were determined with a Zwick 1425 testing machine (Nordrhein Westfalen, Germany). The compressed sheets were cut into dumbbell-shaped specimens with appropriate punching dies with a width of 4 mm. The specimens, with a width of 4 mm, a neck length of 15 mm, and a thickness of 1–1.5 mm, were tested at a crosshead, speed of 50 mm/min and with a load cell of 10–20 according to specification of American Society for Testing and Materials D624 (2007) for determination of physicomechanical properties. These tests were repeated 10 times and the averages and SD of the properties were calculated [13].

The main percent elongation of CPSs was in the range of 10.25–135.30. The elongation of CPSs was also different according to the concentration of chitosan and glycerol. The CPSs had the highest elongation when 3% glycerol was added to 3% chitosan and had the lowest elongation with the composition of 5% glycerol and 3% chitosan (Table 1).

Surgical technique

First, Institutional Review Board approval of the study design and the obtaining of informed consent were performed.

After exclusion of traumatic perforations, large perforation greater than 5 mm, bilateral perforations, previous operated ear, middle ear cholesteatoma, A-B gap greater than 30 dB, eustachian tube dysfunction, and middle ear or other ENT infections, 60 patients were selected randomly from outpatient clinic with small (<3 mm), medium-sized (3–5 mm), safe, and dry perforations.

Table 1 The elongation of chitosan patch scaffold according to the concentration of chitosan and glycerol

Concentration of chitosan (%)	Concentration of glycerol (%)	Elongation (%)
3	1	40.05
3	2	125.43
3	3	135.3
3	5	10.25
5	1	45.32
5	3	90.89
5	5	81.44

Figure 1

Picture shows chitosan powder.

Figure 2

Picture shows chitosan patch scaffold in the Petri dish.

All patients were subjected to preoperative assessment, myringoplasty, and postoperative assessment.

(1) Preoperative assessment
   (a) Detailed history of the disease (onset, course, duration, frequency of exacerbations, vertigo, tinnitus, hearing loss, development of any complications, and history of previous surgery to the ear).
   (b) Nasal and nasopharyngeal examinations were performed.
   (c) Otoscopic and microscopic examination of the ear to exclude infection and granulation tissue and to assess the size of TM perforation using a right-angle otologic hook and was graded as small (<3 mm), medium (3–5 mm), and large (>5 mm).
   (d) Audiological assessment through pure tone audiometry and the A-B gap in dB was calculated. The differences between air conduction and bone conduction at (500–1000–2000–4000) successive frequencies were calculated and divided by 4.
   (e) Assessment of eustachian tube function by tympanometry.
   (f) Imaging study of the temporal bone, sinuses, and nasopharynx.
(2) Operative procedures
   (a) Patients were operated upon through the period from May 2010 to February 2012 (Benha University Hospital).
   (b) The procedures were performed in the operating room under local anesthesia after preparing the patients with 10 mg valium (diazepam) or, if anxiolytic is required, dormicum (midazolam 0.02 mg/kg) intravenously 30 min before surgery.
   (c) Under the operating microscope and through the permeatal approach, a classic quadratic injection was made using a 27-G needle with 2% lidocaine (Xylocaine) with 1: 100 000 epinephrine.
   (d) The rim of the perforation was carefully excised and freshened, using a Rosen needle and a cupped forceps, to encourage migration of the mucosal layer and the epithelium.
   (e) A small piece of the prepared CPS was trimmed to provide a 1 mm overlapping margin of the perforation.
   (f) The CPS was then placed on the lateral surface of the TM with the help of an alligator forceps and an angled hook, making sure that it completely covered the perforation and that it was in close contact with its entire margin.
   (g) Packing of the external ear canal with gel foam.
   (h) Patients were discharged with instruction to avoid getting water in the ear canal and to avoid nose blowing.
(3) Postoperative assessment:
   (a) Weekly examination under the microscope during the first month then monthly up to 3 months for detection of the closure of the perforation.
   (b) Pure tone audiometry was performed after healing of the TM perforation for successful cases.

