Design and Construction of Medical Device for ENT Applications Based on Biodegradable Polymer

Bc. Eva Hnátková

Master thesis
2012

Tomas Bata University in Zlín
Faculty of Technology

Univerzita Tomáše Bati ve Zlíně
Fakulta technologická
Ústav výrobního inženýrství
akademický rok: 2011/2012

ZADÁNÍ DIPLOMOVÉ PRÁCE

(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

Jméno a příjmení:	**Bc. Eva HNÁTKOVÁ**
Osobní číslo:	**T100039**
Studijní program:	**N 3909 Procesní inženýrství**
Studijní obor:	**Konstrukce technologických zařízení**
Téma práce:	**Návrh a konstrukce zdravotnického prostředku pro ORL aplikace na bázi bioresorbovatelného polymeru**

Zásady pro vypracování:

I. Introduction
1.On the basis of literature survey summarize the state of art of the ear ventilation tube as medical device for ENT applications
2.Beside theoretical aspects, focus on the biodegradable polylactic acid as innovative biomaterial
II. Practical part
3.Describe design and method of production of ear tube
4.Determinate experimentally the influence of in vitro degradation on physical properties of PLA
III. Conclusion

Rozsah diplomové práce:

Rozsah příloh:

Forma zpracování diplomové práce: **tištěná/elektronická**

Seznam odborné literatury:

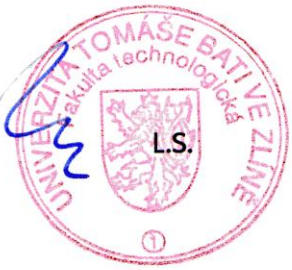
1)WONG, J, Y., BRONZINO J.D. Biomaterials. CRC press: Boca Raton 2007, ISBN 0-8493-4788-5
2)SCOTT, G. Degradable Polymer: Principles and Applications. 2nd edition. Kluwer 2002. ISBN 1-4020-0790-6
3)GREENER, R., WIMBERG, F. R. Precision Injection Molding: process, materials, and applications. Munich: Hanser 2006, ISBN 1-56990-400-6
4)JACSON, CH. Diseases of the Nose, Throat, and Ear. Philadelphia: Saunders 1946
5)Book and journal databases available through the library of Tomas Bata

Vedoucí diplomové práce: **doc. Ing. Zdeněk Dvořák, CSc.**
Ústav výrobního inženýrství

Datum zadání diplomové práce: **13. února 2012**

Termín odevzdání diplomové práce: **18. května 2012**

Ve Zlíně dne 2. února 2012



doc. Ing. Roman Čermák, Ph.D.
děkan

prof. Ing. Berenika Hausnerová, Ph.D.
ředitel ústavu

Příjmení a jméno: Bc. Eva Hnátková Obor: Konstrukce technologických zařízení

P R O H L Á Š E N Í

Prohlašuji, že

- beru na vědomí, že odevzdáním diplomové/bakalářské práce souhlasím se zveřejněním své práce podle zákona č. 111/1998 Sb. o vysokých školách a o změně a doplnění dalších zákonů (zákon o vysokých školách), ve znění pozdějších právních předpisů, bez ohledu na výsledek obhajoby [1)];
- beru na vědomí, že diplomová/bakalářská práce bude uložena v elektronické podobě v univerzitním informačním systému dostupná k nahlédnutí, že jeden výtisk diplomové/bakalářské práce bude uložen na příslušném ústavu Fakulty technologické UTB ve Zlíně a jeden výtisk bude uložen u vedoucího práce;
- byl/a jsem seznámen/a s tím, že na moji diplomovou/bakalářskou práci se plně vztahuje zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, zejm. § 35 odst. 3 [2)];
- beru na vědomí, že podle § 60 [3)] odst. 1 autorského zákona má UTB ve Zlíně právo na uzavření licenční smlouvy o užití školního díla v rozsahu § 12 odst. 4 autorského zákona;
- beru na vědomí, že podle § 60 [3)] odst. 2 a 3 mohu užít své dílo – diplomovou/bakalářskou práci nebo poskytnout licenci k jejímu využití jen s předchozím písemným souhlasem Univerzity Tomáše Bati ve Zlíně, která je oprávněna v takovém případě ode mne požadovat přiměřený příspěvek na úhradu nákladů, které byly Univerzitou Tomáše Bati ve Zlíně na vytvoření díla vynaloženy (až do jejich skutečné výše);
- beru na vědomí, že pokud bylo k vypracování diplomové/bakalářské práce využito softwaru poskytnutého Univerzitou Tomáše Bati ve Zlíně nebo jinými subjekty pouze ke studijním a výzkumným účelům (tedy pouze k nekomerčnímu využití), nelze výsledky diplomové/bakalářské práce využít ke komerčním účelům;
- beru na vědomí, že pokud je výstupem diplomové/bakalářské práce jakýkoliv softwarový produkt, považují se za součást práce rovněž i zdrojové kódy, popř. soubory, ze kterých se projekt skládá. Neodevzdání této součásti může být důvodem k neobhájení práce.

Ve Zlíně 15. 5. 2012

.....

[1)] zákon č. 111/1998 Sb. o vysokých školách a o změně a doplnění dalších zákonů (zákon o vysokých školách), ve znění pozdějších právních předpisů, § 47 Zveřejňování závěrečných prací:

(1) Vysoká škola nevýdělečně zveřejňuje disertační, diplomové, bakalářské a rigorózní práce, u kterých proběhla obhajoba, včetně posudků oponentů a výsledku obhajoby prostřednictvím databáze kvalifikačních prací, kterou spravuje. Způsob zveřejnění stanoví vnitřní předpis vysoké školy.
(2) Disertační, diplomové, bakalářské a rigorózní práce odevzdané uchazečem k obhajobě musí být též nejméně pět pracovních dnů před konáním obhajoby zveřejněny k nahlížení veřejnosti v místě určeném vnitřním předpisem vysoké školy nebo není-li tak určeno, v místě pracoviště vysoké školy, kde se má konat obhajoba práce. Každý si může ze zveřejněné práce pořizovat na své náklady výpisy, opisy nebo rozmnoženiny.
(3) Platí, že odevzdáním práce autor souhlasí se zveřejněním své práce podle tohoto zákona, bez ohledu na výsledek obhajoby.
[2)] zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, § 35 odst. 3:

(3) Do práva autorského také nezasahuje škola nebo školské či vzdělávací zařízení, užije-li nikoli za účelem přímého nebo nepřímého hospodářského nebo obchodního prospěchu k výuce nebo k vlastní potřebě dílo vytvořené žákem nebo studentem ke splnění školních nebo studijních povinností vyplývajících z jeho právního vztahu ke škole nebo školskému či vzdělávacího zařízení (školní dílo).
[3)] zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, § 60 Školní dílo:

(1) Škola nebo školské či vzdělávací zařízení mají za obvyklých podmínek právo na uzavření licenční smlouvy o užití školního díla (§ 35 odst. 3). Odpírá-li autor takového díla udělit svolení bez vážného důvodu, mohou se tyto osoby domáhat nahrazení chybějícího projevu jeho vůle u soudu. Ustanovení § 35 odst. 3 zůstává nedotčeno.
(2) Není-li sjednáno jinak, může autor školního díla své dílo užít či poskytnout jinému licenci, není-li to v rozporu s oprávněnými zájmy školy nebo školského či vzdělávacího zařízení.
(3) Škola nebo školské či vzdělávací zařízení jsou oprávněny požadovat, aby jim autor školního díla z výdělku jím dosaženého v souvislosti s užitím díla či poskytnutím licence podle odstavce 2 přiměřeně přispěl na úhradu nákladů, které na vytvoření díla vynaložily, a to podle okolností až do jejich skutečné výše; přitom se přihlédne k výši výdělku dosaženého školou nebo školským či vzdělávacím zařízením z užití školního díla podle odstavce 1.

