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Tissue engineering and regenerative medicine strategies for the repair of tympanic membrane perforations

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ABSTRACT

Despite the high success rate of autologous grafts in tympanic membrane repair, clinical alternatives are required for the closure of unresponsive chronic perforations that can lead to recurring infection and hearing loss. Tissue engineering and regenerative medicine approaches have emerged as another strategy to repair the eardrum, in addition to negating the need for donor tissue harvest and related surgical iatrogenicities. This review highlights the main approaches using biomaterials, growth factors, and cell therapies towards the healing of complex TM perforations. In addition, we discuss the challenges and advances for the development of reliable animal models, which will allow the optimisation and development of novel techniques. Finally, we indicate technologies that are currently used clinically and others that are closer to the market. The advances here discussed on tissue engineering and regenerative medicine strategies applied to the field of TM perforations will allow otologists, surgeons, and researchers to better bring novel technologies to the bedside as well as to develop new ones.

1. Tympanic membrane perforations

1.1. The anatomical and physiological structure of the tympanic membrane

The tympanic membrane (TM), commonly known as the eardrum, is a semi-transparent membrane composed of three layers, a keratinized squamous epithelial outer layer, fibrous middle layer, and mucosal inner layer [1] (Fig. 1). It separates the middle ear from the outer ear, is oval, with a vertical diameter of 9–10 mm and a horizontal diameter of 8–9 mm [2,3]. The main function of the TM is sound perception and protection of the middle ear [2]. Clinically, the TM can be divided into four parts, with each quadrant separated, by an imaginary line straight down the malleus handle and across the umbo. Additionally, the TM is composed of two portions, the *pars flaccida* and *pars tensa*. The *pars flaccida* occupies the most superior and smaller part of the TM, and is more vascularized than the *pars tensa*. Its flaccid feature is due to a less prominent fibrous layer, lacking the densely arranged collagen fibers present in the *pars tensa*. On the other hand, the *pars tensa* occupies the most inferior an greater part of the TM, as a thin and tense membrane [4] (Fig. 1). Perforations to the TM can be troublesome and still are subject to new technologies and procedures development. The objective of this review is, therefore, to highlight the main approaches using biomaterials, growth factors and cell therapies towards healing of complex TM perforations, including the ones already evaluated clinically as the ones closer to market commercialisation. Prior to analyzing this approaches, we will discuss TM perforations clinically and current surgical interventions. We will also discuss the challenges and advances for the development of reliable animal models of TM perforations.

1.2. Clinical aspects involving tympanic membrane perforations

TM perforations are a common problem most frequently caused by middle ear infections, ventilation tube insertion, trauma, and an increase of pressure on the TM [2,5]. The exact size of TM perforations is rarely known, as ENT surgeons tend to guess the size of the perforations with one quadrant perforation equal to 25% [6]. TM perforations are categorized as either acute or chronic and dry or wet [2]. The closure rate depends on the perforation type, with almost all acute, wet perforations closing spontaneously (77–94%) within a few weeks [7]. The re-

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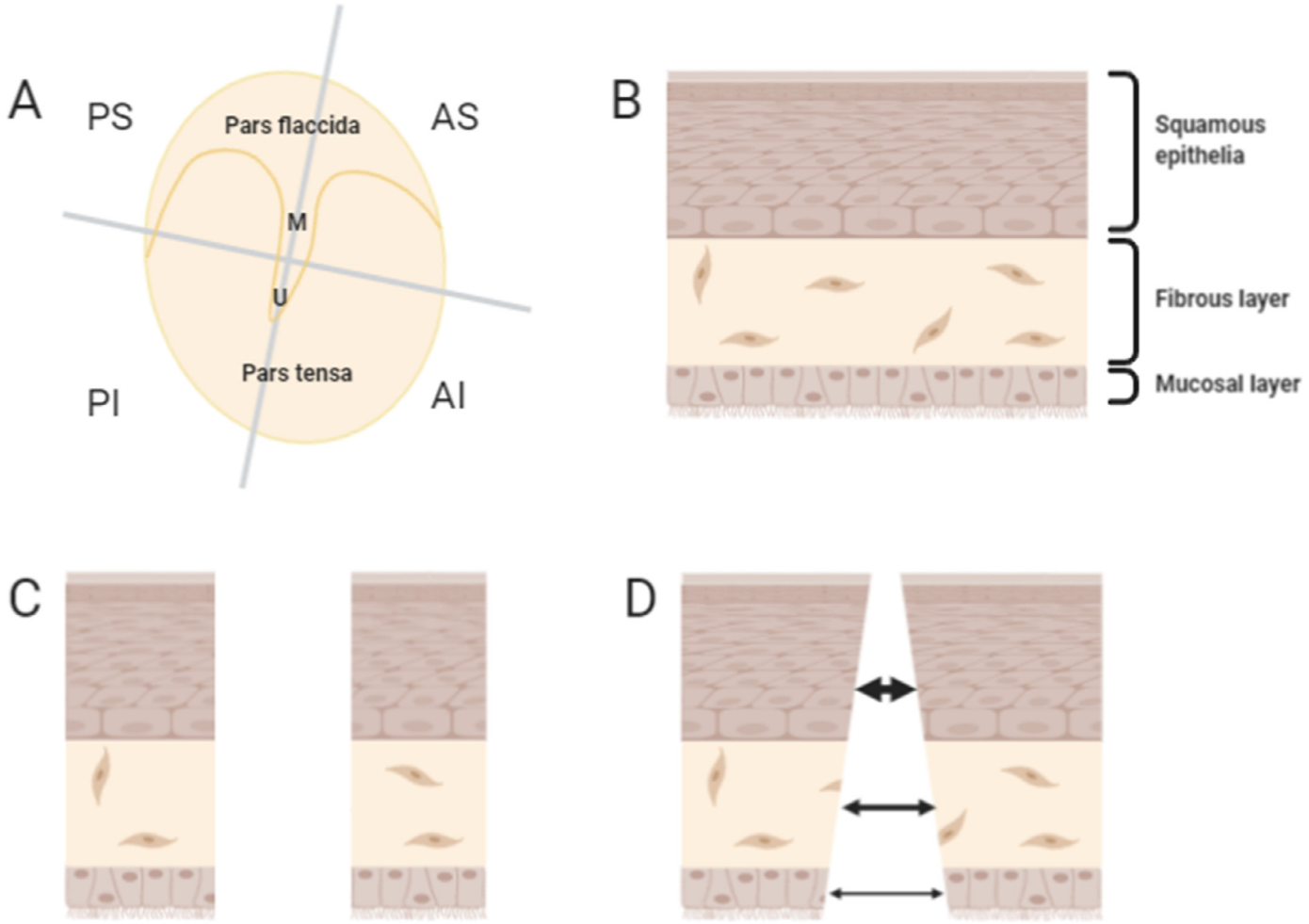

Fig. 1. Tympanic membrane anatomy, histology, perforation, and regeneration: (A) the tympanic membrane can be divided into four parts, posterior superior (PS), anterior superior (AS), posterior inferior (PI), and anterior inferior). The line that divides its anterior and posterior parts passes through the malleus (M) and the umbo (U). (B) Histologically it is divided into three layers: a squamous epithelium, a fibrous layer, and a mucosal layer. (C) As a thin membrane (74–100 μm), TM perforations easily rupture the three layers. (D) TM repair differs from classical wound healing as keratinocytes from the squamous epithelia migrate and bridge the perforation first, followed by the fibrous and the mucosal layers.

generation of the TM follows a process different from that of skin wound repair in that squamous epithelia migrates towards and covers the perforation closing the defect [8,9] (Fig. 1). This process is then followed by the reforming of the fibrous layer, followed by ingrowth of the mucosal layer [8,9]. TM perforations that do not close spontaneously and are present for more than three months are considered chronic [2]. The size of the perforation, which affects the ability of the epithelial cells to span the defect, is one main reason for the development of a chronic TM perforation [9]. As a result, an epithelialised rim is formed around the edge of the perforation, affecting the healing process by preventing the migration of cells across the defect and insufficient supply of growth factors to aid in the repair process [9].