Statistical analysis

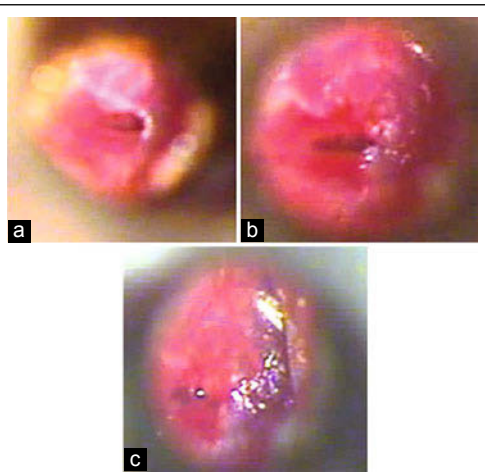
Statistical analysis of the collected data was performed using SPSS version 17 (SPSS version 17; spss Inc., Chicago, Illinois, USA). Quantitative data were analyzed using mean and standard deviation, whereas frequency and percentage were used with qualitative data. The Student *t*-test and Pearson correlation were used to compare means of different groups, whereas *Z*-test was used to compare proportions (Figs. 3 and 4).

Results

Sixty patients were included in this study, 19 with small and 41 with medium-sized perforations. The age ranged from 15 to 55 years with the mean of 35 ± 15.4 years. The study included 33 male patients (55%) and 27 female patients (45%).

The main percent elongation of CPSs was in the range of 10.25–135.30. The elongation of CPSs was also different according to the concentration of chitosan and glycerol. The CPSs had the highest elongation

Figure 3



(a) Small-sized perforation in the posteroinferior quadrant. (b) After trimming of the perforation. (c) The perforation is covered with chitosan patch scaffold.

when 3% glycerol was added to 3% chitosan and had the lowest elongation with the composition of 5% glycerol and 3% chitosan (Table 1).

The preoperative mean of A-B gap for the 60 cases was 20.15 ± 1.904 dB and classified as the following: 16 cases (26.7%) with 11.875 ± 1.89 dB, 17 cases (28.3%) with 17.82 ± 1.46 dB, 25 cases (41.7%) with 23 ± 1.443 dB, and two cases (3.3%) with 28.0 ± 2.82 dB (Table 2).

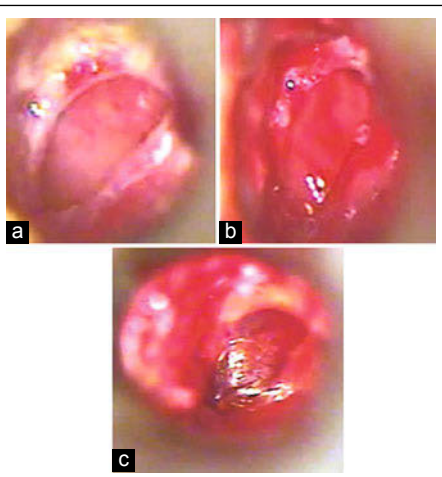
The healing of TM perforation after 3 months of operation was achieved in 14 of 19 with small perforations (73.68%) and 25 of 41 with medium-sized perforations (60.97%). A statistically significant difference was found in the closure rates (Z-test = 2.2 and 1.7 and P = 0.02 and 0.08, respectively); small perforations had the highest closure rate. The final closure rate was 65%, which is statistically significant (Z-test = 3.2 and P = 0.001) (Table 3).

According to tinnitus preoperatively, it was manifested in 33 patients (55%).

The improvement of tinnitus postoperatively was noticed in 23 patients (69.7%) with significant improvement (Z-test = 1.85 and P = 0.035) (Table 4).

The time required for healing was 3–9 weeks for small perforations (average 5.2 weeks) and 4–14 weeks for medium-sized perforations (average 8.4 weeks), with high statistical significance regarding the time required for healing of TM perforation (t-test = 4.24 and P = 0.0008) with the least time required for healing of small perforations (Table 5; Figs. 5 and 6).

Figure 4



(a) Medium-sized perforation in the anteroinferior quadrant. (b) After trimming of the perforation. (c) The perforation is covered with chitosan patch scaffold.

