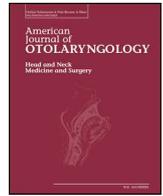




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Bacterial cellulose graft versus fat graft in closure of tympanic membrane perforation[☆]

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ABSTRACT

Object: To compare between results of bacterial cellulose graft myringoplasty and fat graft myringoplasty in patients had mild or moderate size safe perforation.

Methods: 120 patients undergoing myringoplasty due to mild or moderate size perforation were divided into 3 groups: group I: 40 patients undergoing myringoplasty with use of bacterial cellulose graft, group II: 40 patients undergoing myringoplasty with use of fat graft, group III: 40 patients undergoing usual myringoplasty with use of temporalis fascia graft (control group).

Results: Healing in 20 patients with small perforation and 17 patients with moderate perforation in Group I, Healing in 15 patients with small perforation and 10 patients with moderate perforation in Group II, Healing in 18 patients with small perforation and 12 patients with moderate perforation in Group III.

Conclusion: Bacterial cellulose graft myringoplasty would be a good, simple, rapid and safe surgery that could be done under local anesthesia in outpatient clinic with shorter time of surgery than fat graft myringoplasty and temporalis fascia graft myringoplasty, with better hearing and healing.

Summary at glance: 120 patients undergoing myringoplasty were divided into 3 groups to compare between results of bacterial cellulose graft myringoplasty, fat graft myringoplasty and temporalis fascia graft myringoplasty.

1. Introduction

The myringoplasty surgery would be one of the most common operations that could be done in otology because of increased risk of tympanic membrane perforation, there would be different types of graft materials as cartilage, temporalis fascia and fat that could be used in closure of tympanic membrane perforation [1]. Temporalis fascia would be the most used graft materials in this operation but it would need massive manipulation, long time of sedation or general anesthesia that would not be suitable for the era of day care surgery [1].

Fat would be known that it could have the resistances; it would be used like an autogenous graft for several types of operations [2,3]. Ringenberg would be the first otolaryngologist who would use fat in tympanic membrane perforation closure [3]. There would be other studies that could confirm that fat graft would be effective in closure of tympanic membrane perforation [3,4].

The myringoplasty surgery would have some pitfalls like expensive cost, defect in graft sites, some cases might need general anesthesia, accurate surgical skills and non-septic surgical procedures [5]. Many factors like epidermal growth factor, fibroblastic growth factor and hyaluronic acid could be used to allow healing and closure of tympanic membrane perforation [6]. Blake in 1887 used a paper patch as a graft to allow epithelial migration and healing of tympanic membrane perforation [7].

There would be promising treatments that could allow closure of tympanic membrane perforation; these treatments would be done in outpatient clinic, safe, easy, low cost and non-invasive procedures [8,9]. Gel foam, atelocollagen, fibroblastic growth factor, autologous serum and chitin could be used as alternative graft for closure of tympanic membrane perforation [9,10].

Tissue engineering would give alternative materials that could be used in closure of tympanic membrane perforation and could replace

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usual myringoplasty surgery, it should be important to understand the success factors and the failure factors of tympanic membrane perforation closure [10].

Bacterial cellulose would be an alternative material that could be obtained by bacterial synthesis, it would be non-toxic, biocompatible, safe and having the ability to support the cellular growth and differentiation [11]. The bacterial cellulose film would be formed by the fermentation of *Acetobacter xillinum* bacteria. It would be inert, resistant and insoluble. It could be permeable to liquid and gas but would be resistant to traction and stretching. It should be sterile and non-toxic [12].

This study would aim to compare between results of bacterial cellulose graft myringoplasty and fat graft myringoplasty in patients had mild or moderate size safe perforation.

