3D-PRINTING TECHNIQUE FOR FABRICATION OF BIODEGRADABLE PDLLA TYMPANOSTOMY TUBE AND EXAMINATION OF BIOFILM FORMATION

by

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ABSTRACT

3D-PRINTING TECHNIQUE FOR FABRICATION OF BIODEGRADABLE PDLLA TYMPANOSTOMY TUBE AND EXAMINATION OF BIOFILM FORMATION

In this study, it is presented that the new fabrication method for biodegradable PDLLA tympanostomy tube by examining its degradation and swelling behavior, bacterial attachment, and biofilm formation with a comparison of Fluoroplastic one. The novelty of the study lies in the 3D printing fabrication technique. No research work studied the fabrication of samples in these dimensions (2 mm in length) and design. The samples are printed at 190 °C temperature under 7.9 bar pressure with 0.1 mm/min speed. The fabrication of one sample took 9 minutes with a 0.3 mm nozzle, and structural collapsing was able to be prevented. After the fabrication of 3D-printed PDLLA samples, they are examined for the degradation and swelling characteristics at 37 °C in PBS for 5 weeks. The degradation rate of 3D-printed PDLLA samples is 5%, and the swelling ratio is 40 % for 5 weeks. Scanning Electron Microscopy (SEM) was used for the surface examination, and results showed that PDLLA samples have fewer surface faults than control group of Fluoroplastic tubes in microscale level. For examining bacterial attachment on the layer-by-layer surface of the PDLLA sample, a biofilm assessment was done for 4 days. According to colony counting and CFU/mL results, 3D-printed samples had less biofilm formation despite the layered surface structure coming from the fabrication process. Overall, biodegradable tympanostomy tube fabrication by using the 3D-printing technique needs to be improved. However, according to experimental results, if the fabrication method can be improved to produce more precise structures in that dimension, it is possible to further this study to produce more perfectly tympanostomy tubes.

Keywords: 3D-printing, Poly-lactic acid, biodegradable implant, bacterial attachment, tympanostomy tube, otitis media.

ÖZET

ÜÇ BOYUTLU YAZIM TEKNİĞİ İLE BİYOBOZUNUR PDLLA TİMPANOSTOMİ TÜPÜ ÜRETİMİ VE ÖRNEK YÜZEYİNİN BİYOFİLM OLUŞUMU AÇISINDAN DEĞERLENDİRİLMESİ

Bu çalışmada, biyobozunur PDLLA timpanostomi tüpleri için yeni bir üretim metodu sunulmaktadır. Ayrıca, örneklerin degradasyon ve şişme davranışları incelenmiş ve günümüzde kullanılan Floroplastik malzemeden üretilen timpanostomi tüpü ile bakteriyel tutunum açısından karşılaştırılmıştır. Bu çalışmanın özgünlüğü üç boyutlu baskı tekniği ile üretilmesindedir. Daha önce bu boyutlarda (2mm uzunluğunda) ve bu tasarıma sahip ürünlerin üç boyutlu baskı tekniği ile üretilmesi ile ilgili bilimsel çalışma literatürde bulunmamaktadır. Üretim 190 °C derecede, 7.9 bar basınç altında, 0.1 mm/dk hızıyla oda sıcaklığındaki ortamda yapılmıştır. Her bir örneğin üretimi 0.3 mm başlık ile 9 dakika sürerken, örneklerin yapısal bütünlüğü korunmayı başarmıştır. Üretim aşamasını takiben, degradasyon ve şişme deneyleri 37 °C sıcaklıkta PBS içinde yapılmış; örneklerin 5 hafta içinde %5 ağırlığını kaybettiği ve %40 oranında sıvı çektiği gözlemlenmiştir. Sonuçlar literatürle paraleldir. Taramalı elektron mikroskopu ile yapılan yüzey çalışmasında, PDLLA ile üretilen örneklerin mikro düzeyde daha pürüzsüz olduğu gözlemlenmiştir.Yüzey değerlendirilmesinin ardından, örnekler bakteriyel tutunum için dört ünlük biyofilm oluşumu deney protokülüyle test edilmiştir. Üç boyutlu baskı tekniğinin PDLLA örneklerin üzerinde oluşturduģu katmanlara rağmen bu örneklerde Floroplastik malzeme ile üretilenlerden daha az koloni sayılmıştır. Sonuç olarak, eğer üç boyutlu baskı tekniği ile üretim süreci yapısal olarak daha hassas ve tutarlı şekilde örneklerin oluşmasını sağlayacak şekilde geliştirilebilirse, bu çalışmanın daha verimli şekilde çalışacak timpanostomi tüpleri üretilmesi adına ilerletilmesi mümkün görünmektedir.

Anahtar Sözcükler: Üç boyutlu baskı, polilaktik asit, biyobozunur implantlar, bakteriyel tutunum, timpanostomi tüpü, orta kulak iltihabı.

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W_w	Weight of wet sample
W_i	Initial weight of sample
W_f	Final weight of sample

LIST OF ABBREVIATIONS

3D	Three Dimensional
PDLLA	Poly(D,L-lactic acid)
PBS	Phosphate Buffered Saline
SEM	Scanning Electron Microscopy
CFU	Colony Forming Unit
TT	Tympanostomy Tube
OM	Otitits Media
OME	Otitis Media with Effusion
PTTO	Post-Tympanostomy Tube Otorrhea
AOM	Acute Otitis Media
PLGA	Poly(lactic-co-glycolic acid)
FDA	Food Drug Administration
PLA	Poly(lactic acid)
PLA-PCL	Poly(lactic acid)/Polycaprolactone
PLLA	Poly(L-lactic acid)
ISO	The International Standard Organization
CAD	Computer Aided Design
TSB	Tryptic Soy Broth
EtOH	Ethanol
TSA	Tryptic Soy Agar

1. INTRODUCTION

1.1 Motivation

Tympanostomy tube studies were started with the invention of Gustav Lincke and Martell Frank in 1854. Their gold tympanostomy did not have massive success in preventing further infections and side effects [1]. Till then, tympanostomy tubes took their popularity back with polyethylene tympanostomy tubes of Armstrong in 1954, and from that time to today, there have been several attempts to create an ideal one by scientists [2]. The ideal tympanostomy tube should be resistant to bacterial attachment, eliminated from the body without any harm, and readily producible.

The antifouling effect for the surface of tympanostomy tubes is a popular topic in otorhinolaryngology because much of post-operative complications are sourced from bacterial attachment and biofilm formation. Biofilm formation of bacteria has three steps; step one includes initial and reversible attachment of the bacteria on the surface, stage two represents early development and maturation of biofilm structure, and the final step is cell aggregation from the biofilm to attach on another part of the organism for survival [3]. Because the initial step of biofilm formation is reversible attachment and contact, the most efficient way to provide an antifouling effect might come from surfaces able to prevent bacterial attachment in the first place.

Another problem with the tympanostomy tubes is early or late retention and persistent perforation after the treatment. Because tubes may stay for a long time on the eardrum, in most cases, they need to be taken from the drum by a surgical operation. As a side effect of these situations, patients may suffer from perforations; and in a minor part of cases, these perforations might be persistent for a long time [4]. To answer these problems, the use of biodegradable materials might be the solution if the degradation time can match with the length of the treatment time, no need to do any extra surgery to extract it. Also, treatment ends without any extra surgical

intervention because the opening on the eardrum starts to close itself parallel with the degradation of the tympanostomy tube. The poly (D, L-lactic acid) (PDLLA) has around three months of half-life and meets the short-term tympanostomy tube treatment time that is six months that is why PDLLA was used as biodegradable material in this study [5].

To produce biodegradable tympanostomy tube 3D-printing was selected for the fabrication part of this project. In the last few years, the 3D-printing technique has become popular because of its easy process and ability to produce devices that meet each patient's specific needs. Because biodegradable materials are proper to use with this technique, using 3D printing can be beneficial to fabricate tympanostomy tubes. However, some limitations of the printing technique created problems during the process of our design, such as heat-dependent degradation of the material, structure collapsing, and layered structure caused by the nature of 3D printing. Despite these problems, 3D-printed PDLLA samples fabricated and examined for degradation and swelling characteristics. Also, they were compared with the Fluoroplastic tympanostomy tubes (Medtronic, Shepard Grommet Ventilation Tube) in terms of bacterial attachment and surface analysis with SEM scanning.

Overall, this thesis study concentrated on tympanostomy tubes that have minimized bacterial attachment with the characteristic of biodegradability and easy fabrication process while focusing on patient-centric medical usage via the 3D printing technique. This study represents the first try of a 3D-printed PDLLA tympanostomy tube with its fabrication technique which is challenging in these dimensions and comparison with a current use type tympanostomy tube. Based on the achieved results of experiments, the fabrication technique needs to be improved but gives hope about the idea, especially in the aspects of bacterial attachment prevention.

1.2 Objectives

The aim of this study is fabrication of new PDLLA tympanostomy tube via 3D-printing technique and analyzing its characteristics in terms of degradation behavior and bacterial attachment. The main objectives of this study are as follows:

- Fabrication of PDLLA tympanostomy tube via 3D-printing technique
- Examination of degradation and swelling characteristics to confirm literature.
- Assessment of surface characteristics caused from 3D-printing and comparison with Fluoroplastic tympanostomy tube and 3D-printed PDLLA one by using SEM.
- Determining differences and comparison of bacterial attachment between 3D-printed PDLLA and Fluoroplastic samples via biofilm assessment.

1.3 Outline

This thesis is presented as follows: Background information about middle ear physiology, otitis media, history of tympanostomy tubes and their materials, biodegradable material use attempts for fabrication of tympanostomy tubes, 3D-printing technique are given in chapter 2. For the chapter 3 and 4, experimental methods and results are explained respectively. Discussion part of this study presented as chapter 5 and finally, chapter 6 contains conclusion and future aspects for this project.

2. BACKGROUND

2.1 Otitis Media

Otitis media (OM) is an infection placed in the middle ear and generally started with an upper respiratory tract viral infection. Inflammation caused by the viral infection starts edema formation in the nasal cavity and nasopharynx which leads to difficulty in the function of the Eustachian tube and pressure disequilibrium occurs. Because of negative pressure inside the Eustachian tube, bacteria-contained mucus secretion from the upper respiratory tract captured in the middle ear. The entrapped bacteria start to replicate and ensure infection behind the eardrum [6].