The postoperative improvement of hearing that was confirmed by audiometric results was about 12.3 dB hearing gain with mean of the A-B gap for the healed cases of 7.85 ± 3.03, and on comparison between it and the preoperative one, it showed highly significant improvement of the A-B gap (P = 0.0003) (Table 6).

Of the 21 failed cases, there were four cases with postoperative infection and two cases with detached patch.

Discussion

TM perforations present a common otologic problem, usually resulting from trauma, Infection, or an extruded ventilation tube [14].

Healing of TM perforation is considered as a regeneration process. Biological materials for replacing TM perforation achieved remarkable progress through tissue engineering.

Table 2 A-B gap of cases preoperatively

A-B gap (dB)	Size of perforation	*n* (%)	Mean ± SD
10–15	S-10M-6	16 (26.7)	11.875 ± 1.89
16–20	S-6M-11	17 (28.3)	17.82 ± 1.46
21–25	S-3M-22	25 (41.7)	23.0 ± 1.443
26–30	S-0M-2	2 (3.3)	28.0 ± 2.82
Total		60 (100)	20.15 ± 1.904

Table 3 Healing of tympanic membrane perforation after 3 months of operation

Size of perforation	*n* (%)			*Z*-test	*P*
	Close ear	Not	Total		
Small	14 (73.68)	5 (26.32)	19 (100.0)	2.2	0.02 (S)
Medium	25 (60.97)	16 (39.03)	41 (100.0)	1.7	0.08 (S)
Total	39 (65.0)	21 (35.0)	60 (100.0)	3.2	0.001 (S)

S, significant.

Table 4 Tinnitus improvement after 3 months of operation

Tinnitus improvement	Number of cases (%)	*Z*-test	*P* value
Yes	23 (69.7)	1.85	0.035 (S)
No	10 (30.3)		
Total	33 (100)		

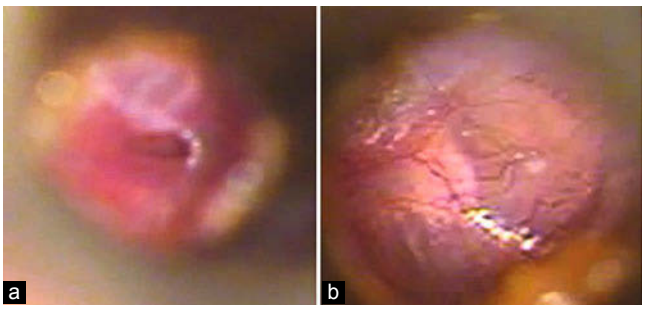
S, significant.

Table 5 Distribution of cases according to the time needed for closure of the perforation regarding the size

Size of perforation (39)	Duration (weeks)	Mean ± SD	*t*-Test	*P* value
Small (14)	3–9	5.2 ± 2.19	4.24	0.0008 (HS)
Medium (25)	4–14	8.6 ± 3.1		

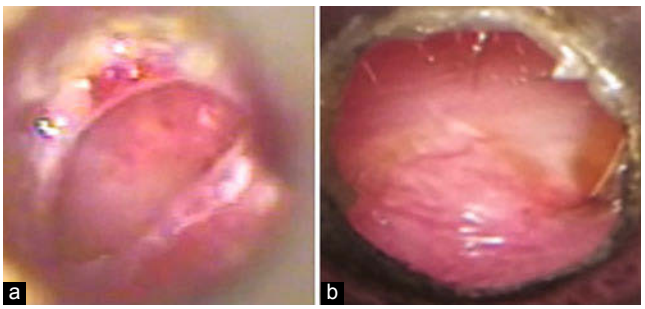
HS, highly significant.

Figure 5



(a) Small-sized perforation. (b) Closure of perforation was observed after 8 weeks.

Figure 6



(a) Medium-sized perforation. (b) Closure of perforation was observed after 14 weeks.

Table 6 Comparison between the mean A-B gap preoperatively and postoperatively for the healed cases

Variables	Groups	Mean ± SD	Paired *t*-test	*P* value
A-B gap	Preoperative	20.15 ± 1.904	26.311	0.0003 (HS)
	Postoperative	7.85 ± 3.08		

HS, highly significant.

