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New Bioengineering Techniques for Tympanic Membrane Repair Using 3D Printing

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ABSTRACT

In nature, when the eardrum is perforated, it is common for the body to heal itself with time. However, in some cases, surgery or grafting is required to repair the membrane. 3-D printing tympanic membrane (TM) scaffolds is a developing field (with great potential) that aims to create a streamlined method to facilitate the repopulation of TM cells and collagen on a damaged TM. Here we analyze and evaluate the different materials, methods of printing, shapes, and resonance that bioengineering researchers tested to optimize the efficacy of the 3-D printed scaffold. Surprisingly, the scaffolds have proven to be not only more structurally consistent, but also structurally stronger. We also suggest that clinical testing on an organic specimen and more developments in synthesizing all the different techniques for these 3-D printed scaffolds would be necessary. Ultimately, the research reviewed here demonstrates that 3-D printed scaffolding is a promising treatment for those with tympanic perforations that may even overtake tympanoplasty or autologous grafting as a more accessible and consistent alternative to TM repair.

Introduction

The Information Age brought about so much change to how things are assembled, particularly in the (relatively new) world of 3-D printing. Originally invented in 1983 by Chuck Hull, 3-D printing's versatile nature allows it to print a wide array of materials from musical instruments to prosthetics [1]. 3-D printing is fundamentally similar to 2-D paper printing: like ink on paper, the 3-D printer interprets computer model files and then proceeds to print, except the 3D printer can fabricate three-dimensional objects based on these computer models.

As of now, the main uses for 3-D printing in the medical field are summed up to making tissues/organoids for research, surgical tools, patient-specific surgical models, and custom-made prosthetics [2]. First, the 3-D printing of tissues/organoids uses pipettes to layer living human cells to "print" out miniature organs or tissues to perform experiments for research. Second, complete organ replacements are in the works with this technique and patients' organs can be indirectly grown and studied outside of the living patient. 3-D printing also produces sterile, precise surgical tools and that drives the production cost of surgical instruments down significantly. Third, 3-D printing is used to create patient-specific models. Using 3-D imaging and other non-intrusive methods, patients' organs and other bodies are made into models. On these models, surgeons can practice procedures beforehand on the "patient" to minimize trauma and safely navigate the patient's body. Lastly, prosthetics are more accessible and made quickly with the help of 3-D printing. Children can take advantage of this as they often need to replace prosthetics they outgrow for a low cost.

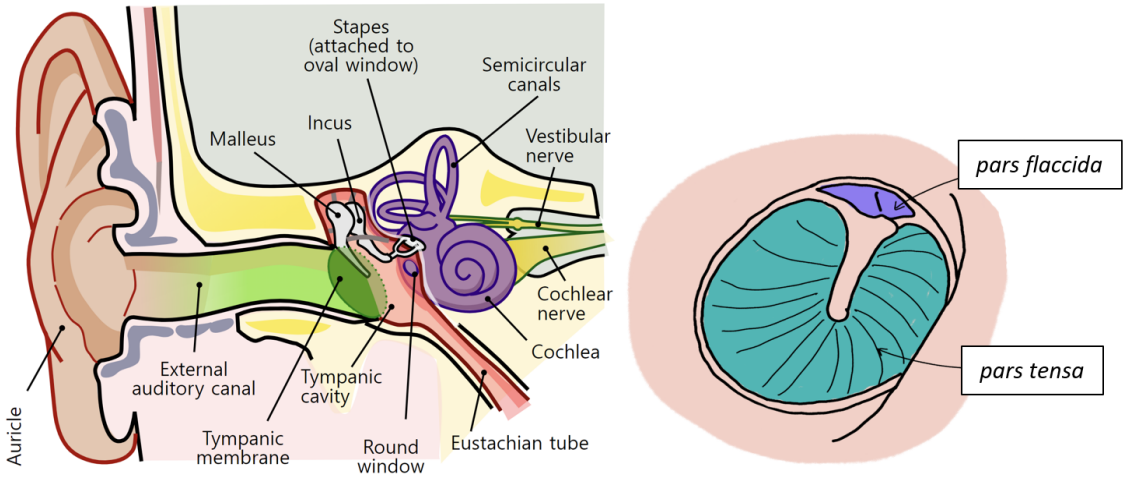

Figure 1. (Left) A diagram of the anatomy of the human ear [21]; (Right) A simplified version of the anatomy of the tympanic membrane showing its components, the *pars tensa* and *pars flaccida*.