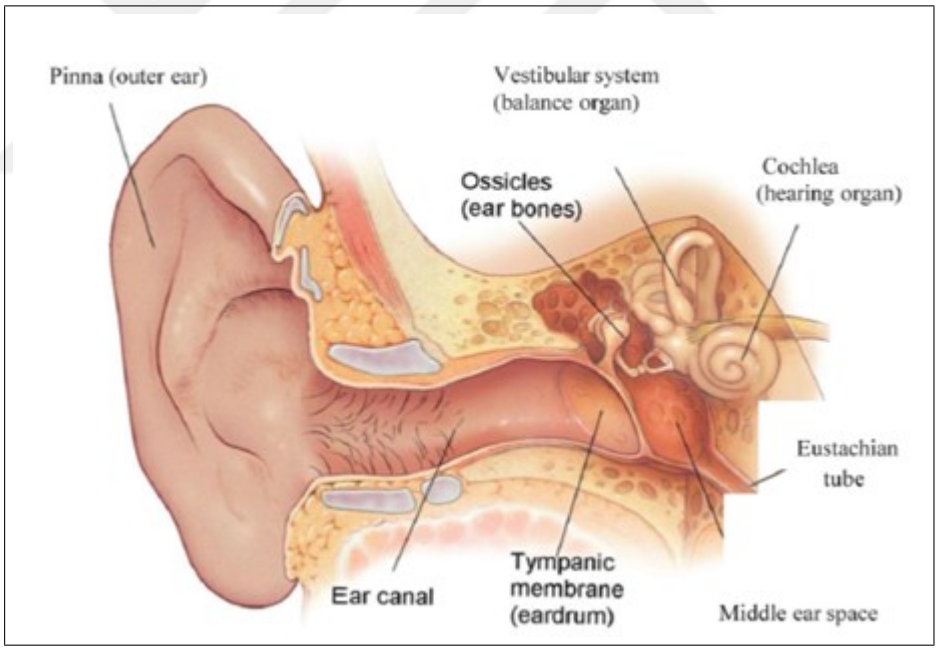

Figure 2.1 The anatomy of middle ear area [7].

The otitis media is one of the most common upper respiratory tract illnesses and tympanostomy tube (TT) replacement is the most common ambulatory surgery performed on children in the United States [1],[8]. By the 3 age, 50-85% of children have had one or more otitis media episodes and 40% of them struggle with it more than six times. The high incidence of OM in young age children is caused by short

and horizontal physiology and poor function of their eustachian tube. With aging, the maturation of the Eustachian tube occurs and the prevalence of the OM in children decreases [9].

2.2 Tympanostomy Tube and Insertion Procedure

The TT insertion occurs because of persistent middle ear effusion and repeated middle ear infections (otitis media) which can be resistant to antibiotic therapy. TTs are inserted on the tympanic membrane which connects the ear canal and middle ear area to create a channel to equalize the pressure [7].

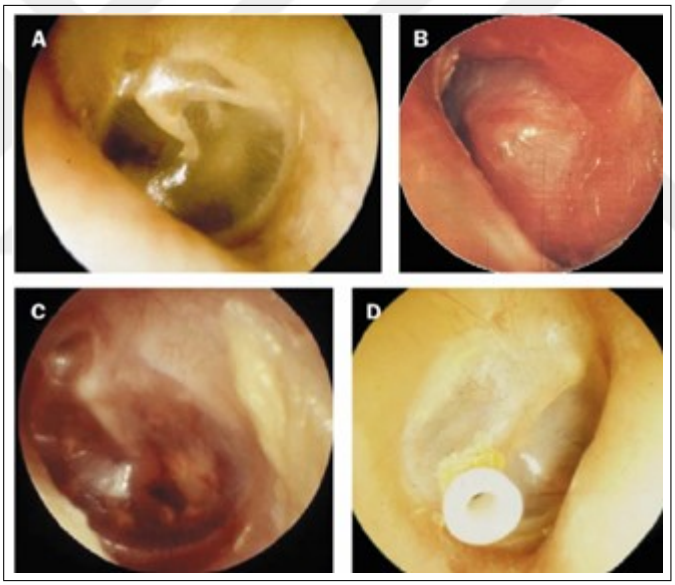

Figure 2.2 (A) Normal tympanic membrane, (B) acute OM, (C) OME, (D) Tympanostomy tube [9].

According to the need and severity of the case, the tubes are divided into two categories as long-term and short-term. Their application times are different; while the short-term tympanostomy tubes are commonly used for 6-18 months, the long-term ones are applicable for years and are generally placed after short-term tube usage. The application time difference is caused by the structural differences between them; the long-term tympanostomy tube has a larger luminal and flange diameter to prevent occlusions during the treatment [4].

After some time, it is expected that the tube should fall from the eardrum by itself; but if it is not the case, an extra surgical operation is required to take off the tube. Also, if the TT does not fall from the eardrum by itself, the TT retention for a long time could cause chronic otorrhea, presence of tissue granulation on the tube, blockage of the lumen, tympanosclerosis, cholesteatoma, and persistent tympanic membrane perforation. These indications can be cleared off only by the removal procedure of TT from the eardrum. However, following the removal of the TT, patients may experience persistent perforation which can cause further middle ear infections, hearing loss, high risk of cholesteatoma on the ear membrane. The surgical removal of the tube increases the rate of incidence of persistent perforation occurrence.

2.3 Post-Tympanostomy Tube Insertion Complications

Otorrhea: Post-tympanostomy tube otorrhea (PTTO) is active drainage through an existing tympanostomy tube caused by a pathogenic bacteria attachment onto the abiotic surface of TT and remaining until reaching a critical mass to detach and produce drainage. Early PTTO occurs within the first 2 weeks after surgery, and it is mainly caused by prior middle ear infection or contamination of the external auditory canal during TT insertion. On the other hand, late PTTO is usually caused by an upper respiratory tract infection after 2 weeks from the surgery [10].

The PTTO can be described as acute (lasting less than 6 or 8 weeks) or chronic (lasting more than 6 or 8 weeks). Under two years of age, the acute PTTO is usually caused by the same pathogen with the acute otitis media (AOM) that are *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. On the other hand, the children older than two years old, the agents of acute PTTO are most likely Staphylococcus aureus and *Pseudomonas aeruginosa*. The antibiotic therapies which include multiple types of drugs contribute to increasing drug resistance of organisms. The challenge of treating antibiotic-resistant infection which causes PTTO creates the need for new developments to prevent PTTO [10].

Tissue Granulation: Granulation tissue formation increases with the oxygen concentration in the middle ear and the incidence of this complication increases parallelly with the prolonged retention time of TT [11].

Tube Occlusion: After TT insertion operation, tubes can turn into a nonfunctional state because of premature occlusion. TT plug is the cause of tube occlusion, and it is composed of mucoid middle ear effusion which is mostly caused by PTTO and there are many studies to break up the plug with solvents, but their results are not promising. In the long run, tube occlusion leads to additional procedures with general anesthesia like replacement of the tube or tube extraction which drastically increases the costs and risks of the cases [12],[13].

The design, surface, and material of the tube could make a difference in terms of plugging it. According to Benjamin et al, the ion-bombarded silicone surface has less occlusion from the untreated silicone tube, and this suggests that the alterations to make the surface smooth can also work for the prevention of tube occlusion [12].

Tympanosclerosis: Tympanosclerosis is an abnormality that is characterized by calcareous deposits on the tympanic membrane and tympanic cavity, and it is the second most common complication seen after TT insertion. In the case of removal and replacement of TT, the appearance of the condition increases, and a couple of years after the procedure, it progresses for 25% of the cases. After this time, its progress stops, and in most cases, the scar becomes stable and permanent [14].

Cholesteatoma: Cholesteatoma is an unusual cyst formation behind the eardrum because of unequal pressure caused by a nonworking eustachian tube. It is treated with surgical operation under general anesthesia. It may cause hearing loss and additional ear infections. The incidence of this complication is reported as 1.1% [14]and this incidence is significantly higher in patients with poor eustachian tube function [15].

Persistent perforation: Persistent perforation of the eardrum is usually caused by the operational removal of a long-term TT. Chronic otorrhea, the presence of granu-

lation tissue, tube occlusion, tympanosclerosis, and cholesteatoma are the main indications for the need for a tube removal procedure. With the extraction of the long-term TT, the incidence of the occurrence of persistent perforation increases from 10% to 47% [4].

As a result of persistent perforation, hearing loss, persistent middle ear infections, and cholesteatoma risks increase. If the persistent perforation remains, tympanoplasty which is a procedure that requires general anesthesia is needed to repair the eardrum [4].

Extrusion and retention times: Extrusion time is commonly between 6 to 18 months for the TTs and normally it occurs spontaneously with the healing of the tympanic membrane but if TT has not fallen in two years, it is considered as retained and should be removed with an extra procedure. Persistent perforation, tissue granulation, cholesteatoma, and otorrhea are the most common complications of retained TT. The removal of the retained TT is advised because of these complications, some of them require surgical repairment and can cause hearing loss. As the retention of the TTs, extrusion into the middle ear is another worrying complication and it is advised that the tubes which are extruded through the middle ear side of the tympanic membrane should be treated as a foreign body and removed even if the patient is asymptomatic [16].

2.4 Materials for the Fabrication of Tympanostomy Tubes

The very first tube insertion on the eardrum was done by two doctors called Martell Frank and Gustav Lincke, in 1845, with a gold tube that keep a perforation open. The invention of new types of tympanostomy tubes continued but these tubes had a very low success in treating otitis media because of pain, plugging of tubes, early extrusion, and further infections. The popularity of the tympanostomy tubes came back with the Armstrong in 1954 [2]. He used a polyethylene tube as a first-timer and after that, it was understood that the fabrication material and the design of the tubes

are more important than it was thought for the fate of the treatment [1].

Tympanostomy tubes have been manufactured from many materials. The important point for a tympanostomy tube material is biocompatibility with the middle ear tissue to prevent the side effects which lead to early extrusion. Because of their compatibility with the body, many metals are suitable as fabrication materials such as gold, titanium, and stainless steel [1].

Other common fabrication materials are fluoroplastic and silicone. The non-adhesive and inert properties of fluoroplastic make the material convenient for medical applications. On the other hand, due to its silicone-oxygen backbone, silicone has excellent flexibility with nonpolar-hydrophobic surface characteristics [1],[17].

However, neither of these materials allows biodegradability. The ideal tube should stand on the eardrum for a required time interval and when the treatment ends, it should not leave scar or tissue damage behind it. These requirements can be met by using a biodegradable polymer as a raw material that can be the solution for wrong-timed falls, surgery requirements for extrusion, and persistent perforation cases.

2.5 Biodegradable Materials for Tympanostomy Tubes

The studies about biodegradable TT fabrication were started with F. Barry Bays in 1987 with a patented PLGA tympanostomy tube [18]. Although the effectiveness of PLGA TT of Bays was proved with an animal study and it is approved by FDA, the biodegradable TT has not been marketed.

Massey et al studied TTs made by PLA and PLGA mixtures in different percentages, their animal study showed that PLA tubes have been resorbed in 86.6 days and the PLGA ones stayed for 19.6 days on average. The result of biocompatibility studies also showed that no inflammation and tissue damage occurred with these materials or their byproducts from the degradation process [19].

One of the other studies that used biodegradable polymer as a fabrication material is Gan et al. They compared commercial TT, PLA-PCL copolymer, and PLA-PCL copolymer with ofloxacin in the guinea pig. It is concluded that the PLA-PCL tube has a smoother surface and could degrade in a predictable period which may prevent the second surgical operation to extract TT in the future [20].

Ludwick et al [21] used PLA as fabrication material for the tympanostomy tube. They fabricated tubes from solid pieces of PLA, and they compared them with the Fluoroplastic tympanostomy tubes in terms of bacterial attachment. They claimed that because of acidic output of degradation reaction, less biofilm formation occurred on them.