Ideal scaffolds for TM regeneration should have biocompatibility, enough flexibility to attach to the eardrum in a concave shape, transparency to see the growing of eardrums, stimulation of TM regeneration, and resistance to infection, not like paper patches that are not biocompatible, transparent, less flexible, and requiring long healing time. Surgical treatments have some problems such as high cost, defective donor sites, the need for general anesthesia in some patients, the complex microsurgical skills of surgeons, and aseptic procedures [14,15].

Chitosan, made from crab chitin, is well known for accelerating the healing of wounds in human [16].

In this study, to our knowledge, we used CPS for the first time in human being for regeneration of TM perforation instead of an autograft or a heterograft, which is composed of chitosan sheet that acts as a scaffold and glycerol that acts as a plasticizer.

Kim *et al.* [11] showed that the CPS had no cytotoxicity and had proliferating effect similar to growth factors.

The selection of an optimum CPS, an optimum concentration of chitosan and glycerol was determined. The mechanical properties were more important than surface properties in determining the optimum CPS, as mechanical properties had significant differences according to glycerol and chitosan concentration. The ideal patch scaffold for artificial eardrums requires high elasticity with proper strength to allow the patch to adapt well to the eardrums. Accordingly, elongation is considered more important than tensile strength because of constant movement of eardrums resulting from sound transmission. The CPSs with high elasticity have a superior attachment to the eardrums than those with high tensile strength. Accordingly, the CPSs with 3% chitosan and 3% glycerol showed suitable tensile strength and high elongation.

In our study, 14 of 19 small-sized perforations (73.68%) were healed; in addition, 25 of 41 medium-sized perforations (60.97%) were closed. Duration of healing varied from 3 to 14 weeks with longer durations for medium-sized perforations.

Previous studies showed that the benefits of paper patching were low and limited to small perforations. Closure rates by rice paper patching after the excision of the perforation margin under local anesthesia for persistent TM perforations were less than 30% [17]. In place of paper patches, a new device of silicone, a TM patcher, was invented to be used as a stable scaffold [18]. Office-based treatment using the TM patcher was effective in small perforations less than 3 mm; five (38%) of 13 small perforations healed and one (8%) became smaller, although perforations larger than 5 mm were not healed ($n = 6$) [18].

Recently, Golz *et al.* [19] reported a closure rate of 55.7% for perforations smaller than 5 mm in diameter by paper patching. They concluded that their higher success rate compared with previous series was due to thorough removal of the perforation rim under local anesthesia and close follow-up seeing patients every week, which enabled immediate replacement of paper patches.

In our study, we used glycerol to adhere the CPS on the TM instead of mupirocin (2%) ointment, which is permitted only for methicillin-resistant *Staphylococcus aureus* infection, and inappropriate usage may induce drug resistance strain of methicillin-resistant *Staphylococcus aureus*.

There was hearing improvement with CPS in all 39 healed cases by about 12.3 dB hearing gain assed with audiometer; no case had A-B gap more than 20 dB. Tos [20] reported improvement of hearing after formal myringoplasty with reduction of A-B gap less than 30 dB in 79% of his patients. Black and Wormald [21] reported improvement of hearing after formal myringoplasty with reduction of A-B gap less than 20 dB in 77.9% of their patients.

There was significant improvement of tinnitus postoperatively in 23 patients (69.7%).

In our study, we had not performed the procedure on children under the age of 15 years and no conclusion can be made with respect to this age group.

Conclusion

CPS was found to be effective in healing, improvement of tinnitus, and reduction of A-B gap.

Although the success rate with CPS is much lower than that usually achieved by formal surgical myringoplasty, CPS has obvious advantages: it is simple, easy, quick, safe, well tolerated, cost effective, transparent, flexible, has good adhesion, has good resistance to infection, with no pain, incision, or sutures, and avoids the risks and morbidity of general anesthesia and formal myringoplasty. In addition, no hospitalization is needed and patients spend less time away from home and work.

Recommendations

More studies on tissue engineering are required to develop other forms of CPS by adding other substances to become more stable and tense to use it in myringoplasty by formal technique.

More studies are needed to compare the results of healing, audiological gain, tinnitus, duration of healing, and causes of failure between this method and the conventional technique of myringoplasty (as a control group) to enhance the advantages of chitosan usage.