2. Patients and methods

This was a prospective, randomized study carried on 120 patients aged between 25 years to 40 years; they had small or moderate sized central safe dry perforation (without any middle ear pathology) due to chronic suppurative otitis media would be undergone myringoplasty at Benha University hospital, Faculty of medicine, ENT department between April 2015 to April 2017 would be included in this study. Local ethical committee approval and informed consent were taken before the onset of the study. The patients would be selected with strict inclusion and exclusion criteria and would be divided into 3 groups.

Group I: 40 patients undergoing myringoplasty with use of bacterial cellulose graft.

Group II: 40 patients undergoing myringoplasty with use of fat graft.

Group III: 40 patients undergoing usual myringoplasty with use of temporalis fascia graft.

2.1.1. Inclusion criteria

1-small size tympanic membrane perforation (< 3 mm), 2-moderate size tympanic membrane perforation (from 3 mm to 5 mm), 3-safe central tympanic membrane perforation, 4-persistent tympanic membrane perforation (> 6 months), 5-Air-Bone gap < 30 dB.

2.1.2. Exclusion criteria

1-history of previous ear operation, 2-middle ear cholesteatoma, 3-traumatic tympanic membrane perforation, 4-large size tympanic membrane perforation (> 5 mm), 5-bilateral tympanic membrane perforation, 6-eustachian tube dysfunction, 7-systemic diseases like (diabetes, hypertension, hepatitis) 8-patients who would be lost during follow up period.

2.1.3. The bacterial cellulose film

It would be produced on Hestrin and Schramm medium (HS medium) [13]. The process of fermentation would be carried out in about 100 ml Erlenmeyer flasks containing 50 ml sterile medium, then it would be inoculated with the content of one tube standard inoculums and to be incubated at 28–30 °C for 7 days as a static culture.

The standard inoculums would be prepared by inoculating of a test tube containing 5 ml of glucose-ethanol acetic medium [14]. With 1 ml of the tested culture, then to be incubated at 28–30 °C about three days, this tube content would be used as a standard inoculums.

The formed bacterial cellulose would be taken from the culture medium and to be washed with distilled water, then it would be transferred to a flask containing 4% NaOH and to be boiled at $100 \pm 5^\circ\text{C}$ about 20 min to remove the bacterial cells, the alkali treated cellulose would be washed with distilled water and to be bleached with 10% of hydrogen peroxide to gain the maximum brightness [15].

The formed bacterial cellulose would be put on a filter paper to remove any solutions, and then was placed in Petri dish to be dried at 45 °C in oven-dry for 3 h to have dry bacterial cellulose graft with fine thickness (0,3 mm). All patients would be subjected to preoperative evaluation, surgical procedures of myringoplasty, postoperative evaluation.

2.1.4. Preoperative evaluation

Full history of the disease, nasal and nasopharyngeal examination (endoscopic), otoscopic and microscopic ear examination, pure tone audiometry, evaluation of Eustachian tube function by tympanometry, imaging of the temporal bone, the Para nasal sinus and the nasopharynx.

2.1.5. Surgical procedures

All patients would be operated under local anesthesia after preparing them with 10 mg valium (diazepam) or anxiolytic like dormicum (midazolam 0.02 mg/kg), with use of operating microscope and through the permeatal approach, the classic quadratic injection would be done by use of a 27-G-needle with 2% lidocaine with 1:100000 epinephrine, in all patients groups the perforations edges would be incised and freshened by use of a Rosen needle and a cupped forceps (Figs. 1–5).

2.1.5.1. *Group I (bacterial cellulose graft myringoplasty)*. The bacterial cellulose graft would be put over the perforation and lateral to the tympanic membrane remnant, the graft would be held in place by its self-adhesion, no gel foam or packing of the external ear canal, the patients would be discharged immediately after these surgical procedures without antibiotics prescriptions.

2.1.5.2. *Group II (fat graft myringoplasty)*. Fat graft would be taken from the medial surface of the ear lobule to be placed through the perforation with great care to contact all parts of the tympanic membrane remnant, gel foam would be put over the graft to keep it in position.