As the tympanic membrane (TM) is an essential part of the ear, it is vitally important to know the different parts of it as shown in Fig 1. The eardrum is composed of two main parts - the *pars tensa* and the *pars flaccida* [3]. The *pars tensa* has an outer coating of keratinizing epithelium, a water-proof layer of dead cells that make up a protective covering. The pars tensa also has a main collagen layer in the middle, made up of radiating and circular collagen fibers (mostly collagen II). Speaking of collagen, the different types of collagen are corresponding to the different functions they have in the body: type I collagen is used for strong hair, skin, nails, and bones, type II collagen is the kind that forms your cartilage, and type III collagen is secreted by fibroblasts and other mesenchymal cell types [3]. The part of the *pars tensa* that is closest to the middle ear is a simple layer of single-layered epithelium. Vessels, nerve endings, and mast cells are located in the connective tissue of the *pars flaccida*. The collagen located in the *pars flaccida* is more 3-dimensional than the flatter *pars tensa* [5].

The three main causes that the eardrum may experience damage of perforation are physical shock, extreme sound, and pressure. Physical shock includes objects in the ear and severe head trauma. Severe head trauma causes dislocation or rupture to the eardrum through changes in the skull, such as a fracture. Extreme sounds like gunshots or explosions can send soundwaves that are too loud for your eardrums to handle, causing a perforation on entry. Barotrauma, or trauma due to pressure, causes perforations due to the difference in pressure between the middle ear and outside atmosphere can cause perforations. For example, going on a plane that is going higher in altitude or scuba diving too quickly.

Luckily, ruptures in the eardrum are not permanent and can heal within a few months if not weeks. However, the complications behind prolonged rupture are why a speedy recovery is necessary. Middle ear cysts and infections are more prone to exist in those with perforated eardrums. All bacteria, skin debris, and miscellaneous foreign objects can easily pass through to the middle ear causing complications. Another side effect that could occur as a result of a perforated eardrum is tinnitus or ringing/buzzing of the ear [6]. As the inner ear is important for balance, a side effect of TM perforation is vertigo and vertigo-induced vomiting.

To avoid the complications of the slow recovery or impossible recovery, grafting was developed. In the early 17th century, TM perforations were repaired with pig bladder. Eventually, grafting autologous or own tissue back into the TM was developed [7]. Although temporalis fascia is considered the gold standard of all the materials used to reconstruct the TM, it could be expanded upon by 3-D Printing. 3-D printing can accelerate and facilitate cell regrowth through 3-D printed scaffolding. This scaffolding allows for cell migration and accelerated healing.

Research Question: The Problem with Eardrum Perforations

The most common way that people get perforated eardrums is through ear infections [8]. Although ear infection is not as common for adults, only 20% of ear infections occur in adults, infants are at high risk [9]. According to the National Institute of Health, 5 out of 6 infants get an ear infection by their third birthday [10]. When an ear is infected, the area behind the eardrum fills up with fluids and pus, which causes a lot of pain. When the eardrum is perforated or forms a hole, the pain from the pus and fluid buildup is lessened. In a study of 1,000 patients in the United States, it was shown that men more commonly had traumatic perforations compared to women, estimated to be at a ratio of 1.49:1. This study in the U.S. was cross confirmed with another study of 529 patients in Nigeria. In the study in Nigeria, it was discovered that the male to female ratio was 2:1, like the United States. Finally, a study with 80 participants showed that the average age of patients who experience TM perforation was from 12.1 to 41.3 years old. Children made up 25% of the sample size of this study [11]. The research question here is: What are the techniques and parameters that are necessary to mimic a TM with artificial materials?