Many future researches are still needed to confirm the versatility and safety of chitosan in human with long term followup.

Acknowledgements

Conflicts of interest

None declared.

References

1 Jeremy DV, Latev MD, Labadie RF, Cohen SM, Werkhaven JA, Hyanes DS. Use of alloderm in type I tympanoplasty: a comparison with native tissue grafts. Laryngoscope 2005; 115:1599–1602.

2 Berthold E. About Myringoplasty. Medical-Chuutgisches central journal. 1878; 14:195–207.

3 Heermann, H. Repare of ear drum with plastic fascia from temporalis muscleafter straightening the frontal wall of the meatus.. Hals Nasen Ohernarzt 1961; 9:136–137.

4 Laidlaw, DW, Costantino PD, Govindaraj S. Tympanic membrane repair with dermal allograft. Laryngoscope 2001; 111:702–707.

5 Chauvin K, Bratton C, Parkins C. Healing large tympanic membrane perforations using hyaluronic acid, basic fibroblast growth factor, and epidermal growth factor. Otolaryngol Head Neck Surg 1999; 121:43–47.

6 Blake CJ. *Transactions of the First Congress of the International Otological Society (abstract).* New York: D. Appleton and Co.; 1887. 125.

7 Park AH, Hughes CW, Jackson A, Hunter L. Cross linked hydrogels for tympanic membrane repair. Otolaryngol Head Neck Surg 2006; 135:877–883.

8 Salen B, Simbach I. Exogenous collagen in the closure of tympanic membrane. J Laryngol Otol 1965; 79:159–256.

9 Konakci E, koyuncu M, Unal R, Tekat A, Uyar M. Repair of subtotal tympanic membrane perforations with Seprafilm. J Laryngol Otol 2004; 111:702–707.

10 Weber DE, Semaan M, Wasman JK, Beane R, Bonassar LJ, Megerian CA. Tissue-engineered calcium alginate patches in the repair of chronic chinchilla tympanic membrane perforations. Laryngoscope 2006; 116:700–704.

11 Kim JH, Choi SJ, Park JS, Lim KT, Choung PH, Kim SW, *et al.* Tympanic membrane regeneration using a water-soluble chitosan patch. Tissue Eng Part A 2010; 16:225–232.

12 Kim JH, Bae JH, Lim KT, Choung PH, Park JS, Choi SJ, *et al.* Development of water-insoluble chitosan patch scaffold to repair traumatic tympanic membrane perforations. J Biomed Mater Res A 2009; 90:446–455.

13 El-Nashar DE, Youssef EAM, Abd El-Ghaffar MA. Modified phosphate pigments as high performance reinforcing materials for rubber vulcanizates. Mater Des 2010; 31:1350–1359.

14 Jh Bae, Y-H Choung. *Development of water-insoluble chitosan patch for regeneration of tympanic membranes.* Korea: Department of Medical Sciences, Ajou University; 2008. 33 .

15 SJ Choi, SW Kim, JH Kim, JB Lee, OS Choo, JH Chung, YH Choung. Efficient treatment of chronic tympanic membrane perforations in animal models by chitosan patch scaffolds. Tissue Eng Regen Med 2011; 8:141–150.

16 Madihally SV, Matthew HWT. Porous chitosan scaffolds for tissue engineering. Biomaterials 2008; 20:1135–1142.

17 Spandow O, Hellstrom S, Dahlstorm M. Comparison of the repair of permanent tympanic membrane perforations by hydrocolloidal dressing and paper patch. J Laryngol Otol 1995; 109:1041–1047.

18 Kartuch JM. Tympanic membrane patcher: a new device to close tympanic membrane perforations in an office setting. Am J Otol 2000; 21:615–620.

19 Golz A, Goldenberg D, Netzer A, Fradis M. Paper patching for tympanic membrane perforations. Otolaryngol Head Neck Surg 2003; 128:565–570.

20 Tos M. Tympanoplasty and age. Arch otolaryngol 1972; 96:493–498.

21 Black JH, Wormald PJ. Myringoplasty effects on hearing and contributing factors. S Atr Med J 1995; 85:41–43.