1.3. Surgical interventions to repair tympanic membrane perforations

Treatment for chronic TM perforations are limited to a surgical procedure known as tympanoplasty and without this surgical intervention, chronic TM perforations can cause hearing loss and reoccurring infections [2]. An autologous graft such as fascia or cartilage is harvested from behind the ear and is placed over or under the TM perforation, by using the transcanal (through the ear canal) or postauricular (incision made behind the ear) approach [10]. Typical graft materials used in tympanoplasty to close TM perforations include temporalis fascia from behind the ear, perichondrium found on either side of the cartilage in the ear, cartilage from the outer ear, and fat from the earlobe [4]. Although the use of graft material to close chronic TM perforations have a high success rate of approximately 80%, the surgery requires the use of general or local anesthesia, harvesting of graft material, can result in surgical complications, such as hearing loss, cholesteatoma and tympanosclerosis, can be very expensive and is the only surgery available for the repair of chronic TM perforations [2,4]. Therefore, there is a need to develop cost-effective and non-surgical alternatives. Tissue engineering has become a new approach to repairing TM perforations using biomaterial scaffolds composed of various materials alone or in combination with growth factors and cells, however, an optimal alternative is yet to be developed [2]. With the increase in the development of biomaterial scaffolds, there is a greater need for an appropriate model in which to accurately assess the new biomaterials and their potential success in humans.

2. Biomaterial-based strategies to repair tympanic membrane perforations

The International Union of Pure and Applied Chemistry (IUPAC) defines a biomaterial as a “material exploited in contact with living tissues, organisms or microorganisms” [11]. Several natural and synthetic biomaterials have been investigated for the healing of TM perforation. Also, the designing process and techniques to fabricate these biomaterials can

Table 1
Current commercially available biomaterials for TM perforations.

Product	Material	Description	Manufacturer
EpiFilm	Hyaluronic acid	2.5 x 2.5 cm lamina	Medtronic (Ireland)
EpiDisk	Hyaluronic acid	Pre-cut, 8 mm spherical discs of EpiFilm® Material	Medtronic (Ireland)
MeroGel	Hyaluronic acid	Packing material	Medtronic (Ireland)
BioDesign Otologic	collagen matrix derived from porcine small intestinal submucosa	Implantable scaffold	Cook Medical (Indiana, USA)
MegaDerm	Dermal allograft	Implantable scaffold	L&C Bio (South Korea)
AlloDerm	Dermal allograft	Implantable scaffold	Allergan (Ireland)
Tutopatch	Bovine pericardium xenograft	Implantable scaffold	Rti Surgical (Illinois, USA)
GelFoam	Gelatin	Packing material	Pfizer (New York, USA)
GelitaSpon	Gelatin	Packing material	Gelita Medical (Germany)

greatly vary, for instance from the more traditional electrospinning and lyophilisation techniques to more recent 3D printing approaches. The main biomaterials currently commercialized are summarized in Table 1.

2.1. Hyaluronic acid

Hyaluronic acid (HyA) is a glycosaminoglycan found throughout the body. HyA plays a role in tissue healing by attracting water molecules to the area, altering its viscoelastic properties enhancing tissue healing, and scaffold organization [12]. It has been shown that hyaluronic acid forms a thin film with keratin that allows migration of epithelial cells towards closure of the perforation and allows proper repair of the fibrous layer of the TM, regulating collagen deposition and avoiding fibrosis [13]. Pre-clinical trials and clinical trials have not only demonstrated safety with the use of HyA for TM perforations but also an acceleration of closure, with thinner scar tissue. Particularly for TM repair, hyaluronic acid is currently commercialised in disk forms by Medtronic as EpiDisc® and EpiFilm® or in injectable packing material form as MeroGel® [13] (all from Medtronic, Ireland).

A review carried out by Daou and Bassim on the use of HyA in otology highlighted the lack of HyA research for the repair of the TM, with only fourteen studies published since 1987 and the two most recent papers in 2013 and 2018 [12]. In 2013, Sayin et al. published a comparative study of spontaneous TM perforation healing against healing with EpiFilm® [14]. No significant difference was observed between the treated and control group with 85.65% of the control group closing with a mean of 10.6 ± 5.23 weeks, the treated group had a closure rate of 94.8% and a mean closure time of 6.61 ± 4.59 weeks. A study from 2018 using HyA for TM repair [15] assessed fifty patients with dry chronic TM perforations of 1−3mm who received a topical application of 1% sodium hyaluronate, four times a week for a month. Each patient was followed up weekly for the first four weeks, then three months after the last application of 1% sodium hyaluronate. Of the fifty TM perforations, twenty-six closed, eighteen were reduced in size and six remained persistent resulting in an 88% closure rate. It is important to highlight though that this trial did not present a control group to compare the application of HyA with. In a rat traumatic acute TM perforation model, Yilmaz et al. results corroborated HyA (EpiFilm®) capacity to induce healing with lesser fibrosis [16].

Of note, HyA can also be used as an additive to other materials or techniques. For instance, Kadah et al. compared temporalis fascia myringoplasty alone or in combination with HyA in a prospective non-randomized controlled trial, although no statistical differences between the groups were observed [17]. On the other hand, HyA used in combination with fat graft has shown encouraging results. For instance, Gun et al. compared HyA fat graft myringoplasty, fat graft myringoplasty, and temporal fascia techniques. Although the authors did not find statistical differences between the three groups' successes in healing TM perforations, they advised the use of HyA fat graft for large perforations and fat graft alone for small perforations, due to technical challenges on using temporalis fascia [18]. Of note, for large perforations, the use of fat graft alone does not result in a flat TM, which can be obtained when HyA is added [19].

To conclude, HyA has been highlighted as a potentially cost-effective alternative for TM perforation repair, which encourages further research in the field [14]. For instance, future studies should focus on the direct comparison of HyA against commonly used autografts. Moreover, the combination of HyA with other materials and techniques opens a great avenue for its application, with a promising perspective.

2.2. Collagen

Another extracellular matrix component with successful use in TM perforation repair is collagen. Of note, collagen is a major constituent of the tympanic membrane, particularly collagen types I, II, and III [20]. Acellular extracellular matrix grafts or tissue-derived allografts, in which collagen is the main component, have been widely used clinically for different applications [21]. Cass et al. reviewed the use of processed human pericardium collagen allografts (Tutoplast® by Rti Surgical, Illinois, USA) with autologous tissue, alone or combinations (collagen allograft alone, fascia, perichondrium, cartilage, cartilage+perichondrium, cartilage+collagen allograft) in tympanoplasty. With an analysis of 255 patients, there were no statistically significant differences in the failure rate between collagen allografts and the analysed autologous tissues. Nevertheless, even not significantly, the chances of success of cartilage and perichondrium were 7.5 times higher than of collagen allografts [22]. Particularly, acellular dermis allograft is commonly used in the field. In 2018, Lee et al. compared the commercially available MegaDerm® (L&C Bio, South Korea) dermis allograft with autologous tragal perichondrium in a prospective randomised controlled study with sixty patients. Both were similarly successful, with regards to significantly lower mean operation time in the dermal allograft group [23]. Another acellular dermis allograft, AlloDerm®, was as beneficial as fascia grafts in chinchilla models, with an added advantage of saving graft procedures [24]. When AlloDerm® (Allergan, Ireland) was compared to fascia and fascia+ cartilage grafts in a clinical trial, again there were no clinical differences between groups despite saving operative time in AlloDerm® group [25]. On the other hand, another clinical trial evidenced statistically significant shortened healing time with AlloDerm® compared to temporalis fascia [26].

Collagen xenografts have also been exploited. Still, in the late 70s, Abbenhaus reported a five-year follow-up with the successful use of reconstituted bovine collagen for tympanic membrane grafting [27]. Most recently, Declau et al. compared a commercially available bovine membrane xenograft extracted from the pericardium (Tutopatch® by Rti Surgical) with collagen allografts in seventy-one patients submitted to tympanoplasty. There were no significant differences between the two materials closure rates (81.6% for xenografts and 78.8% for allografts) [28]. Another collagen-based material used for various otologic procedures is the BioDesign® Otologic Repair Graft (Cook Medical, Indiana, USA). It is derived from porcine small intestinal submucosa (SIS) and has been proposed to achieve closure rates clinically comparable to temporalis fascia [29,30], therefore eliminating donor-site morbidity and saving an average of ten minutes of surgical procedure [31].

Some approaches associate collagen with other components aiming at increased success. For instance, Zhang et al. proposed a collagen mem-

brane integrated with collagen-binding basic fibroblast growth factor. *In vitro*, the controlled release of the growth factor accelerated fibroblasts proliferation, and in vivo, in a rat traumatic TM perforation model, the membrane accelerated healing, promoting early stages of healing rate (~7 days) [32]. In 2016, Choi et al. reported the results of twenty-nine patients with traumatic TM perforations treated with a commercially available collagen-bound fibrinogen sealant (Tachocomb® by Takeda, Japan). The used sealant completely closed the perforations in all patients, although a comparison with a control group was not performed [33].

Allografts and xenografts remain as the main materials in which collagen, as an extracellular matrix constituent, is used for the repair of TM perforations. Nevertheless, for several other regenerative medicine applications, collagen has been used as a biomaterial not only derived from a decellularized matrix but also having been extracted, purified, and polymerized into a new scaffold [21]. Future TM perforation studies should focus on this last, so that collagen biomaterials can be specifically designed for such application, also allowing controlled incorporation of other materials and chemical cues which could further enhance its regenerative potential.