2.6 Physical and Chemical Properties of PDLLA

Kulkarni et al. [22] used polylactic acid as material for surgical implant because of its degradation process occurs with hydrolytic de-esterification and the result product of the process is lactic acid which is a compound that found in animal body naturally. This study is first suggest for PLA use as a fabrication material for inter body devices and their idea relied on nontoxic and non-tissue reacting nature of the polymer [5].

L-lactic acid and D-lactic acids are two isomers of lactic acid and shown in Figure 6.1. The homopolymer of lactic acid has glass temperature (Tg) equals to 55°C and melting temperature (Tm) 175°C. Lactic acid polymer can have pure L-Lactic acid isomer (PLLA) or D-Lactic acid isomer (PDLA), with the structure of hemicrsytalline and crystalline, respectively. On the other hand, with the combination of both isomers in different ratios, the poly (D, L-lactic acid) (PDLLA) can be produced as amorphous polymer. PLLA, PDLA and PDLLA has common solvents as benzene, chloroform, dioxane etc. And all of them degraded by hydrolysis of ester bonds without require any hydrolase. PLA has a half-life for degradation from 6 months to 2 years that change according to environmental conditions. Experiments show PDLLA has about 3 months degradation half-life in 37°C normal saline [5].

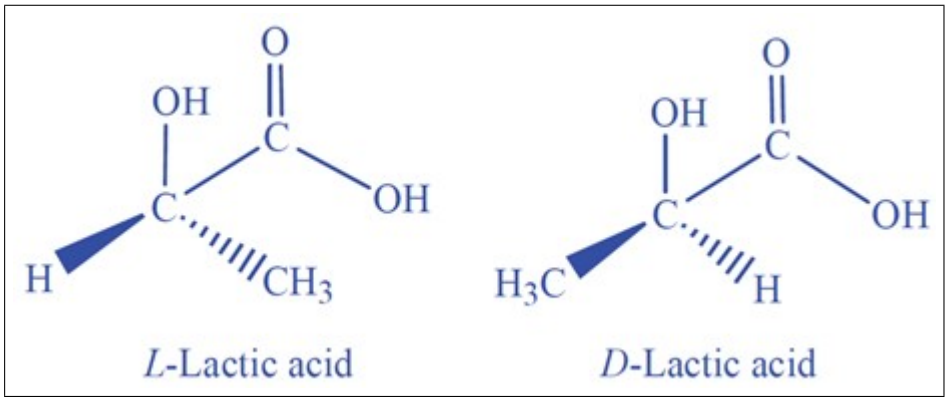

Figure 2.3 L- and D- isomers of lactic acid [5].

2.7 Three-Dimensional Printing Technique and Fabrication of Tympanostomy Tubes

A growing trend towards to meet the anatomical needs of patient drives attention to tailored medical devices and, with the discoveries in engineering and technology, the three-dimensional printing (3D-printing) technique became the most revolutionary and powerful one in terms of being a versatile tool to manufacture patient centered devices. The International Standard Organization (ISO) defines three-dimensional printing as 'fabrication of objects through the deposition of material using a print head, nozzle or another printing technology' [23].

The fabrication process relies on layer-by-layer deposition of the material according to a computer aided design (CAD) and different solidification techniques according to specific printability characteristics of the materials. Firstly, the extrusion-based 3D printing methods typically use cartilage and a pneumatic actuator to feed the nozzle with material. The materials that can be used with this type of system has extensive range and one should choose the right one. Some materials are extensively characterized for medical use and perform better. For example, polycaprolactone may be an excellent candidate for usage in bone cartilage tissue because of its mechanical strength similarity with native tissue and low melting point (60°C). However, if the printing goal is fabrication of a scaffold out of specific cell types, cell embedded hydrogels are good options as bio-inks. After choosing right material, the planned object

should be designed with CAD software. Design of the device is important in terms of compatibility with living tissue and its needs according to where and when it will be used. Finally, the model should be sliced into layers. The material viscosity, printer head speed, nozzle temperature, extrusion rate, and nozzle diameter effects the slicing process in terms of need of layer spacing, rotation in fiber orientations and material deposition in unsupported regions [23],[24].

3. MATERIALS AND METHODS

3.1 TT Fabrication with 3D Printing Technique

The fabrication of the samples was done with the Envisiontec 3D-Bioplotter Manufacturer printer thanks to generosity of the Prof. Dr. Vasıf Hasırcı and Dr. Menekşe Ermiş in Middle East Technical University, BIOMATEN.

Poly (D,L Lactic Acid) (PDLLA)(Purasorb PDL 20, Corbion, Netherland) was chosen according to its biocompatibility, degradation time in body which is more than 6 months based on previous studies, and printability with the 3D-printer [25].

Sample geometry and measures were designed according to industrial tympanostomy tube types. The collar button type has optimum geometry for the printing process without using any support material. Figure 3.1 shows the measures of the 3D-printed PDLLA TT CAD design. The inner diameter was 1.27 mm, the shaft length between the flanges was 1.55 mm, the total length was 2mm and the flange diameter was 2.8 mm. Also, all the dimensions of the sample were consulted by the ear nose and throat specialist.

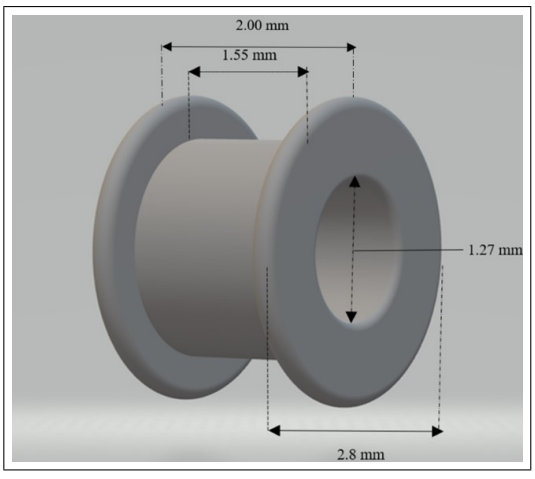

Figure 3.1 Design of the 3D-printed collar bottom type tympanostomy tube with the measures via CAD.

The material was stored at 4 °C as manufacturer advised. Prior to printing the cartridge was heated to the preheating temperature which is independent from the polymer than 2.5 g of polymer added in the cartridge and heated for a few minutes to melted out. Based on the pilot study with PDLLA and the design, the PDLLA samples printed at a temperature of 190 °C for nozzle with a 0.3 mm tip and a printing speed of 0.1 mm/min with the 7.9 bar pressure. Fan or any other cooling effect for the bed was not used to not to prevent adhesion between the fabrication layers. The results of the fabrication process is shown in the Figure 3.2 and Figure 3.3. In the Figure 3.2,the left one is Fluoroplastic and the right one is 3D-printed PDLLA TT. The metal pin on the Fluoroplastic one is used for holding of the tube during the surgery procedure.

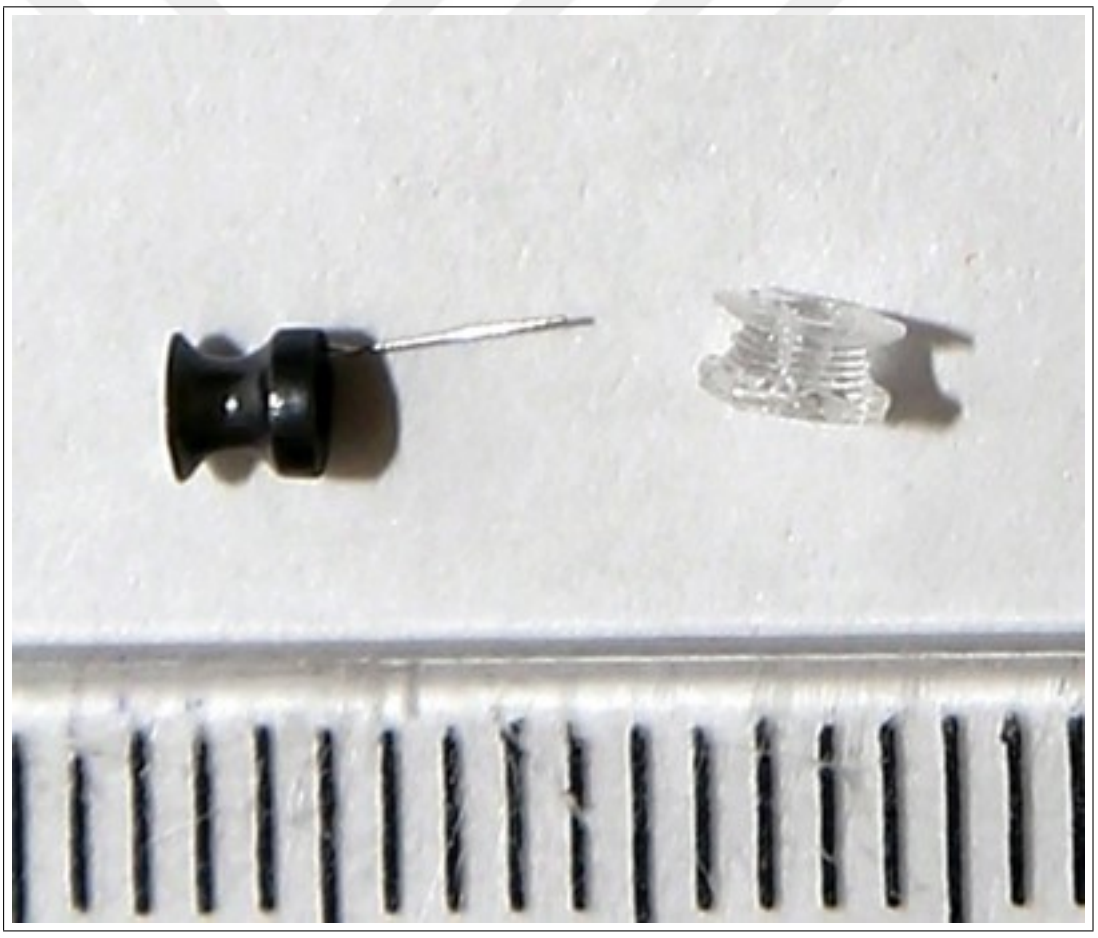

Figure 3.2 The result of fabrication process. The right one is 3D-printed PDLLA tympanostomy tube and the left one is Fluoroplastic tympanostomy tube (Medtronic, Shepard Grommet). Each gap between scale bars represents 1 mm.