Methodology

This Methodology section covers all the major techniques and processes that are related or used to mimic a TM through 3-D printed scaffolding.

Grafts: A component used to help heal injuries when they are too large to heal on their own. In that case, the surgeon can replace tissue with a healthy substitute (Fig 2), which will heal over and merge with the surrounding area. Grafts are often autologous, which means the graft is tissue from one area of a patient's body and is then moved to another.

Scaffolding: In the case of scaffolding, there are two types: the *in vivo* and *in vitro* types of scaffolding. In vivo scaffolding is composed of acellular, or nonliving, material that facilitates growth within the body by letting the cells migrate using the scaffolding. In vitro scaffolding is composed of pre-grown keratinocyte/silk-based material that is initially grown outside of the body and then implanted in to allow for the TM to be able to grow over [7] (Fig2).

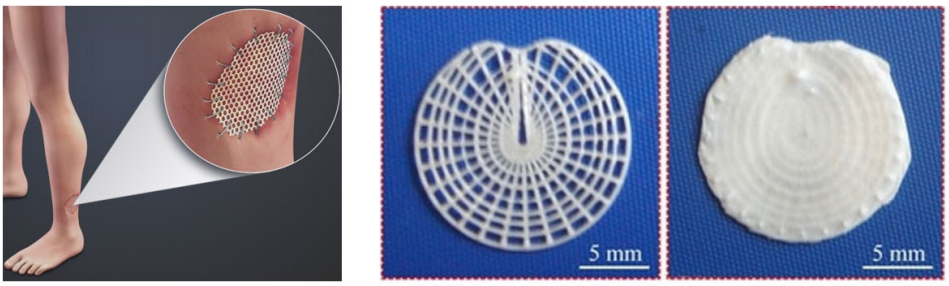

Figure 2. (Left) Grafting process that places fresh skin over a wound with damaged skin [22]; (Right) photographs of onepiece grid layer before and after coating with the electrospun membrane (dual scale TM scaffold) [7].

Laser Doppler Vibrometry (LDV): A non-invasive measuring technique that uses a camera and lasers to find sound-induced velocities. LDV is used to measure acoustic properties such as velocity and surface motions of TM grafts in response to sounds at different frequencies [12].

Electrospinning (ES) [13]: A method to create ultrafine fibers by ejecting a polymer melt or solution through a spinneret under a high-voltage electric field and to solidify or coagulate it to form a filament. These filaments are similar to the extracellular matrix. The high surface area to volume ratio that the meshes of the electrospun scaffolds give cells many areas to attach to, which induces cell growth. Furthering developments in this field may even allow for hybrids of cell and fiber meshes to be created [7].

Additive Manufacturing (AM): A method in which computers are used to fabricate scaffolds. AM is almost synonymous with 3-D Printing [14].

Human Mesenchymal Stromal Cells (MSC): These cells are very capable of differentiating into various cell types such as bone, fat, and cartilage cells. Due to their adaptability, MSCs are commonly used in many treatments involving regenerative medicine, cell therapy, and tissue engineering [15].

What Defines Success?

The overall healing process by 3-D printed scaffolding is based on the setup of the scaffold. Through combined ES and 3-D Printing, a base scaffold is made. Then, mesenchymal stromal cells, source cells for cellular constructs, are placed onto the scaffold. These mesenchymal stromal cells were obtained from human bone marrow, and therefore have properties such as self-renewal and multilineage differentiation potential. Using this, the healing process of the perforated eardrum could be accelerated. Considering the outcomes, the fundamental favorable outcome is one in which the perforated eardrum is healed over. However, multiple factors determine overall success. For example, the eardrum may have different levels of function once it is healed (no to full). Another factor is recurring illness such as infection or rejection of the scaffold after some time. Lastly, the scaffold's ability to effectively outperform the grafts in healing the TM is important. If the scaffolds can prove to be a safer and more consistent method to heal the TM than grafts, it will be accounted as a success.

Analysis and Critiques on State-of-the-Art Research

In this section, we review and critically analyze a few important state-of-the-art techniques necessary to heal TM using scaffolds or grafts, measure their key properties, maximize cell growth, and evaluate TM for vibration and sensitivity characteristics.