2.3. Gelatin

Gelatin, which is obtained from collagen hydrolysis [34], has traditionally been used in tympanoplasty. Gelatin film protects against fibrosis [35], and is harmless to the lining mucosa of the middle ear [36], being superior to other types of films such as silastic [37]. In a technique named GelFilm tympanoplasty, fascia grafts are surrounded by two sheets of gelatin films, as a sandwich, which gives support to the graft [38]. Besides its use as films, gelatin is also traditionally used as a sponge, a haemostatic packing material implanted in the middle ear to give support to a graft in the healing TM perforation [39]. Several commercially available products address this purpose, such as Gelfoam® (Pfizer, New York, USA), GelitaSpon® (Gelita Medical, Germany) [40], and Marbagelan® (Sanofi-Aventis, France) [41]. In small perforations, the fat plug technique can be used, in which adipose tissue is harvested from the lobule of the outer ear or the subcutaneous tissues behind the ear and plugged into the perforation without the need of an additional graft, such as fascia [42]. Gelfoam® plug achieved comparable results to fat graft tympanoplasty in a prospective study of 17 patients with perforations ranging from 2 to 4 mm, with the advantage of a simpler and faster procedure [43].

More recent strategies focused on incorporating growth factors into gelatin sponges. Saeedi et al. evidenced that enriching gelatin sponges with platelet-rich plasma increased the complete healing rate of TM perforation compared to conventional gelatin sponges [44]. Another promising approach by Kanemaru et al. developed a gelatin scaffold with basic fibroblast growth factor (bFGF), which induced complete closure of chronic TM perforations in 98.1% (52/53) of the patients, compared to 10% (1/10) of the patients in the control group without the growth factor [45]. Similarly, in 2015 [46] a human clinical trial on chronic TM perforations in children under the age of 16 was carried out. Their study followed the protocol described by Kanemaru et al. [45]. Initially, they used fibrin glue to fix the gelatin sponge in the TM; however, the first two patients experienced liquefaction of the fibrin glue resulting in the rapid onset of otorrhoea. Therefore, a drop of cyanoacrylate was used, or a drop of blood on the top of the sponge in lieu of fibrin glue. 83% of TM perforation closure was achieved, with only 58% achieved by the first attempt with the fibrin glue. The range of closure time was between two weeks and three months post-treatment. Each of these studies concluded that bFGF delivered to the TM perforation on a gelatin sponge is cost-effective, suitable for children as young as six years old, a considerably shorter procedure of just seven minutes, and the use of less sophisticated instruments. The gelatin sponge can be cut to any size and therefore be used for any size perforation; it is also gradually reabsorbed within three months. One of the limitations of this approach is the need for a sealant. The sealant is required to hold the sponge in place, insulate it from the outside, prevent cells from drying out and reduce the risk of infection [45]. In 2018, the gelatin with bFGF strategy was tested in forty-five patients, of which twenty-five cholesteatomas, three had tumors, and seventeen had severe TM calcification. Complete closure of the TM perforation was achieved in 91% of the patients [47]. Each study also describes the need to repeat the surgery more than once for several patients, implying that the first attempt is not always successful and the patient may have to return to the consultant a couple of times before the gelatin sponge stays in place and the TM perforation closes. Lou et al. recently reviewed the use of bFGF in TM closure and pointed out three studies in which the presence of bFGF in gelatin sponges improved the success rate of chronic TM perforation treatment (83–98.1% with bFGF to 10% without bFGF) [48].

The potential of gelatin to be 3D printed has inspired an approach by Kuo et al. who tested 3D printed TM grafts made of gelatin methacrylate (GelMA) in a chinchilla model. Importantly, the graft shape was based on endoscopic images of the perforation, and using a 3D printed gelatin support that was later washed away, it was possible to place the scaffolds in the lesion site without the need of glues, suture, or any other support material. 100% of treated tympanic membrane perforations healed when the scaffold was embedded with epidermal growth factor (EGF), 75% with the grafts without EGF, while only 25% with no graft [49]. Indeed, 3D printing or additive manufacturing (AM) is often proposed to represent the next frontier in the field of tissue engineering and regenerative medicine as they allow the manufacture of scaffolds with anatomically inspired and complex morphologies [50]. Finally, through the electrospinning technique, Li et al. used gelatin to develop nanofibrous membranes with tensile strength and water-resistance considered suitable for use as TM patches. The patches supported the growth of human umbilical vein endothelial cells and fibroblasts *in vitro* [51].

Despite the traditional use of gelatin as films and sponges, the advent of more advanced techniques for biomaterials fabrication, such as the here mentioned 3D printing, has enabled researchers to further explore the use of gelatin for TM perforations. Rather alone, in combination with other biomaterials or with growth factors, gelatin is being rediscovered in the field, with great promise.

2.4. Silk and cellulose

Silk fibroin-based materials also show potential towards tissue engineering applications due to their biocompatibility and suitable mechanical properties [52]. Silk fibroin scaffolds have shown the capacity to support the growth of human tympanic membrane keratinocytes *in vitro*, as well as structural and mechanical properties, such as transparency, stability, and tensile strength, that promoted them as good candidates for use in otology [53,54]. In 2013, Shen et al. compared silk fibroin scaffolds with acellular collagen scaffolds, paper patches, and untreated TM perforations in guinea pigs. Both silk fibroin and acellular collagen scaffolds induced healing at earlier stages, with histological characteristics of repair tissue that closely resembled a native TM [55]. The same group later implanted the silk fibroin and acellular collagen scaffolds in rat's subcutaneous tissue and middle ear, comparing with paper and Gelfoam®. The scaffolds induced a milder inflammatory response compared to paper. Moreover, Gelfoam® was more associated with fibrosis and osteoneogenesis after implantation in the middle ear compared to the silk fibroin and acellular collagen scaffolds [56]. Lee et al. used silk fibroin in a polycaprolactone/silk-fibroin nanofibrous composite combined with human umbilical cord serum, which presented increased biocompatibility and enhanced healing potential in a guinea pig TM perforation model [57]. In 2016, a clinical prospective cohort study (40 patients) compared perichondrium myringoplasty with silk fibroin patch and evidenced a similar success rate between the groups while the use of silk fibroin was considered an easier and faster procedure [58]. Although the use of silk for TM perforations is still novel, the few studies

in the literature presented promising results. With the support of more pre-clinical and larger clinical trials, silk scaffolds have great potential to become commercially available and come part of the routine clinical use for TM perforations.

Cellulose is another highly versatile natural biomaterial [59]. It can be obtained from plants and bacteria, being designed into biomaterials scaffolds through different techniques, including 3D printing [60]. Kim et al. produced bacterial cellulose nanofibrillar patches for TM repair. The thin and transparent patches were able to support the growth of rat TM cells as well as increase the healing of rat TM perforations *in vivo* compared to spontaneous healing controls [61]. In 2016, Silveira et al. conducted a clinical trial with forty patients comparing bacterial cellulose with autologous fascia in TM perforations secondary to chronic otitis media. Although the closure of perforations was similar between the groups, the use of the cellulose graft significantly reduced the procedure time (5.44 times faster with cellulose) and its costs (12.96 times cheaper with cellulose) [62]. Indeed, the use of cellulose for TM perforations is still in its infancy and should attract the attention of researchers and clinicians due to its high potential.

2.5. Synthetic biomaterials and composites

In a pioneering study using electrospinning and additive manufacturing, Mota et al. developed TM scaffolds inspired by the anatomy of the human tympanic membrane and its collagen fibre arrangement. FDA-approved copolymers were used, and human mesenchymal stromal cells were able to attach, migrate and proliferate on the scaffolds [63]. Kozin et al. 3D printed TM scaffolds with polydimethylsiloxane (PDMS), flex-polylactic acid (PLA) and polycaprolactone (PCL) infilled with a fibrin-collagen composite hydrogel. *In vitro,* acoustic and mechanical tests evidenced the novel scaffold acoustic properties similar to human TM and mechanical properties superior to human temporalis fascia [64].

Seonwoo et al. developed a nanofibrous patch made of PCL capable of sustainably release epidermal growth factor (EGF). Moreover, it was possible to align the patch fibres to facilitate cellular migration. *In vitro* and *in vivo* studies evidenced the success of the novel patches in promoting TM healing. Particularly, in a rat chronic TM perforation model, the regeneration rates of the patches were significantly superior to untreated controls, with a greater superiority of aligned over random fibres [65].