2.1.5.3. *Group III (temporalis fascia graft myringoplasty)*. The underlay method would be used to place the temporalis fascia graft placed under the tympanomeatal flap and medial to the tympanic membrane remnant, gel foam would be put over the graft to keep it in position.

2.1.5.4. *In all groups*. Patients would be discharged with instruction of no nose blowing or water entry into the ear, with antibiotics prescriptions in group II and group III but no antibiotics in group I.

2.1.5.5. *Post operative evaluation*. Every week under the microscope during the first month then every month for six months to detect the



Fig. 1. Wet bacterial cellulose.



Fig. 2. Bacterial cellulose graft.



Fig. 3. Bacterial cellulose myringoplasty.

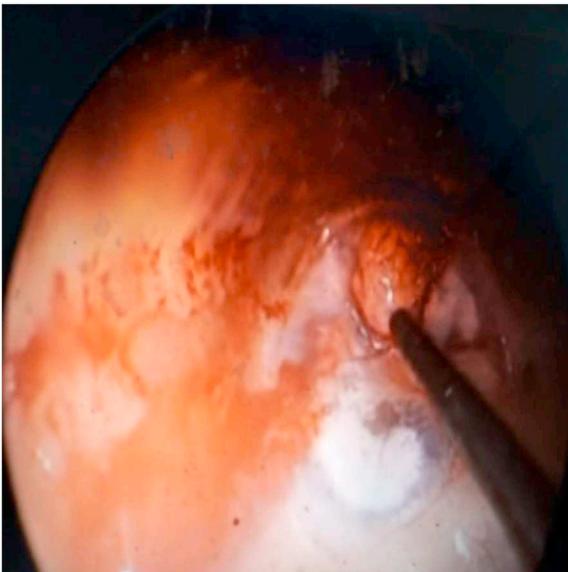


Fig. 4. Fat myringoplasty.

closure of the perforation, pure tone audiogram would be done after perforation healing.

2.2. Statistical analysis

The clinical data would be expressed as mean and standard

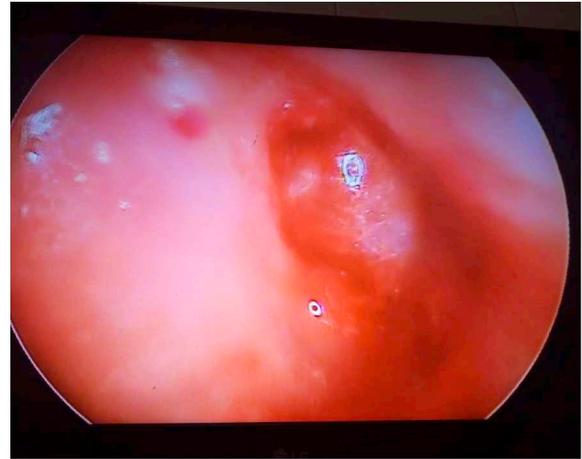


Fig. 5. Healing of bacterial cellulose myringoplasty after 3 weeks.

deviation for quantitative data, Frequency and distribution for qualitative data. Quantitative data would be compared using: -Paired *t*-test and Wilcoxon test (Z-test) All data would be tabulated and analyzed using the computer program SPSS(Statistical package for social science) version 20. *P* value < 0.05 would be considered statistically significant (*) while > 0.05 statistically insignificant *P* value < 0.01 would be considered highly significant (**) in all analyses.

3. Results

120 patients were included in this study and were divided into three groups. **Group I:** 40 patients undergoing myringoplasty with use of bacterial cellulose graft, 28 of them were males while 12 were females. The mean of age was 34.48 ± 0.65 . Small <3 mm tympanic membrane perforation was in 20 patients and moderate >3–<5 mm tympanic membrane perforation was in 20 patients. Healing of small tympanic membrane perforation was in 20 patients while healing of moderate tympanic membrane perforation was in only 17 patients. The mean time of operation was 15.01 ± 0.46 min. Healing of small perforation occurred in 3.11 ± 0.84 weeks while healing of moderate tympanic membrane perforation was 5.03 ± 0.69 weeks. Air–bone gap of successes cases that was 25.25 ± 0.41 dB before the operation became 6.68 ± 0.29 dB after the operation.