Grasping the Shape of the Scaffold

Carlos Mota’s research in [7] is mostly concerned with following the very specific TM geometry for mesenchymal cells to efficiently migrate onto the TM scaffold. TM has a very specific geometry. Through AM and ES, Mota's group was able to make scaffolds that approximated the basic structure of the membrane. As the collagen fiber orientation on the TM is radiate on one side and circular on the opposite, Mota and his team closely modeled their scaffolds after the natural fiber orientation. Using ES and 3D printing, tympanic scaffolds with ultrafine fiber and radial orientation were able to be created. However, this fibrous electrospun product only covered the singular radial portion of the TM, which meant that dual and triple scale exploits of ES with 3D printing would be needed to cover both radial and circular microfiber patterns to try and fully mimic the extracellular matrix of the TM. Dual and triple scale scaffolds include radial, circular, and parabolic structures, which allow for a 3-D shape and structure integrity of the TM (Fig 3).

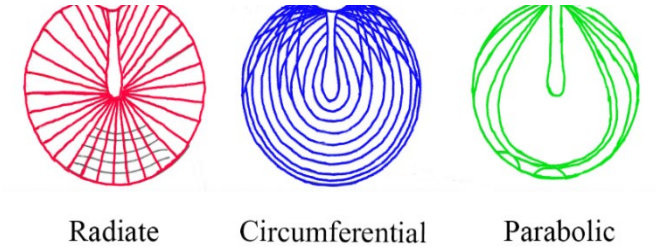

Figure 3. Two-dimensional representation of the different parts of the TM.

Additionally, these scaffolds have grooves to facilitate cell growth and movement. Notably, Mota's research uses human mesenchymal stromal cells, which may be viable and metabolically active, but do not show any depositing of collagen [7]. In his second paper [16], however, Mota addresses the collagen deposition on the scaffold. All in all, this research serves as an important proof of concept that TM scaffolds can be made and cells can live upon them.

Bettering the Scaffold: Testing Acoustical and Mechanical Properties

Mota's research in [16] furthers the previous methods of building scaffolds by using the dual and triple scale exploits and further testing the scaffolds for their structural/acoustic capabilities and ability to facilitate cellular alignment and collagen deposition. Since the transmission of the acoustic vibrations in different frequency ranges is directly affected by the structural architecture of the TM, the researchers looked at what geometrical features had the most impact on our hearing. Testing this hypothesis, the researchers confirmed the computer predictions with LDV. As a result, many different scaffolds were able to be produced and tested *in silico*.

Macro-indentation was used to simulate mechanical stresses on the TM, which revealed that radial components had the most influence over the mechanical behavior and that there was an increasing trend in resonant frequencies with rising stiffness. 3D printing was more successful in cell agglomeration and orientation compared to plain electrospun membranes. Furthermore, higher deposition of collagen was found around the 3D-printed filaments and the collagen successfully followed the alignment of their corresponding cells. As expected in all cases, the closer Mota's team made the scaffold to the real TM, the more success they found in cell and collagen deposition.

One pitfall in [16] was that the acoustical characterization of each of the scaffolds that were made was inconclusive. Even the best of figures seems to have patches lacking collagen and cell distribution, and researchers have yet to test the scaffolds on live subjects.

Maximizing Cell Growth: Cell Viability and Collagen Deposition

In Elif Ilhan's paper, a different method of fixing up the TM was proposed [17]. Instead of the scaffold of an entire eardrum, Ilhan's team decided to continue with the use of grafts but use 3-D printing for these grafts in a method that will allow for proper cell cultivation. Ilhan et al. also explored the different materials that could be used to create the grafts. Testing different combinations of polymers, the researchers reached an optimal combination that would facilitate the production of scaffolds and cell viability. These solutions were best suitable to keep strands separate, not clog the dispensing needle, and get the best drop shape when printing. Pores were a large consideration in this experiment as pores are crucial for tissue formation as they help cell nutrition, migration, proliferation, and permeability. Researchers found the scaffold materials that had the best cell viability overall, and also the addition of biopolymers such as those found in chitin improved its ability to swell, which helped nutrient transport.