Finally, other synthetic polymers, besides the already mentioned PLA and PCL, have been investigated as material sources to manufacture scaffolds for tympanic membrane repair. For instance, Danti et al. developed PEOT/PBT copolymer ultrafine scaffolds (220 ± 56 μm thickness), capable to support the growth of human MSCs and human tympanic membrane keratinocytes *in vitro* [66]. Immich et al. produced through electrospinning meshes of poly (L-lactic acid) and poly (lactic-co-glycolic acid) (PLLA/PLGA). The scaffolds supported a co-culture of human keratinocytes and fibroblasts, with differentiation and stratification of the keratinocytes in an epithelial-like tissue. Co-cultured grafts were implanted in rat TM perforations, which healed faster and with better macroscopic and histologic characteristics compared to untreated perforations [67].

To summarize, synthetic biomaterials have been extensively investigated in the field of tissue engineering and regenerative medicine mainly due to their highly controllable development and manufacturing features. It is not surprising that they would find applicability in the repair of TM perforations. Probably, a commercially available synthetic graft for TM perforation is not far to become a reality. Focusing on FDA-approved materials can accelerate this process.

3. Strategies focusing on growth factors/biomolecules

The repair of TM perforations is stimulated by growth factors [2]. The two common growth factors studied for TM regeneration are epithelial growth factor (EGF) and basic fibroblast growth factor (bFGF) [1]. Most recent studies involving topical applications of regenerative biomolecules for the repair of TM perforations are reviewed in Table 2 below.

3.1. Epithelial growth factor (EGF)

EGF is a polypeptide, made up of 54 amino acids, it is found in numerous biological tissues and fluids and is known for having a role in wound repair by increasing the growth of epithelial and fibroblast cells [75]. EGF expression is parallel to the reparative process as it stimulates the synthesis of DNA, RNA, and proteins. EGF receptors with high affinity are found in the epithelial layer of the TM. In 1995 Ramsay et al. [75] carried out a human clinical trial on chronic TM perforations repair using rice paper patch and sponge soaked in EGF. The results of the study concluded that EGF did not support the regeneration of the TM with only one TM perforation in the placebo group closing. However, today EGF is one of the most researched growth factors for the closure of TM perforations. Recently, two studies [68,69] carried out a similar human clinical trial on chronic and subacute TM perforations. EGF was topically applied to the ear canal, followed by self-administration of EGF drops to the TM by the patients daily. Lou et al. found 61.1% of non-treated perforations healed spontaneously with a mean closure time of 20.6 ± 10.7 days. Whereas, 96.2% of the EGF treated group, achieved complete closure, with a mean closure time of 9.1 ± 3.9 days. Similarly, the study carried out by Lou 2019 saw a 100% closure rate of the 24 TM perforations with a mean closure time of 6.1 ± 2.3 days. However, no control group was included in this study to determine the rate of closure. These studies highlight the potential of topically applied EGF for the repair of TM perforations. The self-administration of the EGF drops by the patients themselves could reduce the waiting time, surgery time, and cost currently associated with TM perforation repair.

3.2. Fibroblast growth factor 2 (FGF-2 or bFGF)

FGF-2 (also known as bFGF) is a polypeptide that stimulates the proliferation of epidermal and connective tissue cells [71]. Clinical studies using topical FGF to close TM perforations have shown promising results, however, there are potential side effects and health and safety concerns with the use of FGF. Some studies have found that FGF is not ototoxic [45,76], whereas others have found it to cause hyperplasia of the ear canal, myringitis, and long-term cholesteatoma [71,77]. In 2018 [71] a human clinical trial was carried out to determine the short and long-term effects of topically applied FGF-2 to treat TM perforations. The total closure rate for the FGF-2 group of 95.5% was found to be significantly different from the control group of 73.4%. The FGF-2 group also had a significantly shorter closure time of 11.9 ± 3.1 days vs 52.6 ± 18.1 days for the control group. There were some short-term side effects in both the control and treated group, with 32% of patients in the treated group developing liquid residue in the ear canal due to misuse of FGF-2 drops, resulting in purulent otorrhoea in 20% of patients. It is worth noting that purulent otorrhoea was also documented in seven patients in the control group. Three patients that experienced purulent otorrhoea developed secondary otitis media effusion as a long-term side effect, resulting in reperforation of the TM. In 2016 [7], a human clinical trial based on the novel therapy using gelatin sponge with bFGF (also known as FGF-2) and fibrin glue developed by Kanemaru et al. was carried out. 88.9% of patients achieved closure of the TMP, with a median closing time of 57 days. However, six of the nine patients required more than one repeat surgery, causing some patients twelve weeks to see complete closure of the TM perforation. There was no control used in this study, the results were compared to a study the group carried out in 2011 [45].

In 2017 Lou et al. [72] carried out a comparative study to evaluate the efficacy of EGF and FGF to repair TMP. In this study EGF or FGF was topically applied to the ear canal followed by self-administration of EGF or FGF drops daily. The study revealed that the closure rates did

Table 2
Recent studies involving regenerative biomolecules for the repair of TMP.

Biomolecule	Results	Model	TM perforation	Refs.
EGF	100% closure rate range 3–12 days	Human	Chronic	[68]
EGF	96.2% closure rate range 3–14 days	Human	Subacute	[69]
Fat graft soaked in PRP	Increase in adipocyte area, decrease in granulation tissue area in comparison to fat graft alone	Rat	Acute	[70]
FGF-2	98.1% closure for non-pathological TMP, 10.1 ± 4.6 days 86.7% closure rate for pathological TMP, 19.1 ± 7.3 days	Human	Not specified	[71]
1% HyA topical application	52% closure 36% TMP size reduction. Time of closure not specified	Human	Acute and chronic, 1–3 mm	[15]
EGF and FGF-2	EGF – 91.11% closure rate 9-16.5 days FGF-2 – 93.18% closure rate 7-15 days	Human	Acute dry	[72]
Human Insulin	Accelerated healing, 100% closure 5-7days.	Rats	Acute	[73]
bFGF	58% closure rate first attempt 83% closure rate overall	Human	Chronic	[46]
Plasminogen	100% closure rate with higher concentration 60% closure rate with lower concentration	Mice	Acute and Chronic	[74]

Table legend: EGF- Epithelial growth factor, PRP- Platelet rich plasma, FGF-2- Fibroblasts growth factor-2, bFGF- Basic fibroblast growth factor, HyA – Hyaluronic acid

not differ significantly between either group. 93.18% of TM perforations treated with FGF closed within 18 ± 8.04 days. Whereas, 91.11% of those treated with EGF closed within 17.36 ± 5.46 days. The work carried out by Lou et al. over the last few years, suggests that EGF topically applied is a prime candidate for an alternative non-surgical and cost-effective way to repair TM perforations. Further research is required on the use of topical FGF as an alternative method for TM perforation repair, due to the side effects associated with FGF and its misuse when self-administered by patients. The need to return for repeat surgery only increases the wait time and cost for both the patient and the consultant.

3.3. Other biomolecules

In recent times the use of biomolecules for TM repair has expanded beyond EGF and bFGF to include different biomolecules such as plasminogen, insulin, and platelet-rich plasma. Plasminogen is a zymogen produced in the liver and activated in the blood by plasminogen activators or urokinase-type PA to support tissue remodeling and wound healing [74]. Shen et al. carried out an animal study with plasminogen deficient mice, with chronic TM perforations treated with local plasminogen injections of varying concentrations into the ear canal. After nine days complete closure of the TM perforations was seen in 100% of mice that received a plasminogen injection of either 0.2 or 0.4 mg, and 60% that received a plasminogen injection of 0.1 mg/day. The chronic model used in this study may not have been appropriate, with the perforations left untreated for only nine days. Therefore, these perforations may have been acute, and the healing rate potentially affected by the natural spontaneous healing associated with acute perforations. Local injection of plasminogen has the potential to be an alternative method for TM perforation repair; however, it requires daily treatment that patients are not capable of performing themselves, and therefore, may not advance to clinical trials.

In recent years, the use of topical insulin has been researched for the application of wound healing. Insulin is known to be involved in the synthesis of lipids, glycogen, and amino acids and plays a role in keratinocyte migration [73]. In a recent study carried out on twenty rats, Araujo et al. [73] applied fifteen units of Regular Novolin R® human insulin (Novo Nordisk, Denmark) topically to eleven perforated TM. Insulin was found to have reduced the size of the TM perforations between day three and five and by day seven all eleven perforations were completely closed, whereas 20% of the nine TM perforations in the control group remained perforated. Treatment began on the day the perforation was created, therefore, it is difficult to directly compare these results for the repair of chronic TM perforations. As the TM does not repair in the same sequence as wound healing, further research on the healing effect of topical insulin, specifically for TM perforations is required, before insulin can become a potential candidate as an alternative approach to TM perforation repair.