Group II: 40 patients undergoing myringoplasty with use of fat graft, 24 of them were males while 16 were females. The mean of age was 30.55 ± 0.55 . Small <3 mm tympanic membrane perforation was in 20 patients and moderate >3–<5 mm tympanic membrane perforation was in 20 patients. Healing of small tympanic membrane perforation was in 15 patients while healing of moderate tympanic membrane perforation was in only 10 patients, the mean time of operation was 35.1 ± 0.83 min. Healing of small perforation occurred in 4.97 ± 0.68 weeks while healing of moderate tympanic membrane perforation was 7.07 ± 0.95 weeks. Air–bone gap of successes cases that was 20.06 ± 0.18 dB before the operation became 7.83 ± 0.05 dB after the operation.

Group III: 40 patients undergoing usual myringoplasty with use of temporalis fascia graft, 20 of them were males while 20 were females. The mean of age was 32.47 ± 0.65 . Small <3 mm tympanic membrane perforation was in 20 patients and moderate >3–<5 mm tympanic membrane perforation was in 20 patients. Healing of small tympanic membrane perforation was in 18 patients while healing of moderate tympanic membrane perforation was in only 12 patients, the mean time of operation was 65.02 ± 0.24 min. Healing of small perforation occurred in 4.05 ± 0.94 weeks while healing of moderate tympanic membrane perforation was 7.07 ± 0.95 weeks. Air–bone gap of successes cases that was 23.72 ± 0.83 dB before the operation became

Table 1
Distribution of study groups according to the results.

Variable	Group I bacterial cellulose graft (40 patients)	Group II fat graft (40 patients)	Group III temporalis fascia graft (40 patients)	F test	P-value
Sex					
Male	28 (70%)	24 (60%)	20 (50%)	X2 = 3.33	0.189
Female	12 (30%)	16 (40%)	20 (50%)		
Age	34.48 ± 0.65	30.55 ± 0.55	32.47 ± 0.65	404.75	< 0.001**
Tympanic membrane perforation size					
Small <3 mm	20 (50%)	20 (50%)	20 (50%)	X2 = 0.0	1.0
Moderate >3–<5 mm	20 (50%)	20 (50%)	20 (50%)		
Healing of tympanic membrane perforation					
Small	20 (100%)	15 (75%)	18 (90%)	FET = 5.73	0.058
Moderate	17 (85%)	10 (50%)	12 (60%)	X2 = 5.71	0.057
Time of operation in minutes	15.01 ± 0.46	35.1 ± 0.83	65.02 ± 0.24	79478	< 0.001**
Time of healing in weeks					
Small perforation	3.11 ± 0.84	4.97 ± 0.68	4.05 ± 0.94	50.33	< 0.001**
Moderate perforation	5.03 ± 0.69	7.07 ± 0.95	5.9 ± 0.50	76.63	< 0.001**
Air–bone gap of successes cases					
Before operation	25.25 ± 0.41	20.06 ± 0.18	23.72 ± 0.83	963.84	0.001**
After operation	6.68 ± 0.29	7.83 ± 0.05	7.05 ± 0.99	4.01	0.021*

7.05 ± 0.99 dB after the operation (Table 1).