ABSTRAKT

Tato práce se zabývá ušními ventilačními trubičkami. Jedná se o medicínské prostředky, které se zavádí na definovanou dobu do ušního bubínku a to v případě chronického zánětu středního ucha. V dnešní době se trubičky vyrábějí zejména z kovů nebo klasických polymerů a ty doprovází celá řada komplikací, zejména tedy s jejich odstraněním. Ušní trubičky vyrobené z biologicky odbouratelného polymeru by mohly nahradit doposud používané materiály. Zvláštní pozornost byla věnována hlavně polymeru kyseliny mléčné (PLA), který je v polední době vyšetřován pro různé medicínské aplikace. Experimentální část se zabývá vyšetřením degradačního procesu za různých podmínek. PLA vzorky byly zkoumány ve fosfátovém pufru a v komoře se 100% relativní vlhkostí, obojí za teploty 37°C. Degradační proces byl sledován po dobu 12 týdnů, pomocí úbytku hmotnosti, diferenční skenovací kalorimetrie, optickým a elektronovým mikroskopem a gelovou permeační chromatografii. Praktická část zahrnuje vývoj nástroje na výrobu trubiček, které vyžadují speciální technologie (rapid prototyping a galvanické pokovování).

Klíčová slova:

Ušní ventilační trubičky, polylaktid, degradace, přetlačovací forma, galvanoplastika

ABSTRACT

This work deals with ear ventilation tubes. This medical device is inserted for prolonged period of time into the eardrum in case of persistent infection of the middle ear. Today, tubes are made of metals and classical polymers that cause many complications, especially with their removal. Ear tubes made from biodegradable polymers could replace currently used materials. Particular attention was given to poly(lactic acid) (PLA), which has recently been researched for many medical applications. In the experimental part the degradation process of PLA under different conditions was investigated. PLA samples were examined in phosphate buffered solution at pH 7.4 and in chamber with 100% relative humidity, both at 37°C. The degradation process was monitored for up to 12 weeks by weight, different scanning calorimetry, optical and electron microscopy, and by gel permeation chromatography. The practical part includes development of the tool for manufacturing tubes, which requires special technologies (rapid prototyping and electroforming technology).

Keywords:

Ear tube, poly(lactic acid), degradation, transfer mold, electroplating

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my supervisor, associated professor Ing. Dvořák Zdeněk, CSc. for his advice, support and patience all the time of the work on my thesis.

I am deeply grateful to associated professor. Ing. Vladimír Sedlařík, Ph.D. and to Ing. Pavel Kucharczyk from Polymer Center at the Faculty of Technology, for creating very good conditions for my research activities.

Furthermore, I am thankful to Ing. Jiří Šálek from Department of Production Engineering at the Faculty of Technology, who helped me with manufacturing of tool components.

My special thanks belong to MUDr. Vladimír Zlínský for his consultation and collaboration from medical point of view.

My acknowledgement also belongs to associated professor Ing. Anežka Lengálová, Ph.D. for her comments and help with a language correlation during the creation of my theoretical part.

Apart from my colleagues and friends, I owe my deepest gratitude to my whole family for their unconditional love and support.

I agree that the results of my Master thesis can be used by my supervisor's decision. I will be mentioned as a co-author in case of any publication.

I declare I worked on this Master thesis by myself and I have mentioned all the used literature.

I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

Zlín, May 15, 2012 .....

Eva Hnátková

CONTENTS

INTRODUCTION

Tympanostomy (ear) tube is a medical device which is used in otolaryngology in case of Eustachian tube dysfunction. This medical device is inserted for prolonged period of time into a tympanic membrane (eardrum) to ventilate and provide pressure equalization to the middle ear. This treatment prevents from chronic ear infections (acute otitis media) of the middle ear or the persistent presence of fluid in the middle ear (otitis media with effusion). Today, tympanostomy tubes are made especially from metals or classical polymeric material.

In recent years, much attention has been focused on biodegradable and biocompatible polymers for biomedical applications, because they have the ability to degrade in the human body and after their roles are dissolved and absorbed by the body. These biodegradable materials have been developed for temporary implants, where the long term persistence is not required. However, there are only few attempts, which have investigated the possible application of this material for the construction of ear tubes. In this way the biodegradable ear tubes can be the promising candidates, because tubes are put into tympanic membrane for a predetermined period of time, usually for several months. In addition, there is a possibility of treating disorders of the middle ear by applying drugs directly into the tube.

One of the most perspective polymers for this application can be poly(lactic acid) (PLA) and its copolymers. PLA is biodegradable thermoplastic polyester, which has an excellent biocompatibility in contact with living tissue and in the body it degrades by enzymatic and hydrolytic mechanism. The chemical structure, morphology and particularly molecular weight, crystallinity degree or distribution of the D- and L-isomers in the polymer chains influences the physical and mechanical properties as well as degradation rate. This means that PLA can be modified for concrete applications. Currently, polymers based on PLA are used in medicine for sutures, orthopedic implants or as supports for delivery systems.

This research follows a project carried out at the Polymer Centre at the Faculty of Technology, Tomas Bata University in Zlín. The project deals with production, modification and testing of PLA material for biomedical use produced at the laboratory of Polymer Centre. Furthermore, the research was done in collaboration with otologist MUDr. Vladimír Zlínský.

In this thesis are presenting preliminary results of many significant changes in physical properties of PLA during in vitro degradation. The measurement of mass loss was done

before testing each sample. Structural changes during degradation were monitored by optical microscopy and inner morphology by scanning electron microscopy (SEM). Decreases in molecular weight were examined by gel permeation chromatography (GPC). Thermal behavior changes and a degree of crystallinity were investigated by using differential scanning calorimetry (DSC). Basic mechanical properties were determined by microhardness and bending test. The practical part of this thesis includes design and development of the tool for a production of ear ventilation tubes. The development of this tool utilizes the rapid prototyping and electroforming technology.

The goal of this work is to find out if the biodegradable PLA can be a suitable material for future construction of ear tubes. A great advantage could be no necessity for additional surgical removing of the tube. This operation is made under general anesthesia and it is very stressful for the patient and expensive for health insurance companies.