Platelet-rich plasma (PRP) is plasma derived from blood with a high concentration of platelets. Within the platelets are alpha granules that contain various growth factors associated with the repair process [70]. The growth factors are released from the alpha granules in response to calcium and PRP, resulting in an increase of growth factors in the area, beginning the repair process [78]. Due to the role of PRP in the healing process, it has been researched as an alternative biomolecule to assist in the repair of TM perforations [70]. In 2018, a study used PRP on fat grafts to repair TM perforations in rats [70]. Fat grafts can be used to repair small central TM perforations, however, a graft size twice as large as the perforation is required as the fat is reabsorbed, resulting in dissatisfaction of graft survival [70]. Many studies have shown the positive effect of applying growth factors such as PRP to the fat graft to prevent reabsorption and adding to the success of the graft for TM perforation repair [70]. The 2018 study found that PRP decreased fat graft reabsorption with the control group of just a fat graph reducing to 63.11% ± 29.86, whereas the fat graph soaked in PRP was not reabsorbed as much with 90.81% ± 12.96 remaining. However, there was no significant difference, with a *P*-value of 0.009 [70]. The ability of the fat graft soaked in PRP to close TM perforations were not discussed by the author of the study. The main purpose of PRP in this study was to prevent the fast absorption of the fat graph before the TM perforation had begun to heal. Considering the ability of PRP to close TM perforations was not discussed in this study, it would suggest that PRP was not used for its known wound healing properties, and therefore, cannot be considered as an alternative topical biomolecule in the same way as EGF or FGF.

4. Strategies focusing on cell therapies

Due to their role in the healing process, either by releasing a range of trophic factors, cytokines, matrix proteins, or by differentiating into local tissue when stimulated [79], stem cells have also been researched in the field of TM repair. There have been a handful of studies carried out using stem cells to repair TM perforations, by either topical application or delivery on a biomaterial scaffold. These studies are listed in Table 3.

In 2003 [80] a study used gerbils to access the ability of embryonic mice stem cells (ESCs) to enhance the repair of acute TM perforations. The ESCs were applied topically to the TM perforation in a sodium chloride solution. All of the TM perforations in the test group healed within five days, with 60% in the control group remaining open. However, after healing the TM underwent a pressure point test, in which 60% of the healed TM ruptured under pressure [80]. This study did not analyse the integration of the stem cells into the TM, which would illustrate the cell

Table 3
Strategies focusing on cell therapies.

Reference	Success rate	Stem cell application	Animal
[80]	100%	Topical application in sodium chloride solution	Gerbil
[81]	100%	Topically applied on gelatin platform	Sprague-Dawley rats
[82]	88.75%	MSC embedded Gelita-Spon and EpiDisc	C57BL/6 mice
[83]	71%	Bio-printed scaffold	Sprague-Dawley rats

type these stem cells would develop into, or if their action was solely paracrine, i.e., by secretion of growth factors and cytokines.

A similar study was carried out in 2007 [81] where a droplet of gelatin was applied to the TM perforation of Sprague-Dawley rats to produce a platform for the ESCs applied in solution to migrate and proliferate on. A second test group only received the topical application of the ESCs in solution with no gelatin platform. No significant difference in TM closure rates was found between the treated or control groups. The potential use of ESCs still depends on cell purity, amplification, and immunogenicity [81]. The study concluded that there was no difference between the ESCs treated ears and the non-treated in terms of TM stiffness and pressure tolerance. However, there was a morphological difference in that those treated with ESCs had a thicker TM at the site of the perforation due to disarrangement of the lamina propria.

In 2016, Goncalves et al. [82] investigated the effectiveness of scaffold-embedded MSCs as a topical treatment for healing TM perforations. Bone marrow-derived MSCs were cultured on Gelita-Spon® (Gelita Medical) and EpiDisc® (Medtronic) scaffolds, before applying to TM perforations in mice. After seven days, 100% of the TM perforations in the control group ($n = 9$) remained open. Partial and complete closure of TM perforations after seven days was observed for both Gelita-Spon and EpiDisc embedded with MSCs. EpiDisc with embedded MSCs was found to have a closure rate of 88.75% and Gelita-Spon a closure rate of 80% compared to 71.69% for the control group.

In 2017, Jang et al. [83] took a similar approach to repair TM perforations with stem cells, by delivering the stem cells to the ear canal in a bio-printed scaffold, composed of polycaprolactone/collagen/alginate-MSC. Subacute TM perforations were created in Sprague-Dawley rats. After four weeks, the edge of the perforation was freshened and the bio-printed scaffold was placed over the perforation and held in place by fibrin glue. 28.5% of the control group and 71% of the test group healed within two weeks, after three weeks, the entire test group had healed. The regeneration at two and three weeks was significant between the control and test group.

Each of these studies heighlighted the potential use of stem cells for the repair of TM perforations, and the lack of research carried out in this area. They also highlight the potential of undifferentiated stem cells to generate tumor cells in the host with further research in this area required. Each of these studies used acute TM perforations and therefore, the benefits of using stem cells to repair chronic TM perforations remains unknown.

5. Future perspectives

5.1. Novel strategies closer to the market

As discussed in this review, current tissue engineering and regenerative medicine strategies to repair TM perforations focus on growth factors, cells, or biomaterials. Regardless of the strategy, the main gaps and needs for the closure of TM perforations reside in the use of off-the-shelf technologies that avoid the harvesting of autologous grafts (cartilage, perichondrium, fascia, etc.), simplifying and reducing the time of surgical procedures, while inducing the closure of the perforations.

Indeed, several biomaterials are already a reality in the clinical practice for TM perforations (Table 1). On the other hand, Table 4 presents some of the most promising biomaterials although not yet commercialised for TM perforations. They are based on silk (ClearDrum by Ear Science Research, Australia), gelatin with bFGF (Kaken Pharmaceuticals, Japan and NobelPharma, Japan), and a light-curable gel (Perf-fix by Tympanogen, Virginia, USA). TymCure (from Israel) does not specify the material by which its implantable scaffold is made of. Nevertheless, the implantation of such scaffold is supposed to be performed using a minimally invasive delivery device.

In regards to growth factors, the only current FDA-approved growth factor for clinical use is the platelet-derived growth factor-BB (PDGF-BB or becaplermin), commercialized as Regranex®, which is indicated for the treatment of lower extremity diabetic neuropathic ulcers [84]. Although EGF and bFGF trials are promising for TM perforations, they would still need to be approved for clinical use. On the other hand, PRP has been widely used clinically for a variety of applications, which eases the route to the market from a regulatory perspective. The FDA already clears the off-label use of PRP, as PRP is not a drug. Manufacturers developing medical devices and kits to obtain PRP usually follow the Premarket Notification (PMN) also known as the 510(k) route [85]. Currently, there are already several commercially available kits to obtain PRP from peripheral blood [86].

Finally, in regards to cell therapies, there are currently seventeen FDA-approved cellular and gene therapy products in the market. Eight of those correspond to umbilical cord blood stem cell therapies approved for haematological disorders. Three products use adult cells designed for soft tissue repair: MACI® for cartilage repair, LAVIV (Azficel-T)® for improvement of the appearance of moderate to severe nasolabial fold wrinkles in adults, and GINTUIT® for wounds of the oral soft tissue. Due to the large, and sometimes uncontrolled, increase of stem cell clinics in the USA [87], the FDA has been warning patients, while clarifying the risks and benefits of stem cell therapy [88]. Therefore, it is unlikely that cell therapies will be soon available for TM perforation procedures beyond the scope of a clinical trial.

To accelerate the process of bringing new strategies to market and clinical scenarios, researchers should also focus on the optimization of the current animal models of TM perforations, as well as on the development of new models more reliable to the clinical practice.

5.2. Standardising animal models

Various animal models have been used to evaluate the safety and efficacy of alternative approaches to the repair of TM perforations, including chinchilla, rat, mouse, and guinea pig[89]. However, the ability to create reproducible chronic TM perforations in an animal model has yet to be successfully achieved. As a result, the majority of animal studies are carried out using acute TM perforations, reducing their clinical relevance to chronic TM perforations due to their ability to heal spontaneously.

The current methods used to reportedly create chronic TM perforations are listed in Table 5. Each of these methods has been reported to create a successful chronic TM perforation. However, the studies that report their success, tend to be missing important validating information, which can confirm the creation of chronic TM perforations, such as, the total number of animals used in a study, an observation time of fewer than eight weeks, and the number of animals having to have repeated procedures [89]. Therefore, current literature based on alternative approaches to the repair of chronic TM perforations with biomaterials is lacking a standardised chronic TM perforation animal model, standardised observation period, and the reporting of accurate statistical results.

Table 4
Promising biomaterials for TM perforations closer to market.