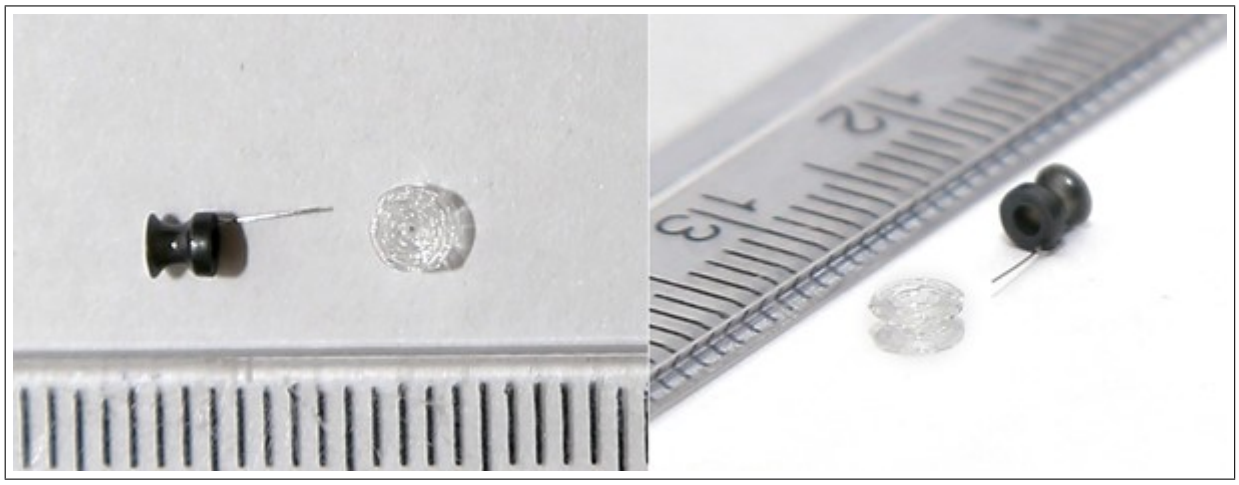

Figure 3.3 The diameter differences of cylindrical hole between 3D-printed PDLLA and Fluoroplastic tympanostomy tube. The black one is Fluoroplastic one, and the white is PDLLA sample.

3.2 Degradation and Swelling Behavior of 3D Printed PDLLA TTs

The degradation and swelling characteristics of the 3D printed PDLLA tympanostomy tubes are analyzed for five weeks with four measurement time points (week 0, 1, 3, and 5), each has six samples.

After time initial weighting (Wi) of samples, PDLLA tubes were put into PBS (pH 7.55) at 37 °C as three groups for measurement time points; one is for week 1, one is for week three, and the last one is for week 5. After each time point, they were removed and slightly wiped with tissue paper to measure their wet weights. After the weight measurement of the wet samples (Ww), they were dried for 24 h at 37 °C in the incubator to take their final weights (Wf) and determine degradation percentage calculated their mass loss and water uptake in percentage [26].

Swelling percentage calculation was done according to the equation of [26],[27]:

$$\%WaterUptake = 100\frac{W_w - W_f}{W_f} \tag{3.1}$$

Mass loss percentage calculation was done according to the equation of [26],[27]:

$$\%MassLoss = 100\frac{W_i - W_f}{W_i} \tag{3.2}$$

3.3 pH Change

pH measurements of the samples (n=6) incubated in PBS were measured twice a week for 21 days via pH meter (Thermo Scientific-Orion Star A211). The pH values were recorded once in three days.

3.4 Surface Characterization and Comparison with Fluoroplastic TT via SEM

The surface morphology of samples that are not subjected to any other experiment was studied via scanning electron microscopy (Philips XL30) at Bogaziçi University to observe the surface and structural differences between fluoroplastic industrial TT and 3D printed PDLLA TT. Samples were coated with platinum and examined under 150X, 500X, 1500x, and 200X magnification.

3.5 Quantitative Biofilm Analysis

3.5.1 Bacterial Preparation

Because *Pseudomonas aeruginosa* infections are one of the most common reasons for otitis media, it was used in this experiment. The cryopreserved bacteria (*Pseudomonas aeruginosa* ATCC 27853) were streaked out onto tryptic soy agar plate, and they were incubated overnight at 37°C. After incubation overnight, colonies were selected and suspended in TSB and incubated overnight with constant agitation at 37°C. After these two preparation steps, the bacteria were transferred to fresh TSB and were grown to the early log phase (optical density of about 0.2 at 640 nm), approximately 1-2x108 CFU/mL L [28],[29].

3.5.2 TT Preparation

For each test group total of 10 TT is used (5 from fluoroplastic TTs and 5 from 3D printed PDLLA TTs), and three separate trials were conducted to evaluate the reproducibility of the protocol and form each trial samples from tubes were inoculated on the agar plate as duplicates [21].

Table 3.1
Experimental Groups of Agar Plates.

Pseudomonas aeruginosa			
Trial	Fluoroplastic	PDLLA	Total
1	5	5	10
2	5	5	10
3	5	5	10
Total TT	15	15	30
Total Plate	30	30	60

TTs were sterilized with EtOH and UV then transferred into 48 well plates,

with the order of one TT for each well. Wells were filled with 600 μL of TSB in 1x108 CFU/mL. After the organization of the wells, TT is incubated at 37 °C for four days with the TSB that was changed every they to prevent nutrient depletion [28],[29].

After four days of incubation, the TSB in the wells was changed with the TSB containing Gentamicin Sulfate 20 μg/mL which is ten times stronger than and incubated for another 24 hours to kill the planktonic bacteria [28],[29].

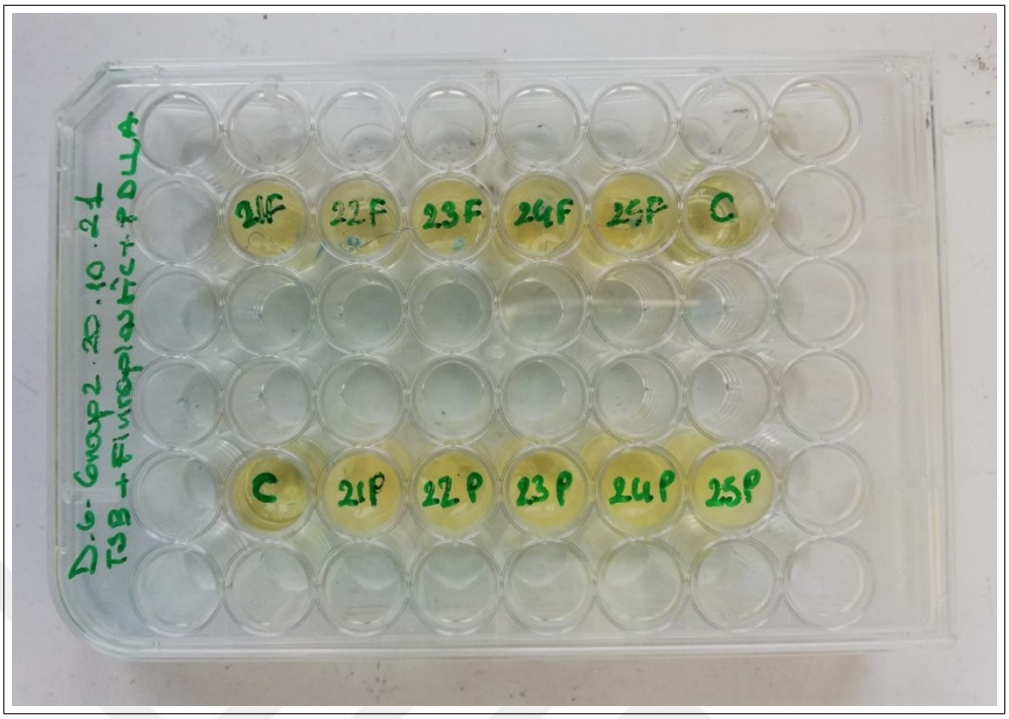

Figure 3.4 The experimental setup after 24 hours incubation with Gentamicin Sulfate 20 μg/mL.

3.5.3 Quantitative Assessment of Biofilm Development

Each group of the tube were taken out from the wells and washed four times for 10 min by adding 150 μL of 1 M PBS. After washing with PBS, each one was placed in 15 mL conical tubes containing 5mL fresh PBS and placed into the water bath at 37 °C and sonicated for 5 min with serial sonication for 1 min exposures separated by 1 min of rest. After sonication, tubes are vortexed in setting 8 for 15 seconds. The supernatants are serially diluted and plated on TSA plates as duplicates [28],[29].

After inoculation on the agar plates, tubes were incubated for 18-24 hours, and colonies were manually counted to determine CFU/mL for *Pseudomonas aeruginosa* [28],[29].

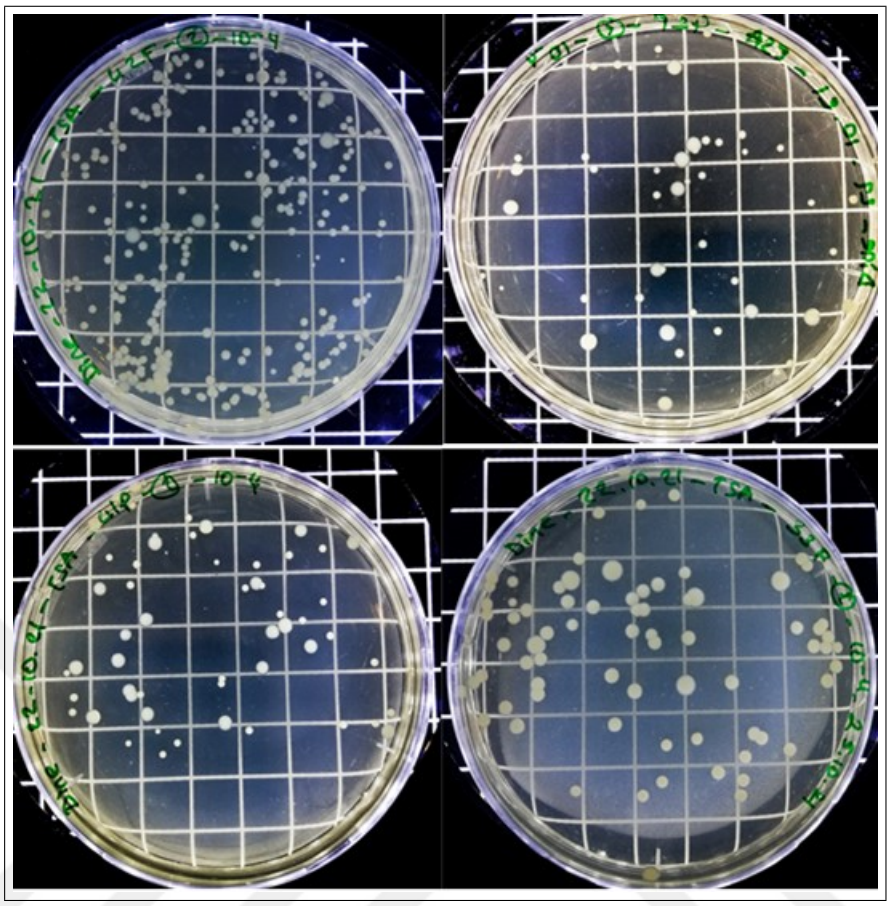

Figure 3.5 Culture plates that are ready for colony counting after 18-24 hours incubation at 37 °C.

3.6 Statistical Analysis

The statistical evaluation of results was performed applying one-way ANOVA analysis with Tukey's post-hoc test for degradation and swelling behavior measurement part and student t-test for quantitative biofilm assessment via SPSS for Windows software, and $p \leq 0.05$ was considered as statistically significant and signed with (*).

4. RESULTS

4.1 Degradation and Swelling Study

Figure 4.1 and Figure 4.2 shows respectively the degradation and swelling characteristics of 3D-printed PDLLA tympanostomy tube samples of incubation for 5 weeks in PBS at 37 °C.