I. THEORY

1 OTOLARYNGOLOGY

Otolaryngology is a medical and surgical specialty which is concerned with diagnosis, management and treatment of diseases and disorders of the ears, nose, and throat. The full name of the specialty is otorhinolaryngology, but commonly is called ENT. This work is focused only on surgical treatment of ear diseases, which may include hearing problems, ear infections and balance disorders. [1]

1.1 The ear

The ear is a part of the auditory system that allows us to hear. The ear can be divided into three anatomical parts, namely the outer, middle and inner ear, shown in Figure 1. Each part performs an important function in the process of hearing. This organ has also another significant role: to keep balance and body position.

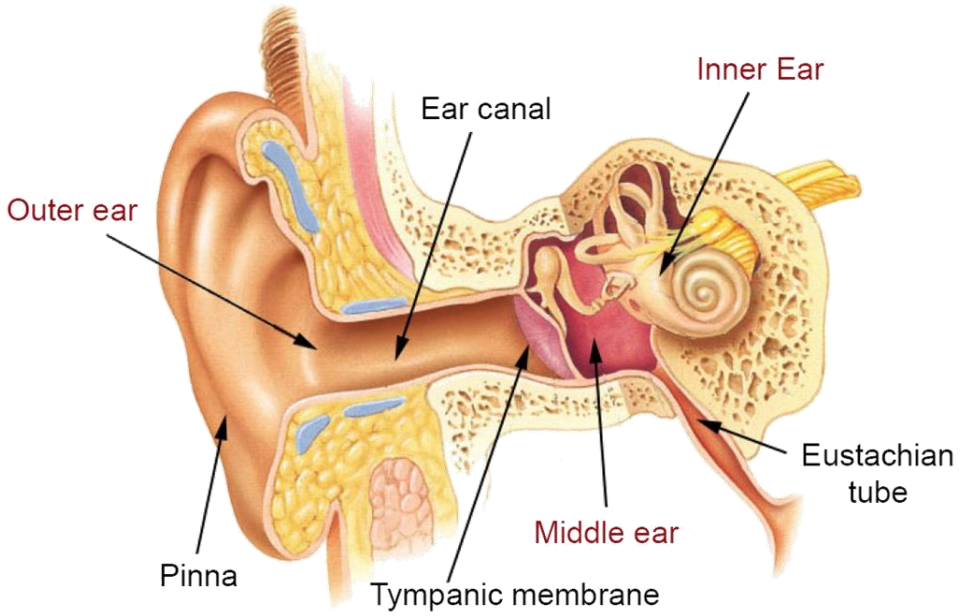


Figure 1 Basic anatomy of the ear [2]

The outer (external) ear

It is composed of the visible part the pinna (auricle) and ear canal (external auditory canal). The pinna is the outside portion of the ear and its shape enables to funnel sound waves into the external auditory canal. The ear canal is a tortuous tube leading to the tympanic membrane. In the adult human it is about 25 mm long. Its outer third is a cartilaginous and the skin here has hairs and ceruminous glands that secrete wax. The walls of the inner two-thirds are bony. The outer ear is a self-cleaning system, because as the skin cells migrate, they carry the wax, and anything trapped inside, out of the ear. [3]

The middle ear

It is an air-filled cavity containing three small bones (ossicles). The three ossicles are known as the malleus (hammer), incus (anvil) and stapes (stirrup) and they are shown in Figure 2. This chain of bones receives sound vibrations from the tympanic membrane and transmits and amplifies them to the inner ear. [4]

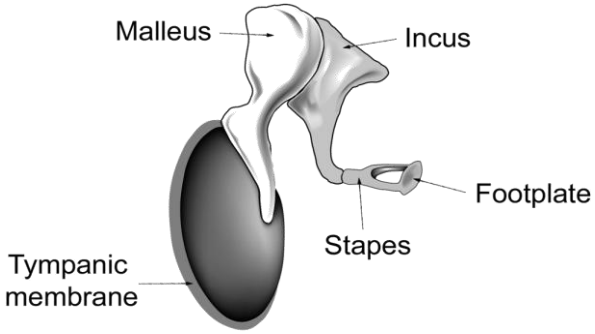


Figure 2 Ossicles [5]

The tympanic membrane also called eardrum is shown in Figure 3. The eardrum is semi-transparent membrane (thick about 0.1 mm) that separates the middle ear cavity from the external ear. In the deeper part of the tympanic membrane is attached malleus and this point is called umbo. The membrane is divided in two distinct parts: the upper pars flaccida and the lower pars tensa, which comprises approximately 80% of eardrum. [6] When the membrane is examined by reflected light, the antero-inferior quadrant is strongly illuminated and this place is known as the cone of light. [7] In adult, the tympanic membrane is angled approximately 140 degrees, the vertical diameter of the tympanic membrane measures from 8.5 mm to 10 mm, and the horizontal measures from 8 to 9 mm. However, there is a considerable variation in size and form, as well as in angle. [8]

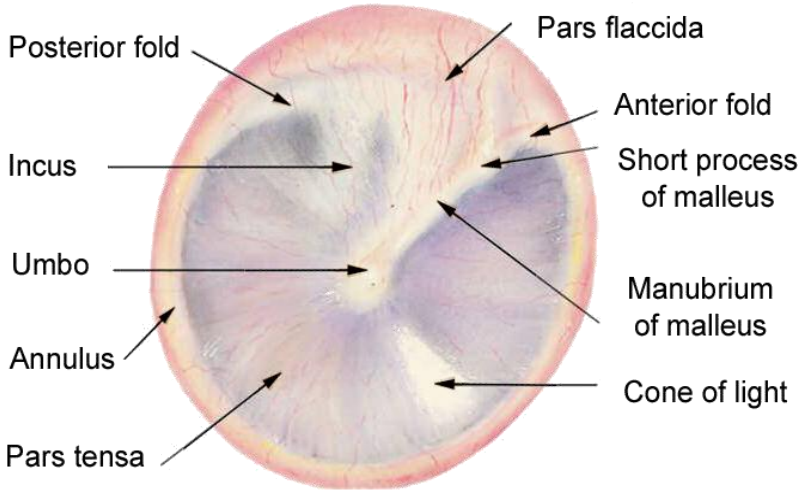


Figure 3 Tympanic membrane

The Eustachian tube is a small canal that connects the middle ear with the upper part of the throat, shown in Figure 6. [9] This canal has three important functions. The first function keeps the middle ear ventilated and maintains ambient atmospheric pressure that allows efficient transferring of sound vibrations in space behind the eardrum. [10] The second function is protection and that is why this tube is normally closed, but the attached muscle pulls the tube open during swallowing, chewing and yawning. Closing of the tube helps protecting the middle ear from bacteria residing in the nose and mouth, loud sounds, and unwanted pressure fluctuations. The third function is drainage of fluids as secretion and mucus that are normally produced in the middle ear. In children, the tube is shorter and more horizontal, and thus opens with more difficulty than in adult.