One thing that seemed somewhat left out was the fact that the pictures taken for the cell viability analysis were overly magnified, making it hard to see whether the cells had evenly developed and spread across the graft for better healing. Overall, Ilhan's method utilized specific materials and formations to create a graft that tried to maximize cell viability, rather than structural integrity or shape.

Testing Tympanic Membrane, Temporalis Fascia, and Grafts

Elliott Kozin's research was all about testing out all the different structure patterns and materials by comparing them with real tissue, such as TM and temporalis fascia (TF) [12]. The first characteristic that was tested was the number of filaments that were used. Using either 8 or 16 radial and circumferential filaments, the researchers were able to determine that a higher filament count would result in higher load and better strength. It is reasonable to conclude that a scaffold is too stiff because the TM still needs to vibrate to operate. Observation methods for the grafts, TF, and TM, were Digital Opto-Electronic Holography to detect sound-induced motion, LDV to see the response to different sound frequencies, and Dynamic Mechanical Analysis to check the tensile strength. Most importantly, the synthetically created TM grafts were far more consistent than the TF. On the other hand, TM was more sensitive to sound frequencies as well as the number of vibrations it could offer.

A shortcoming of this research was that researchers could not capture the conical figure of the TM in the grafts as well as resonate lower frequencies with the graft. Ultimately, this research was conducted simply to prove a point rather than to create realistic grafts. By comparing the previous method of grafting with temporalis fascia, the researchers can show that there are many benefits in developing 3-D printing TM grafts.

Conclusion and Future Work

3-D will have a great positive impact on treating TM perforations in the future. We can expect more developments in the coming decade, especially with 3-D printing techniques developing parallel to their usage in the medical field. What has largely been missing from the research on 3-D printed scaffolds and grafts is *in vivo* testing of the scaffolds and grafts and their efficacy in healing TM perforations. There have been tests with perforation in mice: researchers used a synthetically produced collagen graft instead of 3-D scaffolding to try to treat a TM [18]. Another area of work for this research would be to deal with the problem of removing the TM scaffold from the body. All of the research papers show inconclusive information on what happens to the scaffolds, so it would be ideal for these scaffolds to be either safely taken out or dissolved into the body.

Looking forward, if TMs can be synthetically repaired, it is plausible that other parts of the ear will be repaired as well. Scaffolds have proven to be much more useful than the previous TM repairing methods. Being minuscule and delicate, scaffolds have the potential to be much more successful in avoiding irritation of the middle ear compared to surgical intervention. Additionally, scaffolds are less invasive than autologous grafting, which requires the removal of tissue from one part of the body and attaching it to another. Lastly, scaffolds will eliminate the complications grafts have. Improper placement and scar tissue forming over the graft is no longer a worry in terms of scaffolds.

Ultimately, this biofabrication research is very important to the future of the medical field. For example, there may come a point in which humanity can increase the resonance range of the TM through synthetic material. This may improve the hearing of the noise frequencies that are inaudible from the unenhanced ear. Creating scaffolds and functional substitutes for TMs is a great segue into the possible development of this research. Today, prosthetics hinder the day-to-day lives of patients, such as a cochlear implant, an electric device that needs to be attached to the side of the head for hearing. If innovations such as the TM replacements and 3-D printing continue (as suggested in [19] with 3-D bioprinting or in [20] with multimodal additive manufacturing), it is very possible that replacement or treatment therapy that returns full hearing would be developed.

In conclusion, as the scaffolds have proven to be structurally consistent and stronger, 3-D printed scaffolding is a promising treatment for TM perforations as a more accessible and consistent alternative to TM repair. In the bigger picture, scaffolding could also be used for other body parts in their repairs. It became obvious that 3-D printed scaffolding would need more bioengineering research into different materials, methods of printing, shapes, and resonance to optimize the efficacy of the scaffolds. Also, clinical testing on an organic specimen and more developments in synthesizing all the different techniques would be necessary.

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