According to statistical analysis, there were significant changes in mass of the experiment groups in 5 weeks ($p \leq 0.01$). The mass loss followed an increasing trend through the experiment. The average weight loss in one week is about 1.73±0.47 % and significantly different from the weight loss of week 5 which is about 4.47±0.65 % ($p \leq 0.01$). Also, there is a particular difference in weight loss percentages of week 3 and week 5 that are 2.34±0.29 % and 4.47±0.65 % respectively ($p \leq 0.05$). However, there is no significant difference between week 1 (1.73±0.47 %) and week 3 (2.34±0.29 %) ($p \geq 0.05$).

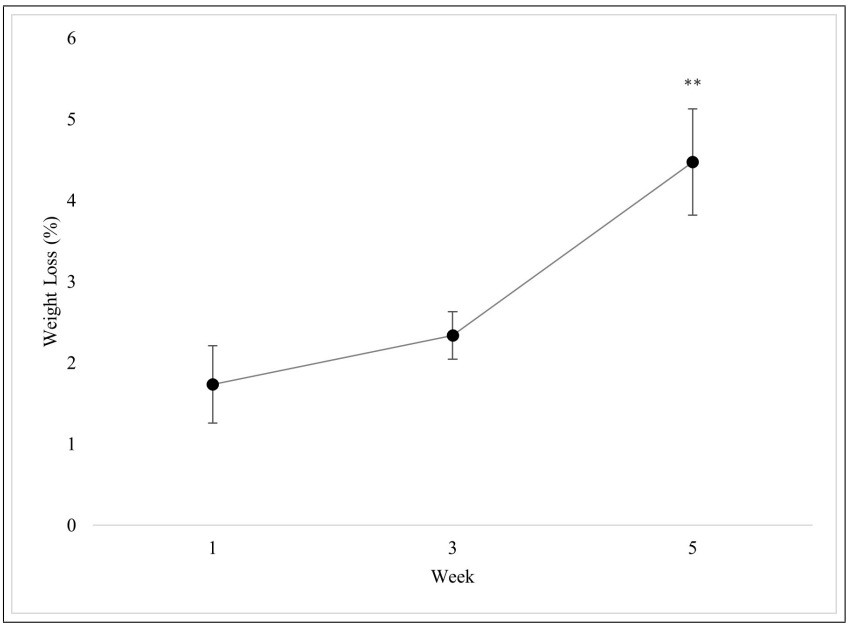

Figure 4.1 Weight loss in percentage through 5-week degradation experiment in PBS. Results are represented with standard errors.

According to statistical analysis, total swelling is around 39.32±4.58 % in 5 weeks. Also, it has increasing trend to swell more with time. Statistically, there is a significant difference between weeks ($p \leq 0.01$).

The average swelling percentage is about 18.25±1.70 % in week 1 and it is significantly different from the week 5 that is about 39.32±4.58 % ($p \leq 0.01$). On the other hand, neither week 1 nor week 5 have the swelling percentage which is significantly different from week 3 (29.07±3.75 %) ($p \geq 0.05$).

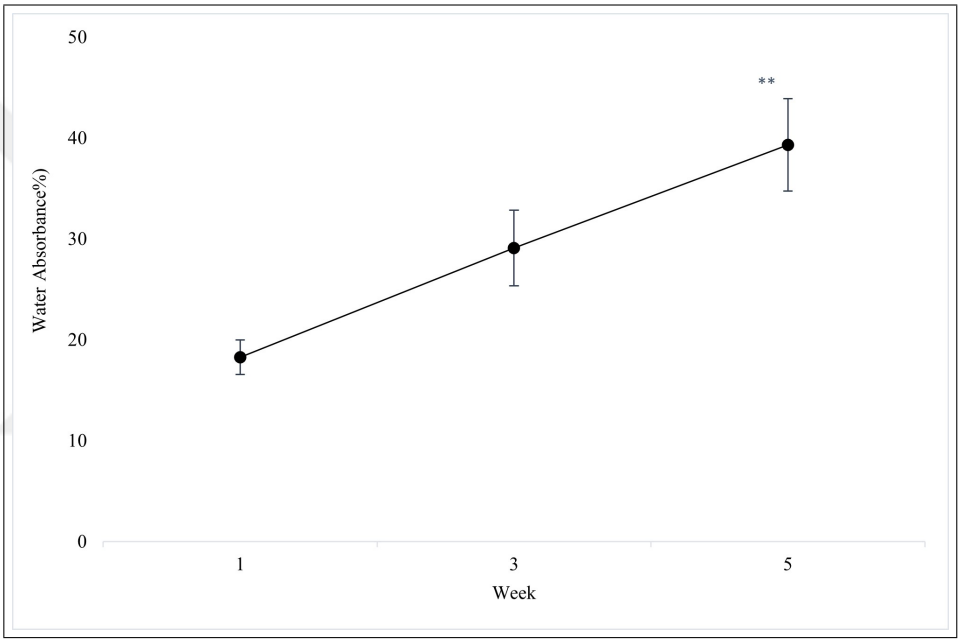

Figure 4.2 Swelling behavior of 3D printed PDLLA Tympanostomy tubes in 5 weeks in PBS. Results are represented with standard errors.

4.2 pH Studies

The measured pH levels for 21 days as 1 in 3 days row are shown in Figure 4.3.

According to statistical analysis, there is no significant difference between time points ($p \geq 0.05$) and at the end of the 5 weeks, the pH level is still around 6.62±0.081.

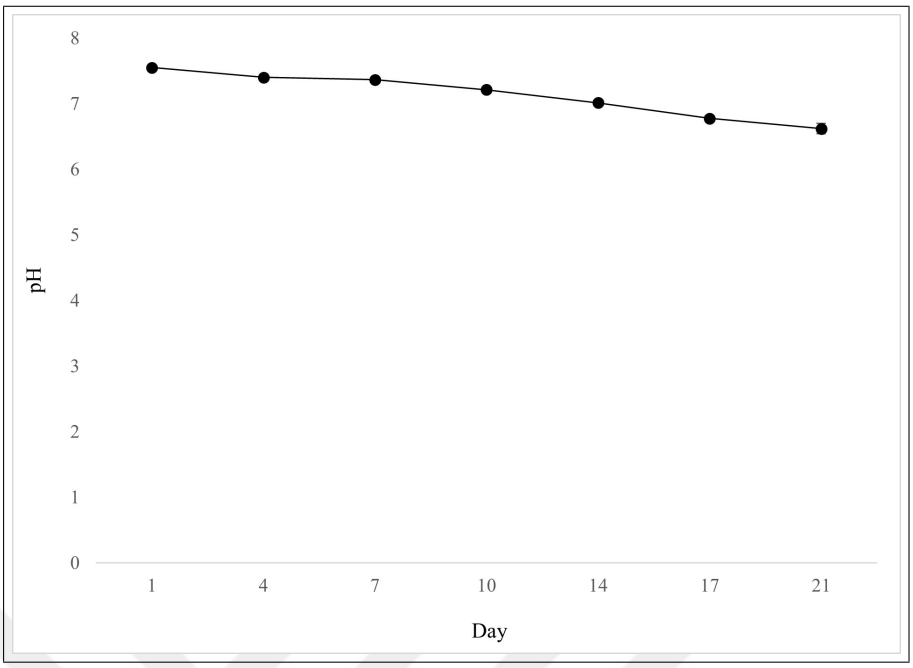

Figure 4.3 pH measurement results of samples after 1,4,7,10,14,17, and 21 days of incubation. Results are represented with standard errors.

4.3 Surface Characterization and Comparison with Fluoroplastic TT via SEM

SEM images of the Fluoroplastic and PDLLA ones are represented with Figure 4.4, Figure 4.5, Figure 4.6, and Figure 4.7.

Figure 4.4 shows us general appearance differences between the two tubes under 150x magnification with scale bar 500 μm. Despite their dimensions are the same, differences between their structures can be seen. The layer-by-layer structure of the 3D printed PDLLA TT is sourced from the fabrication technique. The diameter of the layers is 300 microns, and this is the lower limit for the filament diameter that can handle the physical limits of the PDLLA and structure of the design. (B)

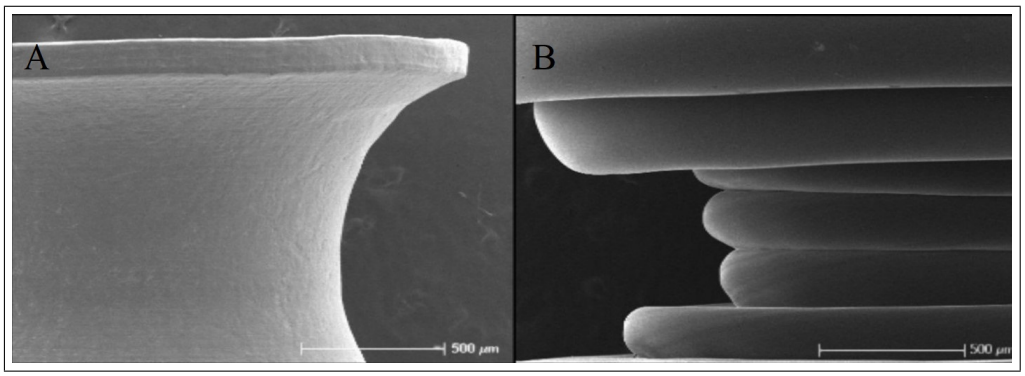

Figure 4.4 (A) Fluoroplastic tympanostomy tube and (B) PDLLA tympanostomy tube under 150x magnification (scale bar: 500μm).

Figure 4.5 shows samples under 500x magnification. It can be seen that there is no gap between the layers of 3D printed PDLLA TT (B). Also, some marks and scratches on the Fluoroplastic one (A) can be seen.

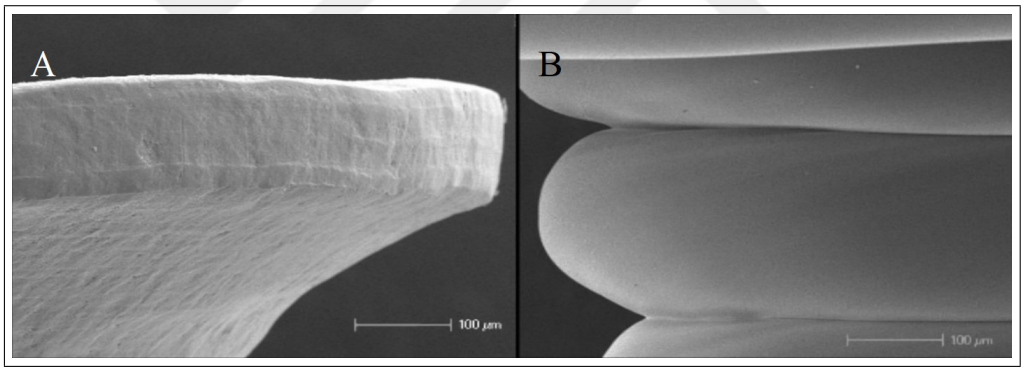

Figure 4.5 (A) Fluoroplastic Tympanostomy tube and (B) PDLLA tympanostomy tube under 500x magnification with the scale bar 100 μm.

Figure 4.6 shows us how layers of the 3D printed PDLLA (B) sample stick to eachother by comparing Fluoroplastic tympanostomy tube.