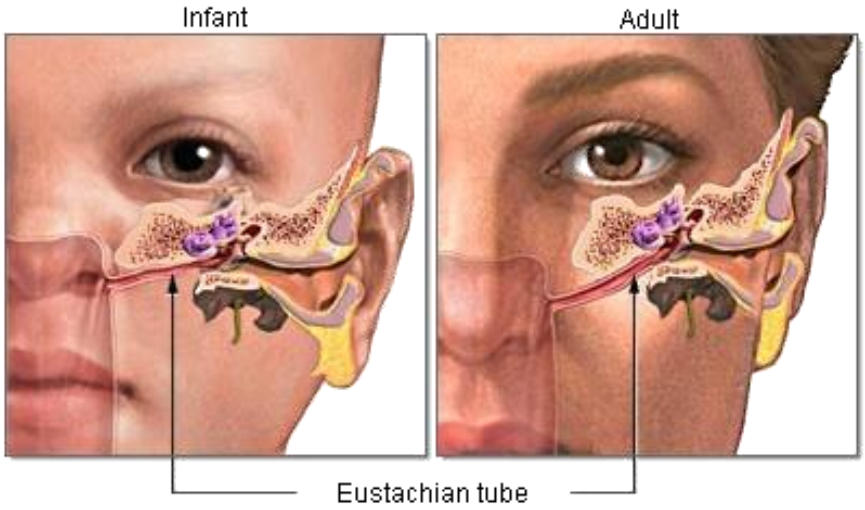


Figure 4 Infant and adult Eustachian tube [11]

The inner ear

It is the most complex part of the ear, which occupies a small bony cavity called labyrinth. The inner ear contains the cochlea for hearing and vestibule and semicircular canals for balance, shown in Figure 5.

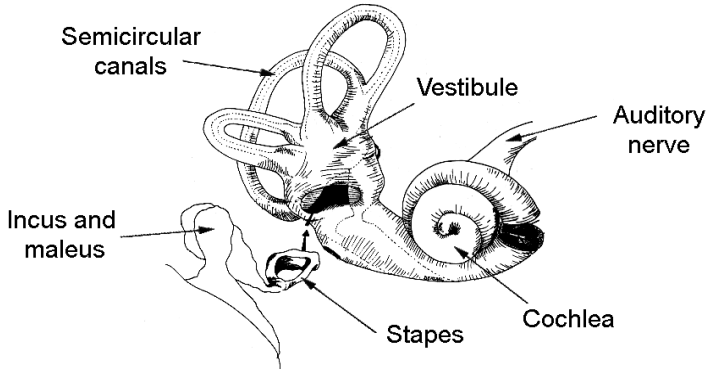


Figure 5 Inner ear [12]

1.2 Otitis media

Otitis media is an inflammation or infection of the middle ear. Otitis media is primarily a disease that affects infants and young children, but it can also affect adults. One of the most important causes of otitis media is Eustachian tube dysfunction. The middle ear inflammation often begins when infections that cause sore throats, colds, or other respiratory problems spread to the middle ear. Bacteria and viruses get inside the ear through the Eustachian tube where can produce the inflammation. [10] The Eustachian tube, which normally allows air to enter in and fluids to drain out from the middle ear cavity, becomes swollen due to infections and remains blocked most of the time. The air present in the middle ear cavity is slowly absorbed into the surrounding tissues and the negative pressure is created behind the eardrum. Increased negative pressure may lead to sucking the fluids into the middle ear and builds up the cavity as can be seen in Figure 6. Although the fluid behind eardrum is usually temporary, persistent fluid (untreatable otitis media) may lead to permanent hearing loss. [13]

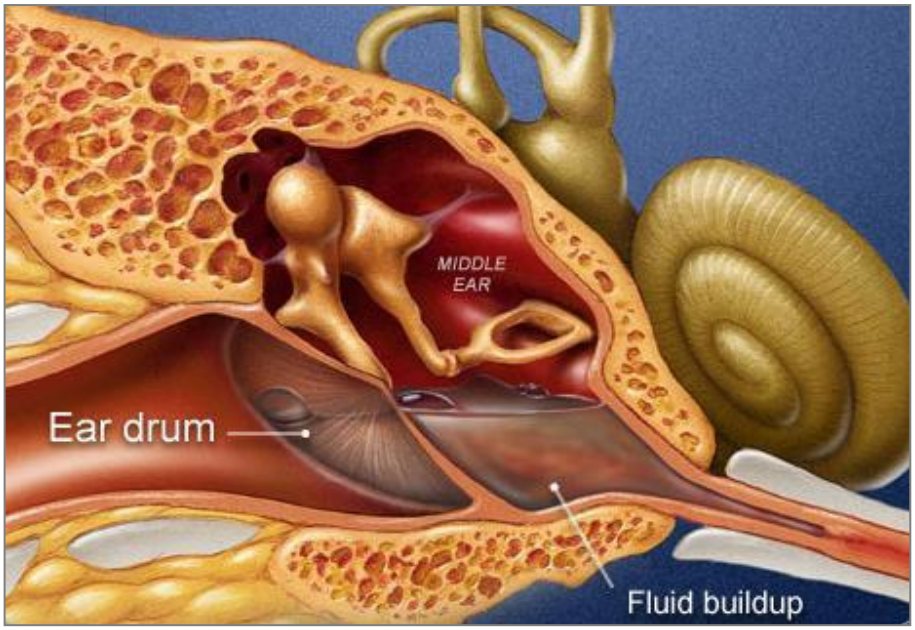


Figure 6 Fluid buidup in the middle ear [14]

Otitis media can be classified in three main types. Each of them has a different combination of symptoms. Acute otitis media is the most common disease in the middle ear with rapid and short onset. Otitis media with effusion is the inflammation with a collection of liquid in the middle ear cavity. The effusion can be serous, mucoid or purulent. Chronic otitis media occurs when the fluid remains for a long time behind the eardrum and does not go away. This may cause permanent damage to the ear as eardrum perforation. [15], [16]

If an infection does not go away with the usual medical treatment and the fluid persists behind the eardrum for three months, or if there are many ear infections over a short period of time associated with a hearing loss, in this case the problem is treated surgically, when a small ventilation tube is inserted into the eardrum. [17]

1.3 Tympanostomy tube

Tympanostomy tube is a small medical device also known as ear tube, ventilation tube, pressure equalization tube or grommet which consists of a length of tube that has a flange at one or both ends, shown in Figure 7. These medical devices are described in the FDA regulations under 21 CFR 874.3880. For medical use the devices are classified into three classes depending upon their risk and criticality. Tympanostomy tubes are included in class II and require special controls before they are applied. [18]

Figure 7 Tympanostomy tubes [19]

Tympanostomy tubes are indicated for: [20]

- Chronic otitis media with effusion (serous, mucoid, or purulent)
- Recurrent otitis media that fails to respond to conventional medical treatment
- A history of persistent high negative middle ear pressure, which may be associated with conductive hearing loss, otalgia, vertigo and/or tinnitus