4. Discussion

Tympanic membrane perforation might be the most common ear problem. It could be due to trauma, infection and extraction of tympanostomy tube [16]. There would be different types of graft materials as cartilage, temporalis fascia and fat that could be used in closure of tympanic membrane perforation [1]. Gelfoam, atelocollagen, fibroblastic growth factor, autologous serum and chitin could be used as alternative graft for closure of tympanic membrane perforation [9,10]. The main goal of myringoplasty might be to regenerate the ear drum, reconstruct the transmission of sound, improve hearing and control infection [17]. The tympanic membrane had significant characteristics like distinct auto-reparative capacity during the healing process that would include the outer squamous epithelial layer migration to the external auditory canal and the supportive matrix absence under the regenerated epithelial layer of the tympanic membrane perforation that might prevent the reparative cells and nutrients movement into the perforation margins [18,19].

The aim of this study would be to compare between bacterial cellulose graft myringoplasty, temporalis fascia graft myringoplasty and fat graft myringoplasty in patients had mild or moderate size safe perforation.

Some factors might affect the operation success like age, site and size of the perforation, Eustachian tube function, the middle ear mucosa status, the used graft and the surgeon experience [20].

Bacterial cellulose graft group in this study showed healing (tympanic membrane perforation closure) in 20 patients with small perforation (100%) and 17 patients with moderate perforation (85%). The mean time of operation was 15.01 ± 0.46 min. Healing of small perforation occurred in 3.11 ± 0.84 weeks while healing of moderate tympanic membrane perforation 5.03 ± 0.69 weeks. Air–bone gap of successes cases that was 25.25 ± 0.41 dB before the operation became 6.68 ± 0.29 dB after the operation.

In Silveira et al. study, bacterial cellulose graft group showed healing (tympanic membrane perforation closure) in 14 patients with small perforation (100%) and 4 patients with moderate perforation (66.7%). The mean time of operation was 14.06 ± 5.23 min [21]. Temporalis fascia graft group in this study showed healing (tympanic membrane perforation closure) in 18 patients with small perforation (90%) and 12 (60%) patients with moderate perforation, the mean time of operation was 65.02 ± 0.24 min. Healing of small perforation occurred in 4.05 ± 0.94 weeks while healing of moderate tympanic membrane perforation 5.9 ± 0.50 weeks. Air–bone gap of successes

cases that was 23.72 ± 0.83 dB before the operation became 7.05 ± 0.99 dB after the operation. In Silveira et al. study, temporalis fascia graft group showed healing (tympanic membrane perforation closure) in 13 patients with small perforation (92.9%) and 3 patients with moderate perforation (50%), the mean time of operation was 76.50 ± 17.92 min [21].

Fat graft group in this study showed healing (tympanic membrane perforation closure) in 15 patients with small perforation (75%) and 10 patients with moderate perforation (50%), the mean time of operation was 35.1 ± 0.83 min. Healing of small perforation occurred in 4.97 ± 0.68 weeks while healing of moderate tympanic membrane perforation 7.07 ± 0.95 weeks. Air–bone gap of successes cases that was 20.06 ± 0.18 dB before the operation became 7.83 ± 0.05 dB after the operation.

In Gün et al. study, fat graft group showed healing (tympanic membrane perforation closure) in 37 patients with small perforation (84.1%) and 13 patients with large perforation (100%), hyaluronic acid graft group showed healing (tympanic membrane perforation closure) in 17 patients with small perforation (85%) and 10 patients with large perforation (90.9%), temporalis fascia graft group showed healing (tympanic membrane perforation closure) in 28 patients with small perforation (100%) and 17 patients with large perforation 85% [22].

5. Conclusion

Bacterial cellulose graft myringoplasty would be a good, simple, rapid and safe surgery that could be done under local anesthesia in outpatient clinic with shorter time of surgery than fat graft myringoplasty and temporalis fascia graft myringoplasty, with better hearing and healing.