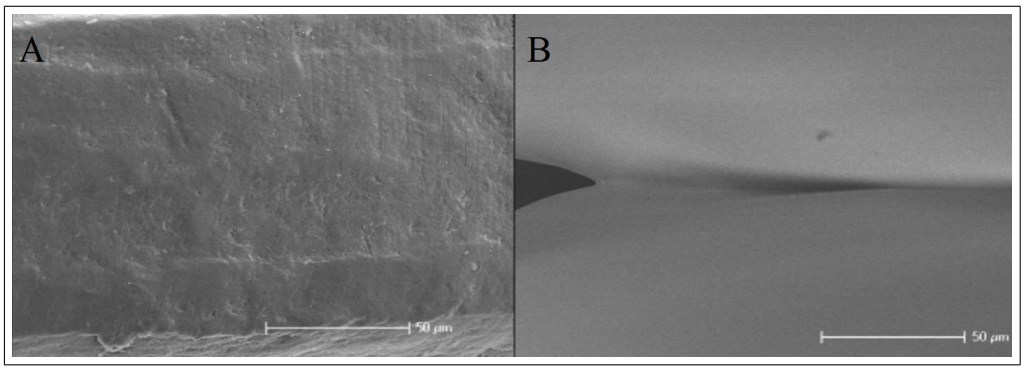

Figure 4.6 Fluoroplastic (A) and PDLLA (B) tympanostomy tubes under 1500x magnification (scale bar: 50 μm).

The surface differences could be easily seen in Figure 4.7. The Fluoroplastic tympanostomy tube has scratches, marks, and material residues on the surface (A). On the other hand, 3D printed PDLLA one (B) has smoother surface with no marks or residues.

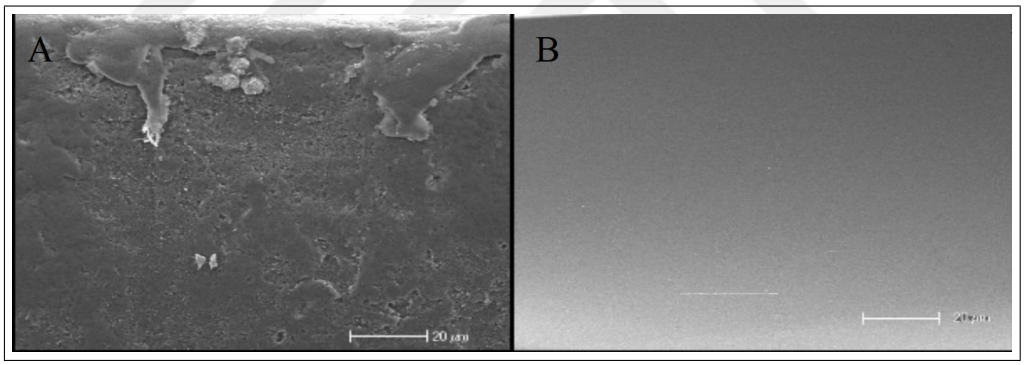

Figure 4.7 Fluoroplastic (A) and PDLLA (B) tympanostomy tubes under 2000x magnification (scale bar: 20 μm).

When we looked at Figure 4.8 with SEM at 10000X magnification results for Fluoroplastic samples, we could see the grooves smaller than the 5.0 micron. However, because the PDLLA samples started to melt in this magnification, we could not observe them in that magnification.

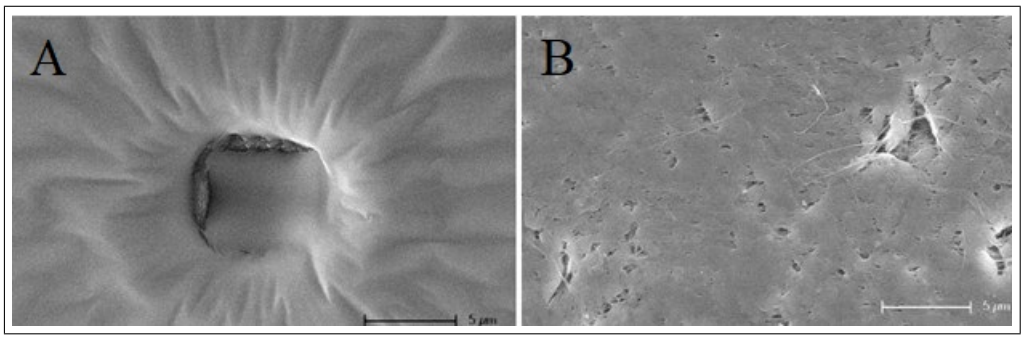

Figure 4.8 The melting on PDLLA samples (A) and grooves on the surface of Fluoroplastic samples (B) in 10000X magnification.

4.4 Quantitative Bacterial Biofilm Analysis

For the comparison of bacterial attachment on fluoroplastic and 3D-printed tympanostomy tubes, the quantitative biofilm assesment with *Pseudomonas aeruginosa* was done.

Table 4.1
Colony counts of fluoroplastic and PDLLA tympanostomy tube samples at the end of the 4 days of biofilm formation assessment with *Pseudomonas aeruginosa*.

Material	Run	Plate	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average	
Fluoroplastic	1	1	88	244	100	50	32	102.8	105.3
		2	72	220	152	45	50	107.8	
PDLLA	1	1	56	64	57	56	98	66.2	74.5
		2	47	56	189	54	68	82.8	
Fluoroplastic	2	1	35	43	249	172	48	109.4	108.5
		2	46	49	259	149	35	107.6	
PDLLA	2	1	37	57	56	45	270	93	84.3
		2	41	56	67	47	167	75.6	
Fluoroplastic	3	1	35	257	76	38	85	98.2	94.1
		2	33	219	88	32	78	90	
PDLLA	3	1	57	44	240	76	40	91.4	86.6
		2	70	40	198	63	38	81.8	

The Colony-Formation Unit/ml calculation was done according to formula which is given in materials and methods part. Table 4.2 shows us the CFU/ml of each experimental group and their summary of statistics.

Table 4.2
Summary of CFU/ml values of each experimental groups and their statistics.

Material	Group	CFU	Mean	Standard Deviation	Standard Error
Fluoroplastic	1	1.05E+07	1.03E+07	7.56E+05	4.37E+05
	2	1.09E+07			
	3	9.41E+06			
PDLLA	1	7.45E+06	8.18E+06	6.43E+05	3.71E+05
	2	8.43E+06			
	3	8.66E+06			

As a result of quantitative biofilm analysis assessment, the average colony formation unit for each ml for fluoroplastic TT's is around $1.03\times10^7\pm4.37\times10^5$ and 8.18×10^6 $\pm3.71\times10^5$ for 3D-printed PDLLA ones. According to statistical analysis 3D-printed PDLLA tympanostomy tubes has less bacterial biofilm formation on them ($p\leq0.01$)

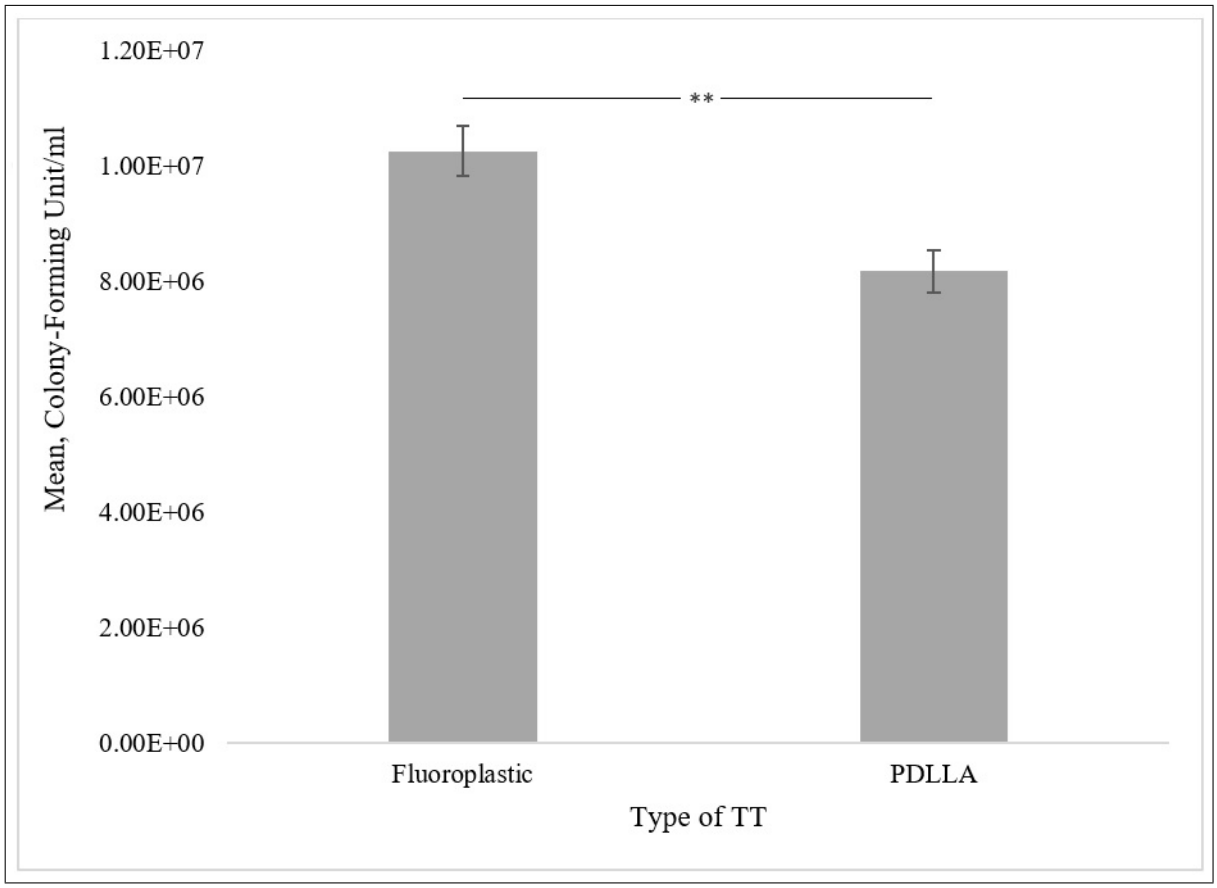

Figure 4.9 Mean colony-formation unit per ml in fluoroplastic and 3D-printed PDLLA tubes for 4-day of biofilm formation assessment with *Pseudomonas aeruginosa*.

5. DISCUSSION

3D printing of a biodegradable tympanostomy tube is the novel part of this thesis, and there is not enough research about samples in the same dimensions as our one in literature to compare. The closest study is a cardiac stent study with PCL/PLA composite, but the printing technique is use of 3D tubular printer with a melted material deposition on a rotating platform [30].

Since 3D printing has some constraints about the temperature and dimensions of the product, there are some structural and fabrication process-related problems. The first problem is requiring a very long time to melt down the polymer and 3D print the scaffolds. Polymer melting in the cartridge before printing takes around 20 minutes and each tympanostomy tube printing with a 200 μm nozzle takes around 13 minutes which is a very long time to prevent temperature exposure caused material degradation [7]. Because high temperature increases the degradation and breaking of structural chains, the viscosity of the material decreases during the printing procedure due to the heat exposure to melt down the polymer. Decreased viscosity increases the fluidity of the polymer during the process, and the time requirement for solidifying increases, which may affect the layer-by-layer structure as mentioned before [7].