1.3.1 Function of tympanostomy tube

This medical device is intended to be implanted into the tympanic membrane for prolonged period of time. The main function of the tympanostomy tube is to ventilate the middle ear and maintain the pressure inside the middle ear cavity equal as outside. The other purpose is to provide drainage and prevent an accumulation of fluids behind the eardrum as is shown in Figure 8. The principal benefit of tympanostomy tube insertion is to keep the middle ear clear of effusion and permits normal transmission of sound. [21]

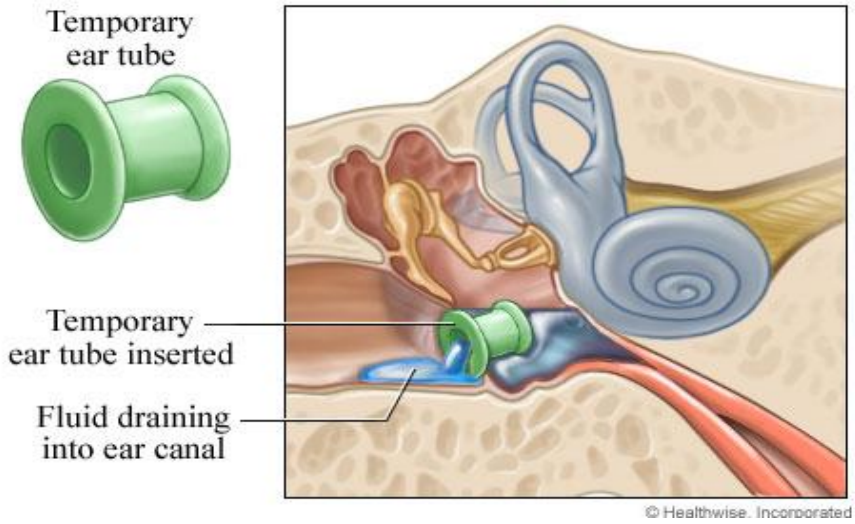


Figure 8 Function of tympanostomy tube [22]

1.3.2 Types of tympanostomy tubes

Since the first ventilation tube, which was introduced by Beverley Armstrong in 1954, many changes have been done. Nowadays tympanostomy tubes are available in a very wide range of models made of various materials, and in several sizes. [23] [24]

Tubes can be divided into two categories depending on duration of staying in the ear:

- Short-term tubes (6 – 12 months)
- Long-term tubes (for more than 12 months)

The fundamental difference between the two categories is the inner diameter of the flange, which is related to the mechanism of tube extrusion. The inserted tube creates in tympanic membrane keratin that pushes the tube out. Tubes with a very large inner flange are more difficult to push them out on their own. Conversely, grommets with a small inner flange are used for very short periods. The lumen diameter and length of tube will determine the ventilation ability of any tube. [15]

Commercially available tympanostomy tubes are shown in Figure 9. They are made mainly from metal as a stainless steel, titanium, gold or silver or from polymers as polytetrafluorethylene (PTFE), silicon rubber and polypropylene (PP). The choice of material is usually based on the preference of individual type. Metals are heavy and have problems with processability and cost. Polymeric tubes have often problems with early obstruction of the lumen and must be coated. PTFE offers very low coefficient of friction, but in general, the conventionally tympanostomy tubes cannot fully satisfy the patient. [25]

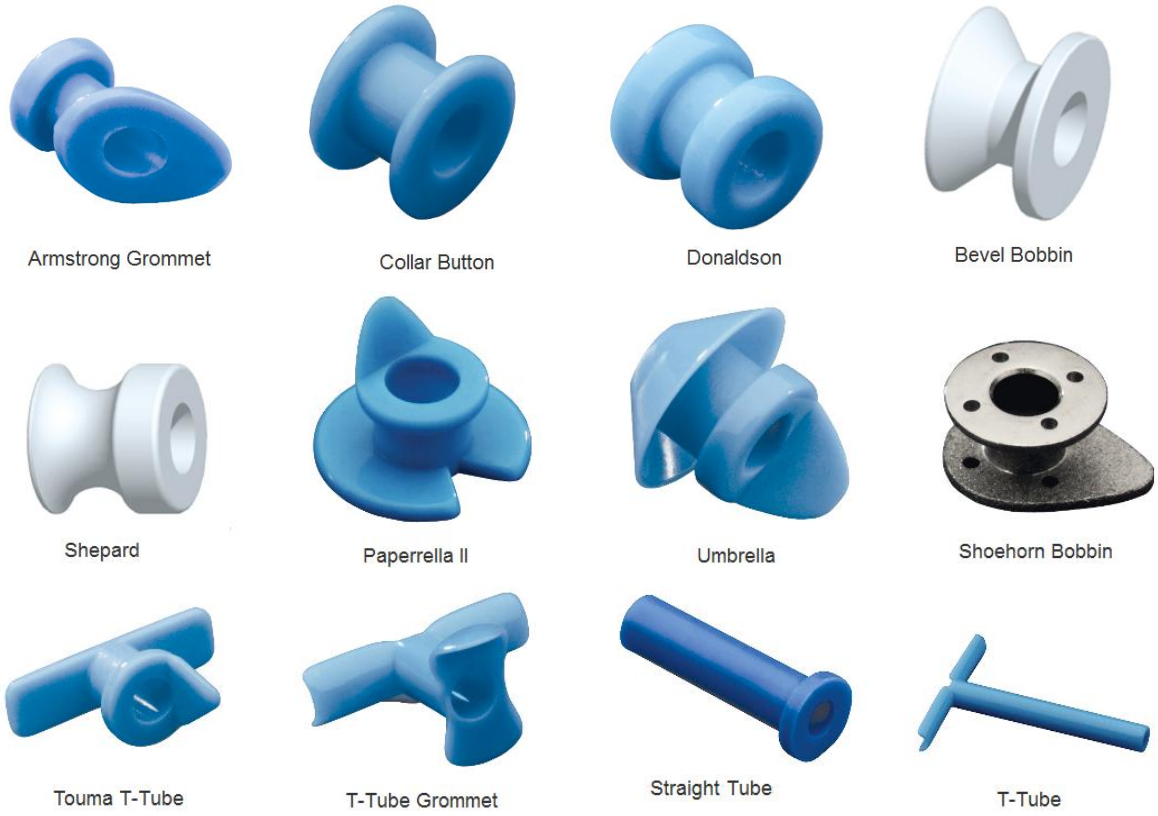


Figure 9 Various commercially available tympanostomy tubes

1.3.3 Myringotomy and tube insertion

The insertion of tympanostomy tubes is one of the most common surgical procedures performed on children. This surgery is done under general anesthesia in children and can be done under local anesthesia in adults.

Myringotomy is a surgical procedure in which a small incision is made into the eardrum by scalpel, shown in Figure 10. It is important to do it in the safe part of tympanic membrane, usually in the anterior-inferior quadrant, because in the other region are located important anatomic structures. Then the fluid is removed and the tympanostomy tube is placed by forceps.