Product	Material	Description	Developer
ClearDrum	Silk	Implantable scaffold	Ear Science Research, Australia
Gelatin sponge w/ bFGF	Gelatin + bFGF	Implantable scaffold	Kaken Pharmaceuticals, Japan and NobelPharma, Japan
TymCure	N/A	Implantable scaffold	TymCure, Israel
Perf-Fix	Light curable gel	Implantable scaffold	Tympanogen, Virginia, USA

Table 5
Chronic tympanic membrane perforation creation in animal models.

Refs.	Animal	Method	Observation time (weeks)	Success rate
[90]	Rat	MC+D+HHA	8	62.5–77.7%
[91]	Chinchilla	CL	8	51%
[92]	Mice	KB-R7785	12	90%
[93]	Rat	MC + D + RM	8	NR
[94]	Chinchilla	IT	6	0%
[95]	Guinea pig	MC+ H +C	6	95%
[10]	Chinchilla	TC	6	68.7%

MC – mitomycin; D –dexamethasone; HHA- hammer handle amputation; CL- co_2 laser; RM- re-myringotomy; IT- infolding technique; TM- thermal myringotomy; H- hydrocortisone; C- ciprofloxacin; TC- thermal cautery; Plg- plasminogen; NR- not recorded

Studies that state the creation of chronic TM perforations, should validate their method with otology images of the presence of the perforation at least eight weeks after the perforation was created and histology of the epithelial rim.

6. Conclusions

Here we highlighted the main biomaterials-based, growth factors/biomolecules, and cell therapy strategies to repair TM perforations. Besides promising advances in all lines, the development of novel biomaterial-based strategies is the closest to market commercialisation. Hyaluronic acid, collagen, and gelatin are currently the main representatives of biomaterials for TM perforations. Future strategies should focus on their combination as well as exploration of other extracellular-matrix components. Although biomaterials combined with growth factors represent a current trend, efficient biomaterials capable of repairing TM perforations without the need for growth factors will be further preferred. Finally, there is a great need to optimise current and develop new and more clinically relevant *in vivo* TM perforation models, so those novel strategies may reach the bedside with better probabilities.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Hong P, Bance M, Gratzer PF. Repair of tympanic membrane perforation using novel adjuvant therapies: a contemporary review of experimental and tissue engineering studies. Int J Pediatr Otorhinolaryngol 2013;77(1):3–12. doi:10.1016/j.ijporl.2012.09.022.

[2] Villar-Fernandez MA, Lopez-Escamez JA. Outlook for tissue engineering of the tympanic membrane. Audiol Res 2015;5(1). doi:10.4081/audiores.2015.117.

[3] Vaidya S, Sharma JK, Singh G. Study of outcome of tympanoplasties in relation to size and site of tympanic membrane perforation. Indian J Otolaryngol Head Neck Surg 2014;66(3):341–6. doi:10.1007/s12070-014-0733-3.

[4] Luers JC, Hüttenbrink KB. Surgical anatomy and pathology of the middle ear. J Anat 2016;228(2):338–53. doi:10.1111/joa.12389.

[5] Saliba I, Abela A, Arcand P. Tympanic membrane perforation: size, site and hearing evaluation. Int J Pediatr Otorhinolaryngol 2011;75(4):527–31. doi:10.1016/j.ijporl.2011.01.012.

[6] Lerut B, Pfammatter A, Moons J, Linder T. Functional correlations of tympanic membrane perforation size. Otol Neurotol 2012;33(3):379–86. doi:10.1097/MAO.0b013e318245cea5.

[7] Omae K, Ichi KS, Nakatani E, et al. Regenerative treatment for tympanic membrane perforation using gelatin sponge with basic fibroblast growth factor. Auris Nasus Larynx 2017;44(6):664–71. doi:10.1016/j.anl.2016.12.005.

[8] Maria PLS, Redmond SL, Atlas MD, Ghassemifar R. Histology of the healing tympanic membrane following perforation in rats. Laryngoscope 2010;120(10):2061–70. doi:10.1002/lary.20998.

[9] O'Reilly RC, Goldman SA, Widner SA, Cass SP. Creating a stable tympanic membrane perforation using mitomycin C. Otolaryngol Head Neck Surg 2001;124(1):40–5. doi:10.1067/mhn.2001.112199.

[10] Wieland AM, Sundback CA, Hart A, Kulig K, Masiakos PT, Hartnick CJ. Poly(glycerol sebacate)-engineered plugs to repair chronic tympanic membrane perforations in a chinchilla model. Otolaryngol Head Neck Surg 2010;143(1):127–33. doi:10.1016/j.otohns.2010.01.025.

[11] Vert M, Doi Y, Hellwich KH, et al. Terminology for biorelated polymers and applications (IUPAC recommendations 2012). Pure Appl Chem 2012;84(2):377–410. doi:10.1351/pac-rec-10-12-04.

[12] Abi Zeid Daou C, Bassim M. Hyaluronic acid in otology: Its uses, advantages and drawbacks – a review. Am J Otolaryngol Head Neck Med Surg 2019:102375 November 2019. doi:10.1016/j.amjoto.2019.102375.

[13] Teh BM, Shen Y, Friedland PL, Atlas MD, Marano RJ. A review on the use of hyaluronic acid in tympanic membrane wound healing. Expert Opin Biol Ther 2012;12(1):23–36. doi:10.1517/14712598.2012.634792.

[14] Sayin I, Kaya KH, Ekizoğlu O, Erdim I, Kayhan FT. A prospective controlled trial comparing spontaneous closure and Epifilm® patching in traumatic tympanic membrane perforations. Eur Arch Oto Rhino Laryngol 2013;270(11):2857–63. doi:10.1007/s00405-012-2331-x.

[15] Sahni D, Singh B, Uppal KS, Bhagat S, Sharma D, Dogra G. Study of effect of 1% sodium hyaluronate application on tympanic membrane perforation. Int J Otorhinolaryngol Head Neck Surg 2018;4(5):1246. doi:10.18203/issn.2454-5929.ijohns20183695.

[16] Yilmaz MS, Sahin E, Kaymaz R, et al. Histological study of the healing of traumatic tympanic membrane perforation after vivosorb and epifilm application. Ear Nose Throat J 2019. doi:10.1177/0145561319854320.

[17] Kadah SMS, Abd-elgaber FM, Mekhiemar SM. Hyaluronic acid temporalis fascia graft myringoplasty versus temporalis fascia graft myringoplasty. Res Otolaryngol 2018;7(1):15–19. doi:10.5923/j.otolaryn.20180701.04.

[18] Gün T, Boztepe OF, Atan D, İkincioğulları 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. doi:10.5152/iao.2016.1938.

[19] Saliba I. Hyaluronic acid fat graft myringoplasty: how we do it. Clin Otolaryngol 2008;33(6):610–14. doi:10.1111/j.1749-4486.2008.01823.x.

[20] Stenfeldt K, Johansson C, Hellström S. The collagen structure of the tympanic membrane: collagen types I, II, and III in the healthy tympanic membrane, during healing of a perforation, and during infection. Arch Otolaryngol Head Neck Surg 2006;132(3):293–8. doi:10.1001/archotol.132.3.293.

[21] Parenteau-Bareil R, Gauvin R, Berthod F. Collagen-based biomaterials for tissue engineering applications. Materials 2010;3(3):1863–87 *(Basel)*. doi:10.3390/ma3031863.

[22] Cass ND, Patten L, Cass SP. Collagen allografts compared with autologous tissue in tympanoplasty. Otol Neurotol 2019;40(6):767–71. doi:10.1097/MAO.0000000000002254.

[23] Lee JM, Seo YJ, Shim DB, Lee HJ, Kim SH. Surgical outcomes of tympanoplasty using a sterile acellular dermal allograft: a prospective randomised controlled study. Acta Otorhinolaryngol Ital 2018;38(6):554–62. doi:10.14639/0392-100X-1839.

[24] Downey TJ, Champeaux AL, Silva AB. AlloDerm tympanoplasty of tympanic membrane perforations. Am J Otolaryngol Head Neck Med Surg 2003;24(1):6–13. doi:10.1053/ajot.2003.5.

[25] Vos JD, Latev MD, Labadie RF, Cohen SM, Werkhaven JA, Haynes DS. Use of AlloDerm in type I tympanoplasty: a comparison with native tissue grafts. Laryngoscope 2005;115(9):1599–602. doi:10.1097/01.mlg.0000172042.73024.ad.