References

- [1] Ayache S, Braccini F, Facon F, Thomassin JM. Adipose graft: an original option in myringoplasty. *Otol Neurotol* 2003;24(2):158–64.
- [2] Storrs L. Myringoplasty with the use of fascia grafts. *Arch Otolaryngol Head Neck Surg* 1961;74:45–9.
- [3] Ringenberg JC. Closure of tympanic membrane perforations by the use of fat. *Laryngoscope* 1978;88:982–93.
- [4] Mitchell RB, Pereira KD, Lazar RH. Fat graft myringoplasty in children a safe and successful day-stay procedure. *J Laryngol Otol* 1997;111:106–8.
- [5] Laidlaw DW, Costantino PD, Govindara JS. Tympanic membrane repair with dermal allograft. *Laryngoscope* 2001;111:702–7.
- [6] 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;121:43–7.
- [7] Blake CJ. Transactions of the first congress of the international otological society (abstract). New York: D. Appleton and Co.; 1887. p. 125.
- [8] Kanemaru S, Umeda H, Kitani Y, Nakamura T, Hirano S, Ito J. Regenerative

- treatment for tympanic membrane perforation. *Otol Neurotol* 2011;32:1218–23.
- [9] Kakehata S, Hirose Y, Kitani R, Futai K, Maruya S, Ishii K, et al. Autologous serum eardrops therapy with a chitin membrane for closing tympanic membrane perforations. *Otol Neurotol* 2008;29:791–5.
- [10] Hakuba N, Hato N, Okada M, Mise K, Gyo K. Tympanic membrane regeneration therapy. *JAMA Otolaryngol Head Neck Surg* 2014;23:E1–7.
- [11] Castro CMMB, Aguiar JLA, Melo FAD, Silva WTF, Marques E, Silva DB. Sugar cane biopolymer cytotoxicity. *An Fac Med Univ Fed Pernamb* 2004;49:119–23.
- [12] Costa HO, Souza FC. Avaliação da regeneração da membrana timpânica após a colocação de Biotissue. *ACTA ORL* 2005;23(3):23–7.
- [13] Hestrin S, Schramm M. Synthesis of cellulose by *Acetobacter xylinum*. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochem J* 1954;58:345–52.
- [14] Hanmoungjai W, Chakeatirote E, Pathmaree W, Yamoda Y, Lumyoung S. Identification of acid tolerant acetic acid bacteria isolated from Thailand sources. *Res J Microbiol* 2007;2(2):194–7.
- [15] Surma-Slusarska B, Presler S, Danielewicz D. Characteristics of bacterial cellulose obtained from *Acetobacter xylinum* culture for application in paper making. *Fibers Text Ilesin East Eur* 2008;16(4):108–11.
- [16] Saliba I. Hyaluronic acid fat graft myringoplasty: how we do it. *Clin Otolaryngol* 2008;33:607–28.
- [17] Bhat NA, De R. Retrospective analysis of surgical outcome, symptom changes, and hearing improvement following myringoplasty. *J Otolaryngol* 2000;29:229–32.
- [18] Stenfors LE. Repair of tympanic membrane perforations using hyaluronic acid: an alternative to myringoplasty. *J Laryngol Otol* 1989;103:39–40.
- [19] Prior M, Gibbins N, John G, Rhys-Williams S, Scott P. Hyaluronic acid ester in myringoplasty. *J Laryngol Otol* 2008;122:3–5.
- [20] Spiegel JH, Kessler JL. Tympanic membrane perforation repair with a cellular porcine submucosa. *Otol Neurotol* 2005;26:563–6.
- [21] Silveira Fabio Coelho Alves, Pintob Flavia Cristina Morone, Neto Slvioda Silva Caldas, Leal Mariana de Carvalho, Cesario Jéssica, Lamartine de Andrade Aguiar José. Treatment of tympanic membrane perforation using bacterial cellulose: a randomized controlled trial. *Braz J Otorhinolaryngol* 2016;82(2):203–8.
- [22] Gün T, Boztepe OS, Atan D, İkinçioğullar A, Dere H. Comparison of hyaluronic acid fat graft myringoplasty, fat graft myringoplasty and temporal fascia techniques for the closure of different sizes and sites of tympanic membrane perforations. *J Int Adv Otol* 2016;12(2):137–41.