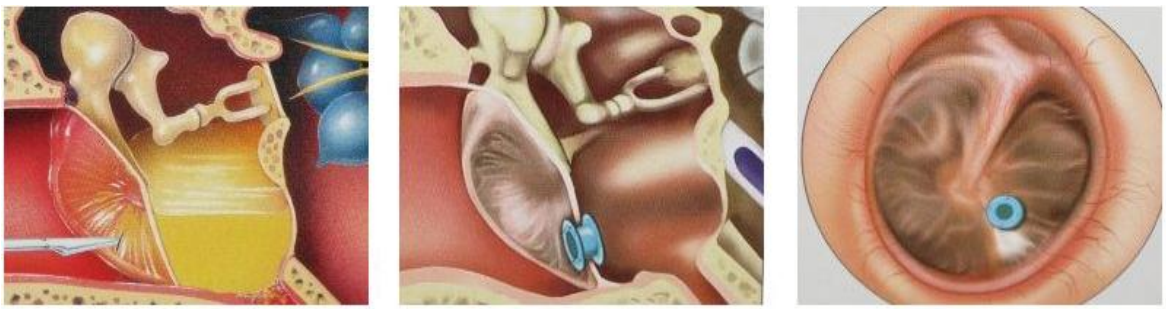


Figure 10 Myringotomy and tube insertion [26]

Tubes usually remain in eardrum for about 6 months to 12months and sometimes they fall out on their own. If they stay in longer than 2 years, they must be surgically removed by an otologist to prevent perforation of the eardrum. This procedure is not pleasant, and the person has to pass another trauma from anesthesia. [18]

1.3.4 Complications

The main complications of tympanostomy tubes may be divided into two groups. The first group includes complications during the insertion of the tube and while the tube is in place. The other group includes late complications that occur after the tube has spontaneously extruded or was surgically removed. The placement itself or surgical removal of devices requires general anesthesia, which may cause problems like allergic reactions or respiratory depression. [27]

Early complications:

- cutting the outer ear causing pain and bleeding
- post-operative infection
- persistent drainage from the ear (otorrhea)
- occlusion of the tubes
- premature extrusion.

Late complications:

- formation at the myringotomy site of granuloma due to inflammation
- formation of a mass of skin cells in the middle ear that can grow and damage surrounding bone (cholesteatoma)
- permanent perforation of tympanic membrane
- inward movement of tube into middle ear
- prolonged tube ventilation (over 2 years).

The most common complication of tube placement is post-operative otorrhea, which can cause tube occlusion. Susceptibility to occlusion depends on the material of the tube and diameter of lumen. This problem could be solved by coating the tubes with an anti-clogging substance. Large lumen and tube, which are intended for long-terms, have higher incidence for permanent perforation of the tympanic membrane. If tympanic devices extrude before the resolution of symptoms, this involves the replacement of the tube. [28]

2 BIOMATERIALS

There are many definitions of biomaterials in literature:

"Materials of natural as well as synthetic origin in contact with tissue, blood, and biological fluids, and intended for use for prosthetic, diagnostic, therapeutic, and storage applications without adversely affecting the living organism and its components." (Bruc, 1980) [29]

"A nonviable material used in medical device, intended to interact with living systems." (Black, 1992) [29]

"A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic of diagnostic procedure, in human or veterinary medicine" (Williams, 2009) [30]

In generally, biomaterials are synthetic or natural origin materials that are intended for medical applications such as medical disposable supplies, prosthetic materials, dental and orthopedic implants, polymeric drug delivery systems, products of tissue engineering etc. These materials must possess a lot of specific characteristics. The most important requirement is related with a biocompatibility. Thus, biomaterials must always be considered in their final fabricated and sterilized form. Typical applications of biomaterials include: syringes, blood bags, contact lens, suture wires, bone plates, knee joins, dental implants, vascular graft and others. Some of them are shown in Figure 11.

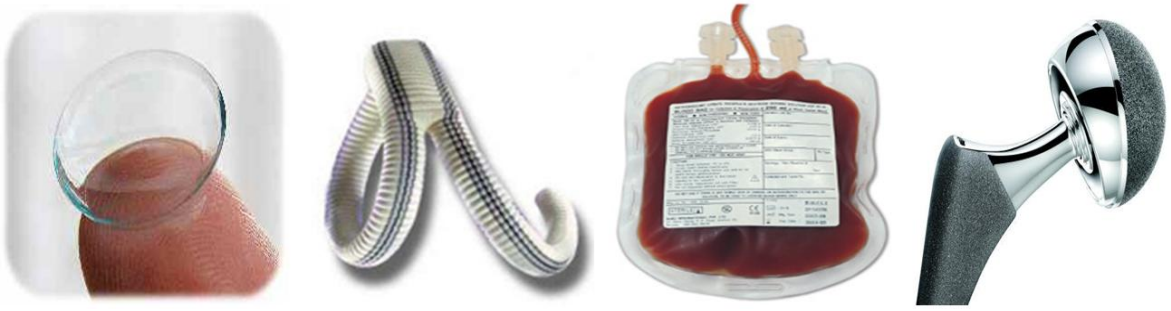


Figure 11 Typical applications of biomaterials in medicine

These medical devices can be utilized for a single, temporary or permanent use, in contact with appropriate biological system in the body. And it is important to mention that material, which was found to be save for specific device might not be safe for different use. [31]

2.1 Classes of biomaterials

Biomaterials can be classified in many ways. They can be considered from the point of view of the problem area, or on tissue level in which they will be in contact and how long. However the main division is into four major classes of material is in Figure 12.

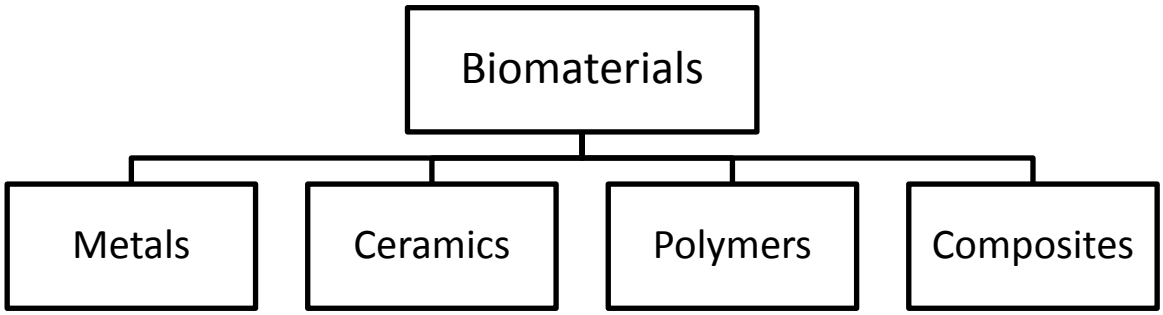


Figure 12 Major classes of biomaterials

Metals

The first materials used as biomaterials were metals. They are known for high strength, ductility and wear resistance. Most common are stainless steel, cobalt-chromium alloys as well as titanium alloys. However, stainless steel and cobalt-chromium alloys are sensitive to corrosion and there is a considerable infection risk which may cause an allergic reaction in the body. Metals are used usually for load-bearing implants, such as knee and hip prostheses or fracture fixation as wires, pins, screws, and plates. [32]