[26] Fishman AJ, Marrinan MS, Huang TC, Kanowitz SJ. Total tympanic membrane reconstruction: AlloDerm versus temporalis fascia. Otolaryngol Head Neck Surg 2005;132(6):906–15. doi:10.1016/j.otohns.2004.12.013.
[27] Abbenhaus JI. The use of reconstituted bovine collagen for tympanic membrane grafting. Otolaryngology 1978;86 3 Pt 1. doi:10.1177/019459987808600320.
[28] Declau F, Van Der Gucht K, Wuyts FL. Uncomplicated type I tympanoplasty with bovine xenografts versus human allografts : functional results and prognostic factors for graft success. B ENT 2017;13:167–76.
[29] D'Eredità R. Porcine small intestinal submucosa (SIS) myringoplasty in children: a randomized controlled study. Int J Pediatr Otorhinolaryngol 2015;79(7):1085–9. doi:10.1016/j.ijporl.2015.04.037.
[30] James AL. Endoscope or microscope-guided pediatric tympanoplasty? Comparison of grafting technique and outcome. Laryngoscope 2017;127(11):2659–64. doi:10.1002/lary.26568.
[31] De Zinis LOR, Berlucchi M, Nassif N. Double-handed endoscopic myringoplasty with a holding system in children: preliminary observations. Int J Pediatr Otorhinolaryngol 2017;96:127–30. doi:10.1016/j.ijporl.2017.03.017.
[32] Zhang D, Huang Z, Sun P, et al. Acceleration of healing of traumatic tympanic membrane perforation in rats by implanted collagen membrane integrated with collagen-binding basic fibroblast growth factor. Tissue Eng Part A 2017;23(1-2):20–9. doi:10.1089/ten.TEA.2016.0265.
[33] Choi SH, Song HY, Il SC. Fibrinogen-based collagen fleece graft myringoplasty for traumatic tympanic membrane perforation. J Audiol Otol 2016;20(3):139–45. doi:10.7874/jao.2016.20.3.139.
[34] Liu D, Nikoo M, Boran G, Zhou P, Regenstein JM. Collagen and gelatin. Annu Rev Food Sci Technol 2015;6(1):527–57. doi:10.1146/annurev-food-031414-111800.
[35] McGhee M, Dornhoffer JL. The effect of gelfilm in the prevention of fibrosis in the middle ear of the animal model. Am J Otol 1999;20(6):712–16.
[36] Holzer F. The fate of gelatin film in the middle ear. Arch Otolaryngol 1973;98(5):319–21. doi:10.1001/archotol.1973.00780020331009.
[37] Falbe-Hansen J, Tos M. Silastic and gelatin film sheeting in tympanoplasty. Ann Otol Rhinol Laryngol 1975;84(3):315–17. doi:10.1177/000348947508400305.
[38] Karlan MS. Gelatin film sandwich in tympanoplasty. Otolaryngol Head Neck Surg 1979;87(1):84–6. doi:10.1177/019459987908700120.
[39] Vadiya SI. Technique and results of cartilage shield tympanoplasty. Indian J Otol 2014;20(4):196–8. doi:10.4103/0971-7749.146938.
[40] Goncalves S, Chiossone-Kerdel JA, Bianco AS, Ercolino JM, Hernandez-Rojas J. Effect of absorbable gelatin sponge in the middle ear: *in vitro* and in vivo animal model. Acta Otolaryngol 2015;135(1):14–25. doi:10.3109/00016489.2014.951455.
[41] Mertens J, Ullman U. Antimicrobial action of rolitetracycline in gelatin sponge packs (Marbagelan) in tympanoplasty. HNO 1987;35(6):262–4.
[42] Ringenberg JC. Fat graft tympanoplasty. Laryngoscope 1962;72(2):188???192. doi:10.1288/00005537-196202000-00005.
[43] Niklasson A, Tano K. The Gelfoam® plug: an alternative treatment for small eardrum perforations. Laryngoscope 2011;121(4):782–4. doi:10.1002/lary.21451.
[44] Saeedi M, Ajallouеian M, Zare E, et al. The effect of PRP-enriched gelfoam on chronic tympanic membrane perforation: a double-blind randomized clinical trial. Int Tinnitus J 2017;21(2):108–11. doi:10.5935/0946-5448.20170021.
[45] Kanemaru SI, Umeda H, Kitani Y, Nakamura T, Hirano S, Ito J. Regenerative treatment for tympanic membrane perforation. Otol Neurotol 2011;32(8):1218–23. doi:10.1097/MAO.0b013e31822e0e53.
[46] Acharya AN, Coates H, Tavora-Vièira D, Rajan GP. A pilot study investigating basic fibroblast growth factor for the repair of chronic tympanic membrane perforations in pediatric patients. Int J Pediatr Otorhinolaryngol 2015;79(3):332–5. doi:10.1016/j.ijporl.2014.12.014.
[47] Kanemaru SI, Kanai R, Yoshida M, Kitada Y, Omae K, Hirano S. Application of regenerative treatment for tympanic membrane perforation with cholesteatoma, tumor, or severe calcification. Otol Neurotol 2018;39(4):438–44. doi:10.1097/MAO.0000000000001701.
[48] Lou ZC, Lou ZH, Xiao J. Regeneration of the tympanic membrane using fibroblast growth factor-2. J Laryngol Otol 2018;132(6):470–8. doi:10.1017/S002221511800083X.
[49] Kuo CY, Wilson E, Fuson A, et al. Repair of tympanic membrane perforations with customized bioprinted ear grafts using chinchilla models. Tissue Eng Part A 2018;24(5-6):527–35. doi:10.1089/ten.tea.2017.0246.
[50] Wu GH, Hsu SH. Review: polymeric-based 3D printing for tissue engineering. J Med Biol Eng 2015;35(3):285–92. doi:10.1007/s40846-015-0038-3.
[51] Li L, Zhang W, Huang M, et al. Preparation of gelatin/genipin nanofibrous membrane for tympanic member repair. J Biomater Sci Polym Ed 2018;29(17):2154–67. doi:10.1080/09205063.2018.1528519.
[52] Kundu B, Rajkhowa R, Kundu SC, Wang X. Silk fibroin biomaterials for tissue regenerations. Adv Drug Deliv Rev 2013;65(4):457–70. doi:10.1016/j.addr.2012.09.043.
[53] Levin B, Redmond SL, Rajkhowa R, Eikelboom RH, Marano RJ, Atlas MD. Preliminary results of the application of a silk fibroin scaffold to otology. Otolaryngol Head Neck Surg 2010;142 3 SUPPL. 1. doi:10.1016/j.otohns.2009.06.746.
[54] Levin B, Redmond SL, Rajkhowa R, Eikelboom RH, Atlas MD, Marano RJ. Utilising silk fibroin membranes as scaffolds for the growth of tympanic membrane keratinocytes, and application to myringoplasty surgery. J Laryngol Otol 2013;127 SUPPL. 1. doi:10.1017/S0022215112001661.
[55] Shen Y, Redmond SL, Teh BM, et al. Tympanic membrane repair using silk fibroin and acellular collagen scaffolds. Laryngoscope 2013;123(8):1976–82. doi:10.1002/lary.23940.
[56] Shen Y, Redmond SL, Papadimitriou JM, et al. The biocompatibility of silk fibroin and acellular collagen scaffolds for tissue engineering in the ear. Biomed Mater 2014;9(1):015015. doi:10.1088/1748-6041/9/1/015015.
[57] Lee H, Jang CH, Kim GH. A polycaprolactone/silk-fibroin nanofibrous composite combined with human umbilical cord serum for subacute tympanic membrane perforation; an *in vitro* and *in vivo* study. J Mater Chem B 2014;2(18):2703–13. doi:10.1039/c4tb00213j.
[58] Lee JH, Kim DK, Park HS, et al. A prospective cohort study of the silk fibroin patch in chronic tympanic membrane perforation. Laryngoscope 2016;126(12):2798–803. doi:10.1002/lary.25980.
[59] Hickey RJ, Pelling AE. Cellulose biomaterials for tissue engineering. Front Bioeng Biotechnol 2019;7:45 MAR. doi:10.3389/fbioe.2019.00045.
[60] Sultan S, Siqueira G, Zimmermann T, Mathew AP. 3D printing of nano-cellulosic biomaterials for medical applications. Curr Opin Biomed Eng 2017;2:29–34. doi:10.1016/j.cobme.2017.06.002.
[61] Kim J, Kim SW, Park S, et al. Bacterial cellulose nanofibrillar patch as a wound healing platform of tympanic membrane perforation. Adv Healthc Mater 2013;2(11):1525–31. doi:10.1002/adhm.201200368.
[62] Silveira FCA, Pinto FCM, Caldas Neto SS, Leal MC, Cesário J, Aguiar JLA. Treatment of tympanic membrane perforation using bacterial cellulose: A randomized controlled trial. Braz J Otorhinolaryngol 2016;82(2):203–8. doi:10.1016/j.bjorl.2015.03.015.
[63] Mota C, Danti S, D'Alessandro D, et al. Multiscale fabrication of biomimetic scaffolds for tympanic membrane tissue engineering. Biofabrication 2015;7(2). doi:10.1088/1758-5090/7/2/025005.
[64] Kozin ED, Black NL, Cheng JT, et al. Design, fabrication, and *in vitro* testing of novel three-dimensionally printed tympanic membrane grafts. Hear Res 2016;340:191–203. doi:10.1016/j.heares.2016.03.005.
[65] Seonwoo H, Shin B, Jang KJ, et al. Epidermal growth factor–releasing radially aligned electrospun nanofibrous patches for the regeneration of chronic tympanic membrane perforations. Adv Healthc Mater 2019;8(2). doi:10.1002/adhm.201801160.
[66] Danti S, Mota C, D'alessandro D, et al. Tissue engineering of the tympanic membrane using electrospun PEOT/PBT copolymer scaffolds: a morphological *in vitro* study. Hear Balance Commun 2015;13(4):133–47. doi:10.3109/21695717.2015.1092372.
[67] Immich APS, Pennacchi PC, Naves AF, et al. Improved tympanic membrane regeneration after myringoplastic surgery using an artificial biograft. Mater Sci Eng C 2017;73:48–58. doi:10.1016/j.msec.2016.12.007.
[68] Lou Z. The effect of epidermal growth factor on the pseudo-healing of traumatic tympanic membrane perforations. Braz J Otorhinolaryngol 2019 xx. doi:10.1016/j.bjorl.2019.06.011.
[69] Lou ZC, Dong Y, Lou ZH. Comparative study of epidermal growth factor and observation only on human subacute tympanic membrane perforation. Am J Otolaryngol Head Neck Med Surg 2019;40(2):209–12. doi:10.1016/j.amjoto.2018.11.011.
[70] Aksoy MA, Açıkalın MF, Gürbüz MK, et al. Efficacy of platelet-rich plasma on fat grafts in the repair of tympanic membrane perforations: an experimental study. J Int Adv Otol 2018;14(1):60–4. doi:10.5152/iao.2017.3845.
[71] Zheng-Cai L, Zi-Han L. The short- and long-term adverse effects of FGF-2 on tympanic membrane perforations. Acta Otorhinolaryngol Ital 2018;38(3):264–72. doi:10.14639/0392-100X-1480.
[72] Lou Z, Lou Z. A comparative study to evaluate the efficacy of EGF, FGF-2, and 0.3% (w/v) of loxacin drops on eardrum regeneration. Medicine 2017;96(30) *(United States)*. doi:10.1097/MD.0000000000007654.
[73] Araujo MM, Murashima AAB, Alves VM, Jamur MC, Hyppolito MA. The topical use of insulin accelerates the healing of traumatic tympanic membrane perforations. Laryngoscope 2016;126(1):156–62. doi:10.1002/lary.25300.
[74] Shen Y, Guo Y, Wilczynska M, Li J, Hellström S, Ny T. Plasminogen initiates and potentiates the healing of acute and chronic tympanic membrane perforations in mice. J Transl Med 2014;12(1):1–9. doi:10.1186/1479-5876-12-5.
[75] Ramsay HA, Heikkonen EJ, Laurila PK. Effect of epidermal growth factor on tympanic membranes with chronic perforations: a clinical trial. Otolaryngol Head Neck Surg 1995;113(4):375–9. doi:10.1016/S0194-5998(95)70071-4.
[76] Kase KI, Iwanaga T, Terakado M, et al. Influence of topical application of basic fibroblast growth factor upon inner ear. Otolaryngol Head Neck Surg 2008;138(4):523–7. doi:10.1016/j.otohns.2007.11.025.
[77] Hakuba N, Hato N, Omotehara Y, Okada M, Gyo K. Epithelial pearl formation following tympanic membrane regeneration therapy using an atelocollagen/silicone membrane and basic fibroblast growth factor: our experience from a retrospective study of one hundred sixteen patients. Clin Otolaryngol 2013;38(5):394–7. doi:10.1111/coa.12164.
[78] Erkilet E, Koyuncu M, Atmaca S, Yarim M. Platelet-rich plasma improves healing of tympanic membrane perforations: experimental study. J Laryngol Otol 2009;123(5):482–7. doi:10.1017/S0022215108003848.
[79] Maharajan N, Cho GW, Jang CH. Application of mesenchymal stem cell for tympanic membrane regeneration by tissue engineering approach. Int J Pediatr Otorhinolaryngol 2020;133:109969 November 2019. doi:10.1016/j.ijporl.2020.109969.
[80] Von Unge M, Dirckx JJJ, Olivius NP. Embryonic stem cells enhance the healing of tympanic membrane perforations. Int J Pediatr Otorhinolaryngol 2003;67(3):215–19. doi:10.1016/S0165-5876(02)00371-3.
[81] Rahman A, von Unge M, Olivius P, Dirckx J, Hultcrantz M. Healing time, long-term result and effects of stem cell treatment in acute tympanic membrane perforation. Int J Pediatr Otorhinolaryngol 2007;71(7):1129–37. doi:10.1016/j.ijporl.2007.04.005.
[82] Goncalves S, Bas E, Goldstein BJ, Angeli S. Effects of cell-based therapy for treating tympanic membrane perforations in mice. Otolaryngol Head Neck Surg 2016;154(6):1106–14 *(United States)*. doi:10.1177/0194599816636845.
[83] Jang CH, Ahn SH, Lee JW, Lee BH, Lee H, Kim GH. Mesenchymal stem cell-laden hybrid scaffold for regenerating subacute tympanic membrane perforation. Mater Sci Eng C 2017;72:456–63. doi:10.1016/j.msec.2016.11.094.
[84] Yamakawa S, Hayashida K. Advances in surgical applications of growth factors for wound healing. Burn Trauma 2019;7. doi:10.1186/s41038-019-0148-1.