The first printing was completed with the 200μm nozzle diameter, and the procedure took a very long time, as mentioned before; although the design improves with fine layers, their structures were not identical to each other because of viscosity change since the degradation of PDLLA inside the cartridge. After that, fabrication continued with a 300 μm nozzle, and the process took 9 minutes rather than 13. The structures of samples were very close to each other, nearly identical.

To prevent the cooling problem mentioned before, the bed temperature may be arranged to cooler temperatures, but, for our samples, it does not work correctly. Because dimensions are tiny (2mm in length), layers solidified too quickly, and the

fast-cooling situation affected proper adhering between layers. To prevent both fast solidification and collapsing of the structure, printing was done at room temperature, and less material was filled out in the cartridge for each printing process as soon as possible to prevent the degradation caused by temperature exposure. The adhering between layers is represented in SEM results, which demonstrated that the layers adhered well to each other.

Also, the printing procedure was done in three steps by cutting the design into three parts as flanges and shaft which are apart from each other. The time passed between each part allowed some cooling and solidification before the next layered on.

From the final product, we could see that the hole inside the cylindrical structure of the PDLLA sample shrank. Through the middle of the cylinder, the hole is getting narrower than upper and lower parts, and a collapsing situation might cause this problem through the printing process. This narrowing situation may cause occlusion of the tube. Figure 5.1 shows the structural differences in terms of hole diameter between Fluoroplastic and 3D-printed PDLLA samples. This narrowing situation may cause occlusion of the tube.

For the degradation results of our samples, they were compared with the literature. Yanfeng et al. [31] studied different polymerization reaction techniques on PDLLA, and for the melt-opening ring, polymerized polylactic acid equals our PDLLA degrade around 5% in five weeks at 37 °C in PBS solution, and it is parallel with our result. For the swelling part, Li et al. [32] had 40% water uptake for five weeks at 37 °C in water, and our one has a swelling of about 39.3% in PBS at the end of 5 weeks.

In terms of the bacterial attachment and biofilm formation minimizing goal, 3D-printed PDLLA samples have significantly less bacterial attachment than the Fluoroplastic tympanostomy tubes, which may result from the surface pattern, surface charge, and hydrophilic/hydrophobic state of the materials [33].

The aim of minimizing bacterial attachment on surfaces and even on tympanostomy tubes have a long history. Ion bombardment, antimicrobial coatings, or antibiotic-loaded material usages all had the same goal as preventing bacterial infections post-operatively [4]. Bacteria infections are occured on medical implants because of two reasons. One is the compromised immune system of patients due to foreign particles on the implant, and the second is the intrinsic tendency of bacteria to attach solid surfaces [33]. Bacterial attachment and biofilm formation have more advantages than the sessile lifestyle in terms of metabolic needs and survival [34]. The initiation of bacterial attachment starts with a few planktonic bacteria that behave like primary colonizers, and with the following others, biofilm formation occurs [33]. To prevent this process, ion bombardment, nitinol coating, and silver oxide usage as antimicrobial coating are successful strategies to decrease bacterial attachment. However, they do not solve the problems about cholesteatoma, persistent perforation, early and late retention times, and extra surgery to take of the tube [35],[36],[37],[38]. Also, antibiotic loading of the tubes is another way to prevent microorganism attachment, but their use increases antibiotic resistance and, as a result, expands the burden on the health system [20]. Turning face to biodegradable materials that have degradation time parallel with the needed time for recovery can be the solution for the problem.

The surface topography in different scales affects the bacterial attachment, but it is not clear that its mechanism is a complex process that requires a strong interplay between bacteria, the surface, and the surrounding medium [34]. More recently, micro-scale surface roughness is more effective in studies with *Pseudomonas aeruginosa* and *Staphylococcus aureus* than the nano-scale ones because of the more air-water interface that prevents bacteria from forming stable anchors the surface of the material and the high surface tension of the water stops bacteria from penetrating the air-liquid interface [39]. Because of this reason, surface topographies capable of stabilizing air-liquid surface interphase, which is much larger than the bacteria size, have a more antifouling effect on the bacteria than the smaller sized topographies [38],[39]. Also, the surface topography near the dimensions of bacteria (1-2 μm) was recognized as a positive contributor to the bacterial attachment process [38],[40]. One study done with silica samples with a defined surface topography shows that bacteria attachment

and contact area are positively correlated. However, when the topographic features are more significant than the dimensions of bacteria, attachment and contract area become independent from each other. After that point, bacterial attachment is not affected by groove width because it seems primarily flat from the point of bacteria. However, there is a difference between motile and non-motile bacteria; whereas non-motile bacteria cannot find and reach the larger grooves, the motile ones can [41].

To comment on the surface topography of our samples, there should be AFM analysis, but we could not do any surface analysis with AFM because of the small dimensions of the samples. From this perspective, we have only SEM analysis to observe the surface topography of the materials. From the images of SEM, we can say that the material has minor roughness on a microscale which is close to bacteria size (1.5 to 3.0 μm) than the Fluoroplastic one. However, it has more microscale roughness sourced from the layered structure that came from the fabrication process, and this type of roughness is bigger than the bacterial size and may contribute to forming air-liquid surface barriers to prevent bacterial attachment, as mentioned before [39].

When we looked at Figure 4.8 10000X magnification in SEM for Fluoroplastic samples, we could see the grooves smaller than the 5.0 micron. However, because the PDLLA samples started to melt in this magnification, we could not compare them with the Fluoroplastic ones but in any way the grooves dimensioned around 2-5 micron may be the reason for more bacterial attachment on fluoroplastic samples.

Also, because of the collapsing situation in the hole structure of the middle of the cylindrical structure of the 3D-printed PDLLA sample, which can be seen in Figure 3.3, the surface area of the samples might be decreased number of attached bacteria on the surface reduced.

Surface charge is another crucial factor for bacterial attachment on the surfaces, and it also affects biofilm formation. Most bacteria cells are negatively charged, and positively charged surfaces are more prone to attachment than negatively charged ones. Practically, this antifouling approach does not work in static systems because dead cell

accumulation on the surface may change the negative charge barrier. Also, some recent studies showed that the surface charge of the material might affect the biofilm structure in the long term [42]. The study which explored the biofilm formation difference between PLA and PLA-Mg samples claims that both samples have negative zeta potential, but PLA-Mg samples have more negatively charged according to measurements, and it is expected that it is helpful to prevent early biofilm formation. However, the free Mg^{+2} ions temporarily make the surface positively charged and facilitate the bacteria's early colonization on the surface [43]. In our study, the negatively charged surface characteristic of the PLA may affect the bacterial attachment on the 3D-printed samples and this might be the reason of less amount of biofilm formation during the short time incubation period because of decreased tendency of bacteria to attach on the negative charged surfaces since its surface also negative. However, because of the static environment effect, as mentioned before, the preventing effect of negative charge may be a temporary situation. The middle ear with effusion creates mainly a static environment.

Recent studies showed that superhydrophobic and superhydrophilic surfaces might both prevent biofilm formation. Francolini et al. 2015 [44] suggest that the antifouling activity is shown, especially when the materials are hydrophilic, electrically neutral, and accept hydrogen bonds. PEG is the most well-known one, and its antifouling characteristic is related to the ability to produce tight bound with water molecules and create a physical barrier on the surface that prevents protein adsorption (conditioning layer) and bacterial attachment. From this idea, polymers usually offer the best performances with good surface wettability and high-water swelling ability. Their study used double-phased materials with PU on one side (the hard part) and PCL, POO, and PLA on the other side (the soft part) to verify the surface wettability and hydrophilicity to check their antifouling characteristics. Mainly, PLA swelled up to 40% in 24 h, and the best antifouling results came from PLA samples. For our samples, PDLLA is a very hydrophobic and amorphous polymer; and the high swelling amounts are also seen from the swelling examination. Despite the hydrophobic character of the material, the high swelling ability might reduce the attachment of the bacteria.

The other way to prevent bacterial attachment is by adjusting the pH [33]. Because the degradation process of PDLLA samples has lactic acid as the output of the chemical reaction, the pH level starts to decrease after a short time. Although the pH measurement results from our experiment had no statistically significant difference at the end of the 21 days, the little amount of change in pH may affect the bacterial metabolism. The Ludwick et al. 2005 [21] quantitatively compared Fluoroplastic and PDLLA (70/30 DL racemic mixture) tympanostomy tubes in terms of bacterial attachment and found PLA ones more successful in preventing bacterial attachment parallel with our result. They discussed the result in terms of the acidic nature of PLA that may prevent biofilm formation [21].

6. CONCLUSION AND FUTURE WORK

Tympanostomy tubes fabricated from biodegradable materials are a new concept, especially fabrication via the 3D-printing technique. There were some holdbacks in terms of 3D printing because of the dimensions of the tympanostomy tubes, heat-related degradation of fabrication material, and the layer-by-layer structure of the final product.

The structural problems of the product, such as shrinking in the middle of the cylindrical structure and layered surface, might create further problems in vivo usage. Also, the pH decrease because of the formation of lactic acid since material degradation may stimulate an immune response. The bacterial attachment in the long term is also unpredictable from four days experiment because of the static environment effect, which is discussed in the discussion part.

The ideal tympanostomy tube should be easily fabricated according to the patient's need with a biodegradable material with proper degradation time to prevent extrusion and persistent perforation problems. Moreover, the bacterial attachment on the surface of the tube is another critical issue for producing an ideal tympanostomy tube because nearly all post-operative problems are caused by bacterial contamination and biofilm formation on the surface.

In conclusion, despite several problems about the fabrication process and unknowns about bacterial behavior on the material surface, this study has importance in its novelty. No study worked about the 3D-printing technique in this dimensional range. In addition, no research worked on tympanostomy tubes about bacterial attachment and printing techniques. Because 3D printing creates a layer-by-layer structure on the product, it should be studied in this aspect.

Our 3D-printed PDLLA samples need to be improved to be studied in vivo in the future. The layered structure may affect the attachment mechanism of the bacteria in either positive or negative way. It is expected that the layers may increase the biofilm formation because of expanding in the surface area. However, bacterial attachment results give hope that layers may affect attachment in a negative way. Because layered structure seems to affect less than we expected in biofilm formation, this study can be the first step to fabricate an ideal tympanostomy tube if the fabrication technique may be developed into a more precise method.

REFERENCES

1. Sandra, H., and D. Kay, "Tympanostomy tube selection: A review of the evidence," *International Journal of Head and Neck Surgery*, Vol. 7, pp. 17–22, 2016.

2. Armstrong, B., "A new treatment for chronic secretory otitis media," *AMA archives of otolaryngology*, Vol. 59, no. 6, pp. 653–654, 1954.

3. Francolini, I., G. Donelli, C. Vuotto, F. A. Baroncini, P. Stoodley, V. Taresco, A. Martinelli, L. D'Ilario, and A. Piozzi, "Antifouling polyurethanes to fight device-related staphylococcal infections: synthesis, characterization, and antibiofilm efficacy," *Pathogens and disease*, Vol. 70, no. 3, pp. 401–407, 2014.

4. Brown, C., and P. Behar, "Factors affecting persistent tympanic membrane perforation after tympanostomy tube removal in children," *International journal of pediatric otorhinolaryngology*, Vol. 130, p. 109779, 2020.