Ceramics

Ceramics are known for their good biocompatibility, corrosion resistance, and high compression and temperature resistance. On the other hand, their drawbacks include brittleness, poor tensile properties and difficulty in fabrication. The most common are aluminum oxide, zirconia and calcium phosphates. Ceramics can be bioinert, bioactive or biodegradable. In otolaryngology, ceramics are not used for commercial ventilation tubes. [32]

Polymers

There are hundreds of polymers, but only ten to twenty are mainly used for medical applications, shown in Table 1. The main advantage of polymers is that they do not cause corrosion and allergy. Compared to metal or ceramic materials, polymers are easy to manufacture and can be produced in various shapes. They have a reasonable cost and are available with desired mechanical and physical properties. Polymeric biomaterials can be divided into biostable, bioabsorbable (biodegradable), and partially bioabsorbable material. [29]

Table 1 Common synthetic polymers used for biomedical applications [29]

PVC	Blood and solution bags, surgical packaging, dialysis devices, catheters
PE	Pharmaceutical bottles, catheter, flexible containers, orthopaedic implants
PP	Disposable syringes, blood oxygenator membrane, artificial vascular grafts
PMMA	Blood pump, implantable ocular lens, bone cement
PS	Tissue culture flask, rollers, bottles and filter wares
PET	Implantable suture, mesh, artificial vascular grafts and heart valve
UHMWPE	Orthopedic implants, total hip and knee joints
PTFE	Catheter, artificial vascular grafts
PU	Film, tubes, prosthetics and other components
PA	Packaging, catheters, sutures, and mold parts

2.2 Criteria for selection of biomaterials

The success of biomaterials in the body depends on many factors such as the material properties, design, and biocompatibility of the material. Other factors can include the surgery technique and health condition of patient. [32] Biomaterials are quite different from other non-medical, commercial products in many aspects. For instance, the industrial manufacturing of biomaterials or the sale of medical devices, are allowed only if they clear strict governmental regulatory issues. The minimum requirements on the biomaterial for such governmental approval include non-toxicity, sterilizability, and effectiveness. [33]

Biocompatibility

Biocompatibility is defined "as the ability of a material, device or system to perform without a clinically significant host response in a specific application". [31] When any biomaterial is introduced into the body, two effects are produced: the effect of the body tissue on the material and the effect of the material on the body tissue. If the material is biocompatible, no long-term toxicity or inflammation reaction appears. The level of biocompatibility depends on the application within the human body and how long it will be in contact with living tissue. [34] A new biomaterial must be tested prior to implantation and biocompatibility is the first perquisite. Testing of biocompatibility of implants is very a detailed and time and money consuming process. Material is tested extensively in vitro, in animal experiments, and in clinical studies with long post-operative follow-up periods. The minimum time from the first proposal of the new implant material to its approval for release on the market is about 10 years. [32]

Sterilizability

Sterilization is a term referring to any process that eliminates or kills all forms of microbial life, including transmissible agents such as fungi, bacteria, viruses, etc. Sterilization of biomaterials is an important aspect and can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration. Commonly used sterilization techniques are dry heat, autoclaving, radiation and ethylene oxide gas. Polymeric biomaterials have lower thermal and chemical stability than metals or ceramics and they cannot be sterilized by conventional techniques. Ethylene oxide (EO or EtO) gas is commonly used to sterilize objects sensitive to temperatures greater than 60 °C if they cannot be adequately sterilized by other methods. It is the most common sterilization method, used for 50% of all disposable medical devices. Radiation sterilization (gamma rays or x-rays) are very penetrating and are commonly used for sterilization of disposable medical equipment too. [29]

Processability

For the processing of biomaterials many technologies are used, which depends on the type of material. The medical device must be reproducible from sample to sample. Generally,

- the products must fulfill required properties and
- the selected technology should be economically advantageous.

Materials intended for biomedical use, must not demonstrate the presence of impurities and pharmacologically active trace components. This is not acceptable for industrial manufacturing of biomaterials.

Mechanical properties

Mechanical properties are chosen in dependence on the function of an implant device and should be adequate. The goal in applying biomaterials for implants is to maintain biofunctionality over extended periods of time. There are many levels of testing a new biomaterial. The basic level is in “in vitro” testing, that means outside of a living body under biological conditions. For example the corrosion in saline fluids or strength reduction is measured in vitro. These basic laboratory tests are performed in a manner that is slightly different from any other engineering material. In vitro testing is probably the cheapest and is widely used to exclude unsuitable materials from further testing. [32]

2.3 Biodegradable Polymers in Medicine

Applications of biodegradable polymers in medicine have received a large amount of attention in the lately. These materials degrade in the body by simple hydrolysis or by enzymes mechanisms. After some period of time, macromolecular chains are broken down into biologically acceptable monomers that can be metabolized and then excreted by the body as carbon dioxide and water without leaving a trace. The most popular and commercially available are absorbable sutures based on poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers, which were developed in the 1960s, so they have a long history for their excellent safety and biocompatibility as biomaterials. Nowadays, biodegradable materials are starting to be used as implantable medical devices, predominantly in orthopedic and dental applications, because they do not need a second operation for their removal. [35] [36]

2.3.1 Classification of biodegradable polymers

Biodegradable polymers can be of either synthetic or natural origin and both of them are used as biomaterials. Generally, synthetic polymers offer greater advantages. They can be tailored to give a wider range of properties and also represent a more reliable source of raw materials, which can be manufactured in a reproducible manner with better quality control. Natural polymers are more suitable for applications like tissue engineering and are less prone to produce toxic effects, but they suffer from some limitations such as immunogenicity, difficulty in processing, and a potential risk of transmitting original pathogens. [36]

Examples of natural polymers are fibrin, collagen, chitosan, gelatin and hyaluronan. The most often used synthetic polymers then are:

- Poly(lactic acid) (PLA)
- Poly(glycolic acid) (PGA)
- Poly(lactide-co-glycolides) (PLGA)
- Poly(ε-caprolactone) (PCL)
- Polyorthoesters
- Poly(dioxanone)
- Poly(anhydrides)
- Poly(trimethylene carbonate)
- Polyphosphazenes.