[85] Beitzel K, Allen D, Apostolakos J, et al. US definitions, current use, and FDA stance on use of platelet-rich plasma in sports medicine. J Knee Surg 2015;28(1):29–34. doi:10.1055/s-0034-1390030.
[86] Fitzpatrick J, Bulsara MK, McCrory PR, Richardson MD, Zheng MH. Analysis of platelet-rich plasma extraction: variations in platelet and blood components between 4 common commercial kits. Orthop J Sport Med 2017;5(1). doi:10.1177/2325967116675272.
[87] Knoepfler PS, Turner LG. The FDA and the US direct-to-consumer marketplace for stem cell interventions: a temporal analysis. Regen Med 2018;13(1):19–27. doi:10.2217/rme-2017-0115.
[88] Marks PW, Witten CM, Califf RM. Clarifying stem-cell therapy's benefits and risks. N Engl J Med 2017;376(11):1007–9. doi:10.1056/NEJMp1613723.
[89] Wang AY, Shen Y, Wang JT, Friedland PL, Atlas MD, Dilley RJ. Animal models of chronic tympanic membrane perforation: a "time-out" to review evidence and standardize design. Int J Pediatr Otorhinolaryngol 2014;78(12):2048–55. doi:10.1016/j.ijporl.2014.10.007.
[90] Esteban T, Atucha NM, Moraleda JM, García-Estañ J, Insausti CL, Moraleda-Deleyto J. Chronic model of tympanic perforation in rats with mitomycin C and dexamethasone. J Otol 2019;14(1):12–16. doi:10.1016/j.joto.2018.11.002.
[91] Rubinstein BJ, Ranney JD, Khoshakhlagh P, Strasnick B, Horn-Ranney EL. A novel gel patch for minimally invasive repair of tympanic membrane perforations. Int J Pediatr Otorhinolaryngol 2018;115:27–32 September. doi:10.1016/j.ijporl.2018.09.012.
[92] Santa Maria PL, Maria CS, Kim S, Yang YP. Single administration of a sustained-release formulation of KB-R7785 inhibits tympanic membrane regeneration in an animal model. J Int Adv Otol 2016;12(3):237–40. doi:10.5152/iao.2016.3124.
[93] Wang AY, Shen Y, Liew LJ, et al. Searching for a rat model of chronic tympanic membrane perforation: healing delayed by mitomycin C/dexamethasone but not paper implantation or iterative myringotomy. Int J Pediatr Otorhinolaryngol 2015;79(8):1240–7. doi:10.1016/j.ijporl.2015.05.020.
[94] Emami N, Schloss MD, Daniel SJ. Chronic tympanic membrane perforation in an animal model. Int J Pediatr Otorhinolaryngol 2014;78(8):1250–2. doi:10.1016/j.ijporl.2014.04.042.
[95] Jang CH, Cho YB, Yeo MG, et al. Regeneration of chronic tympanic membrane perforation using 3D collagen with topical umbilical cord serum. Int J Biol Macromol 2013;62:232–40. doi:10.1016/j.ijbiomac.2013.08.049.