5. Xiao, L., B. Wang, G. Yang, and M. Gauthier, "Poly (lactic acid)-based biomaterials: synthesis, modification and applications," *Biomedical science, engineering and technology*, Vol. 11, pp. 247–82, 2012.

6. Rettig, E., and D. E. Tunkel, "Contemporary concepts in management of acute otitis media in children," *Otolaryngologic Clinics of North America*, Vol. 47, no. 5, pp. 651–672, 2014.

7. Rosenfeld, R. M., S. R. Schwartz, M. A. Pynnonen, D. E. Tunkel, H. M. Hussey, J. S. Fichera, A. M. Grimes, J. M. Hackell, M. F. Harrison, H. Haskell, *et al.*, "Clinical practice guideline: tympanostomy tubes in children," *OtolaryngologyâHead and Neck Surgery*, Vol. 149, no. 1_suppl, pp. S1–S35, 2013.

8. Lavere, P. F., J. F. Ohlstein, S. P. Smith, W. Szeremeta, and H. S. Pine, "Preventing unnecessary tympanostomy tube placement in children," *International journal of pediatric otorhinolaryngology*, Vol. 122, pp. 40–43, 2019.

9. Rovers, M. M., A. G. Schilder, G. A. Zielhuis, and R. M. Rosenfeld, "Otitis media," *The lancet*, Vol. 363, no. 9407, pp. 465–473, 2004.

10. Wang, J. C., A. N. Hamood, C. Saadeh, M. J. Cunningham, M. T. Yim, and J. Cordero, "Strategies to prevent biofilm-based tympanostomy tube infections," *International journal of pediatric otorhinolaryngology*, Vol. 78, no. 9, pp. 1433–1438, 2014.

11. El-Bitar, M. A., M. T. Pena, S. S. Choi, and G. H. Zalzal, "Retained ventilation tubes: should they be removed at 2 years?," *Archives of Otolaryngology–Head & Neck Surgery*, Vol. 128, no. 12, pp. 1357–1360, 2002.

12. Tsao, B. A., G. R. Stevens, and P. J. Antonelli, "Opening plugged tympanostomy tubes: effect of tube composition," *OtolaryngologyâHead and Neck Surgery*, Vol. 128, no. 6, pp. 870–874, 2003.

13. Sherman, E. G., P. J. Antonelli, and R. Tran-Son-Tay, "In vitro testing of tympanostomy tube occlusion," *Otolaryngology–Head and Neck Surgery*, Vol. 141, no. 5, pp. 598–602, 2009.

14. Đorđević, V., B. Bukurov, N. Arsović, S. Ješić, J. Milovanović, and V. Nešić, "Long term complications of ventilation tube insertion in children with otitis media with effusion," *Vojnosanitetski pregled*, Vol. 72, no. 1, pp. 40–43, 2015.

15. Golz, A., D. Goldenberg, A. Netzer, L. M. Westerman, S. T. Westerman, M. Fradis, and H. Z. Joachims, "Cholesteatomas associated with ventilation tube insertion," *Archives of Otolaryngology–Head & Neck Surgery*, Vol. 125, no. 7, pp. 754–757, 1999.

16. Michel, M., G. Nahas, and D. Preciado, "Retained tympanostomy tubes: Who, what, when, why, and how to treat?," *Ear, Nose & Throat Journal*, p. 0145561320950490, 2020.

17. Karlan, M. S., B. Skobel, M. Grizzard, N. J. Cassisi, G. T. Singleton, P. Buscemi, and E. P. Goldberg, "Myringotomy tube materials: bacterial adhesion and infection," *Otolaryngology–Head and Neck Surgery*, Vol. 88, no. 6, pp. 783–795, 1980.

18. Bays, F. B., R. L. Dunn, S. Marchand, and R. W. Treharne III, "Biodegradable prosthetic device," Mar. 17 1987. US Patent 4,650,488.

19. Massey, B. L., X. Wen, L. R. Rohr, P. A. Tresco, L. Dahlstrom, and A. H. Park, "Resorption rate and biocompatibility characteristics of two polyester ventilation tubes in a guinea pig model," *Otolaryngology-Head and Neck Surgery*, Vol. 131, no. 6, pp. 921–925, 2004.

20. Gan, C. W., W. H. Chooi, H. C. A. Ng, Y. S. Wong, S. S. Venkatraman, and L. H. Y. Lim, "Development of a novel biodegradable drug-eluting ventilation tube for chronic otitis media with effusion," *The Laryngoscope*, Vol. 123, no. 7, pp. 1770–1777, 2013.

21. Ludwick, J. J., S. N. Rossmann, M. M. Johnson, and J. L. Edmonds, "The bacteriostatic properties of ear tubes made of absorbable polylactic acid," *International journal of pediatric otorhinolaryngology*, Vol. 70, no. 3, pp. 407–410, 2006.

22. Kulkarni, R., K. Pani, C. Neuman, and F. Leonard, "Polylactic acid for surgical implants," *Archives of surgery*, Vol. 93, no. 5, pp. 839–843, 1966.

23. Jamróz, W., J. Szafraniec, M. Kurek, and R. Jachowicz, "3d printing in pharmaceutical and medical applications–recent achievements and challenges," *Pharmaceutical research*, Vol. 35, no. 9, pp. 1–22, 2018.

24. Placone, J. K., and A. J. Engler, "Recent advances in extrusion-based 3d printing for biomedical applications," *Advanced healthcare materials*, Vol. 7, no. 8, p. 1701161, 2018.

25. Middleton, J. C., and A. J. Tipton, "Synthetic biodegradable polymers as orthopedic devices," *Biomaterials*, Vol. 21, no. 23, pp. 2335–2346, 2000.

26. Özalp, Y., N. Özdemir, and V. Hasirci, "Vancomycin release from poly (d, l-lactide) and poly (lactide-co-glycolide) disks," *Journal of microencapsulation*, Vol. 19, no. 1, pp. 83–94, 2002.

27. Grizzi, I., H. Garreau, S. Li, and M. Vert, "Hydrolytic degradation of devices based on poly (dl-lactic acid) size-dependence," *Biomaterials*, Vol. 16, no. 4, pp. 305–311, 1995.

28. Ojano-Dirain, C. P., R. C. Silva, and P. J. Antonelli, "Biofilm formation on coated silicone tympanostomy tubes," *International journal of pediatric otorhinolaryngology*, Vol. 77, no. 2, pp. 223–227, 2013.

29. Antonelli, P. J., E. M. Sampson, and C. Ojano-Dirain, "Biofilm formation on silicone tympanostomy tubes with polyvinylpyrrolidone coating," *Archives of Otolaryngology–Head & Neck Surgery*, Vol. 137, no. 1, pp. 19–23, 2011.

30. Guerra, A. J., P. Cano, M. Rabionet, T. Puig, and J. Ciurana, "3d-printed pcl/pla composite stents: Towards a new solution to cardiovascular problems," *Materials*, Vol. 11, no. 9, p. 1679, 2018.

31. Luo, Y., Y. Wang, X. Niu, C. Fu, and S. Wang, "Synthesis, characterization and biodegradation of butanediamine-grafted poly (dl-lactic acid)," *European polymer journal*, Vol. 43, no. 9, pp. 3856–3864, 2007.

32. Li, X., H. Yu, Y. Jiao, M. Q. Shahid, J. Wu, and X. Liu, "Genome-wide analysis of dna polymorphisms, the methylome and transcriptome revealed that multiple factors are associated with low pollen fertility in autotetraploid rice," *PloS one*, Vol. 13, no. 8, p. e0201854, 2018.

33. Khalid, S., A. Gao, G. Wang, P. K. Chu, and H. Wang, "Tuning surface topographies on biomaterials to control bacterial infection," *Biomaterials Science*, Vol. 8, no. 24, pp. 6840–6857, 2020.

34. Cheng, Y., G. Feng, and C. I. Moraru, "Micro-and nanotopography sensitive bacterial attachment mechanisms: a review," *Frontiers in microbiology*, Vol. 10, p. 191, 2019.

35. Jang, C. H., Y. B. Cho, and C. H. Choi, "Effect of ion-bombarded silicone tympanostomy tube on ciprofloxacin-resistant pseudomonas aeruginosa biofilm formation," *International journal of pediatric otorhinolaryngology*, Vol. 76, no. 10, pp. 1471–1473, 2012.

36. Joe, H., and Y. J. Seo, "A newly designed tympanostomy stent with tio2 coating to reduce pseudomonas aeruginosa biofilm formation," *Journal of biomaterials applications*, Vol. 33, no. 4, pp. 599–605, 2018.

37. Park, A. H., D. Hoyt, D. Britt, S. Chase, K. Tansavatdi, L. Hunter, L. McGill, X. Sheng, A. Skardal, and G. D. Prestwich, "Cross-linked hydrogel and polyester resorbable ventilation tubes in a chinchilla model," *The Laryngoscope*, Vol. 123, no. 4, pp. 1043–1048, 2013.

38. Tatar, E. C., F. Ö. Ünal, I. Tatar, H. H. Celik, and B. Gursel, "Investigation of surface changes in different types of ventilation tubes using scanning electron microscopy and correlation of findings with clinical follow-up," *International journal of pediatric otorhinolaryngology*, Vol. 70, no. 3, pp. 411–417, 2006.

39. Nguyen, D. H., V. T. Pham, V. K. Truong, I. Sbarski, J. Wang, A. Balčytis, S. Juodkazis, D. E. Mainwaring, R. J. Crawford, and E. P. Ivanova, "Role of topological scale in the differential fouling of pseudomonas aeruginosa and staphylococcus aureus bacterial cells on wrinkled gold-coated polystyrene surfaces," *Nanoscale*, Vol. 10, no. 11, pp. 5089–5096, 2018.

40. Medilanski, E., K. Kaufmann, L. Y. Wick, O. Wanner, and H. Harms, "Influence of the surface topography of stainless steel on bacterial adhesion," *Biofouling*, Vol. 18, no. 3, pp. 193–203, 2002.

41. Hsu, L. C., J. Fang, D. A. Borca-Tasciuc, R. W. Worobo, and C. I. Moraru, "Effect of micro-and nanoscale topography on the adhesion of bacterial cells to solid surfaces," *Applied and environmental microbiology*, Vol. 79, no. 8, pp. 2703–2712, 2013.

42. Song, F., H. Koo, and D. Ren, "Effects of material properties on bacterial adhesion and biofilm formation," *Journal of dental research*, Vol. 94, no. 8, pp. 1027–1034, 2015.

43. Fernández-Calderón, M. C., D. Romero-Guzmán, A. Ferrández-Montero, C. Pérez-Giraldo, J. L. González-Carrasco, M. Lieblich, R. Benavente, B. Ferrari, M. L. González-Martín, and A. M. Gallardo-Moreno, "Impact of pla/mg films degradation on surface physical properties and biofilm survival," *Colloids and Surfaces B: Biointerfaces*, Vol. 185, p. 110617, 2020.

44. Francolini, I., G. Donelli, and P. Stoodley, "Polymer designs to control biofilm growth on medical devices," *Reviews in Environmental Science and Biotechnology*, Vol. 2, no. 2, pp. 307–319, 2003.