These biodegradable polymers are a promising class of biomaterials that can be engineered to meet specific requirements for their biomedical applications. Polymers can be selected on the basis of characteristics such as mechanical resistance, degradability, permeability, solubility, and transparency in which all can influence manufacturing and performance of device. In addition, the choice of polymer requires a thorough understanding of the surface and bulk properties as well as degradation process. In particular aliphatic polyesters appear the most attractive, because of their variable biodegradability and versatile physical, chemical and biological properties. [36]

2.3.2 Biodegradation mechanisms

Degradation of polymeric biomaterials in the body is a very complex process that includes hydrolytic and enzymatic mechanisms within the polymer matrix. These mechanisms can act separately or simultaneously and they result from the contact with living elements as tissue, cells, body fluids or enzymes. Degradation takes place mainly through scission of the main chains or side chains of macromolecules, which involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion. [37]

Hydrolytic degradation

In hydrolytic degradation, water is the agent that causes hydrolysis. Hydrolysis is a chemical reaction, in which a molecule of water is added to a substance, resulting in the split of that substance into two parts, shown in Figure 13. The hydrolytic reaction can be catalyzed by both acids and bases. [36]

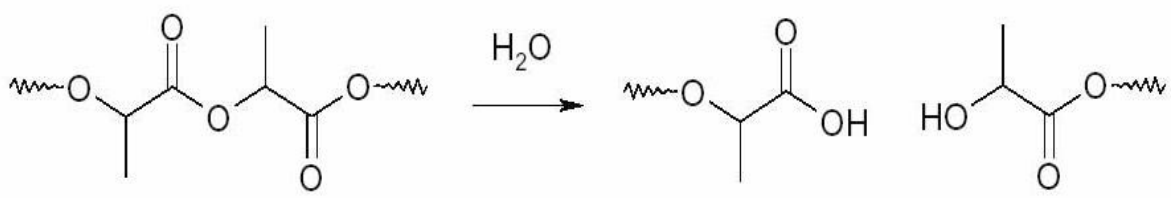


Figure 13 Hydrolysis of ester bonds

There are many parameters that can effect degradation, but water penetration rate is the most important factor. Considering the diffusion of water into the polymer matrix the degradation process can be divided into bulk and surface eroding polymer. The term degradation means bond cleavage while erosion means depletion of material. [38]

If water molecules penetrate easily throughout the matrix, degradation occurs rapidly and most probably homogenously within the bulk. In this case, ingress of water is faster than

the rate of degradation. The mass loss of the hydrophilic polymer is uniform and erosion rate is dependent on the volume of the polymer rather than on the wall thickness as is shown in Figure 14. [39]

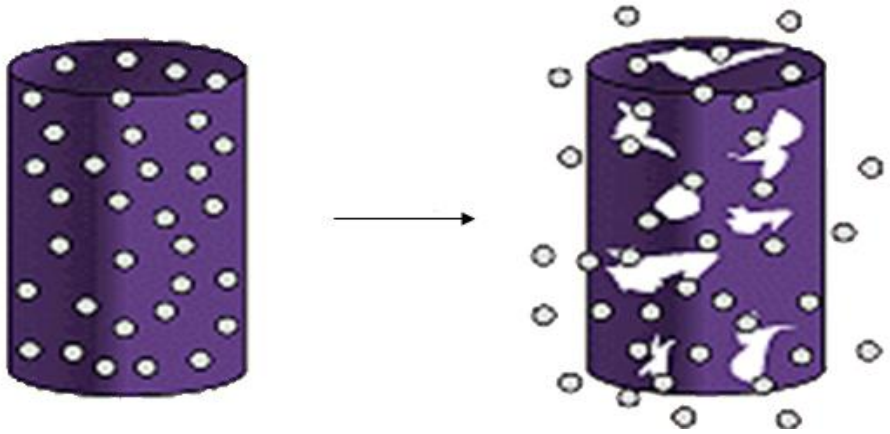


Figure 14 Bulk degradation [39]

Hydrophobic polymers do not allow water to penetrate into the material and these samples are eroded much more slowly and only from the surface as is shown in Figure 15 . In this case, the mass loss of polymer is faster than ingress of water into the bulk. The erosion rate is dependent on the surface area of the polymer rather than on its volume. [39]

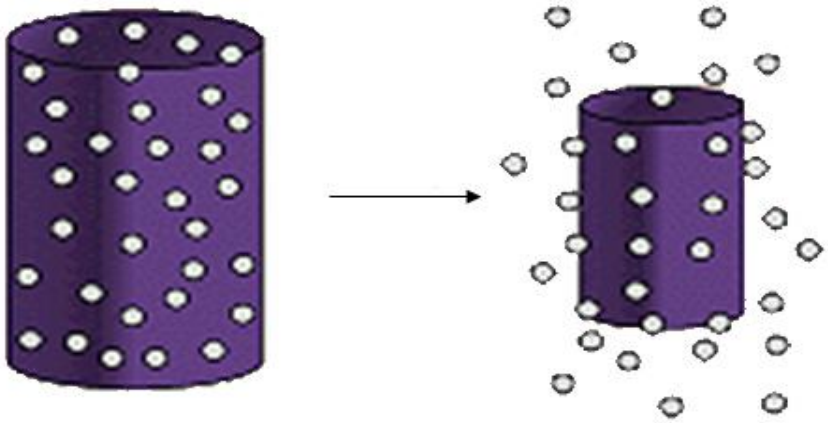


Figure 15 Surface degradation [39]

Enzymatic degradation

Natural polymers are generally degraded in the body by enzymatic degradation. Enzymes are large protein molecules and therefore cannot penetrate within the polymeric matrix, which leads to degradation only on the surface. Enzymatic degradation rate is difficult to pre-evaluate because of the differences of concentration in different parts of the body. In some specific cases enzymatic degradation may be as fast as hydrolysis, but this is an exception. [36]

The physical process of degradation in the body can be divided into 4 steps:

- water absorption into the polymer
- chain scission
- reduction of mechanical properties (modulus and strength)
- weight loss

In the first step water penetrates into the polymer and long chains are broken down into shorter chains, shown in Figure 16.

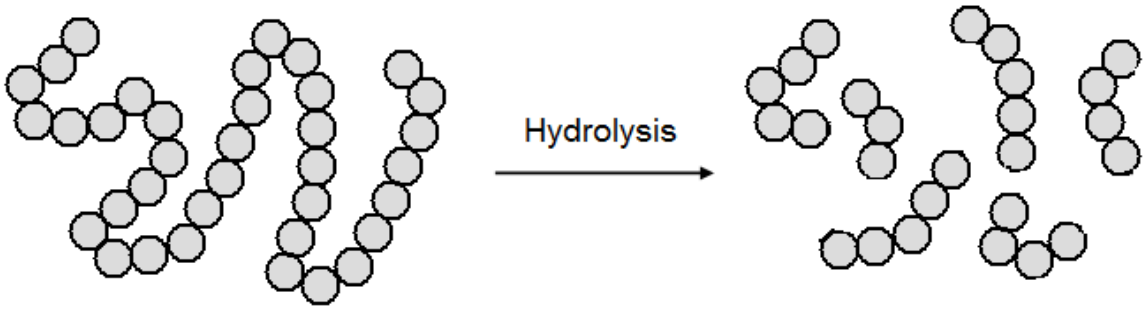


Figure 16 Chain scission

As can be seen in Figure 17, the chain scission results in rapidly molecular weight decrease. If the polymer is semicrystalline, there is a reduction of molecular weight without a loss in mechanical properties, because the polymeric matrix is held together by crystalline regions. As a reduction in molecular weight continues, soon is followed by reduction of mechanical properties. At the end when the polymer is hydrolyzed to the water-soluble fragments, there is rapid loss of polymer mass. [40]

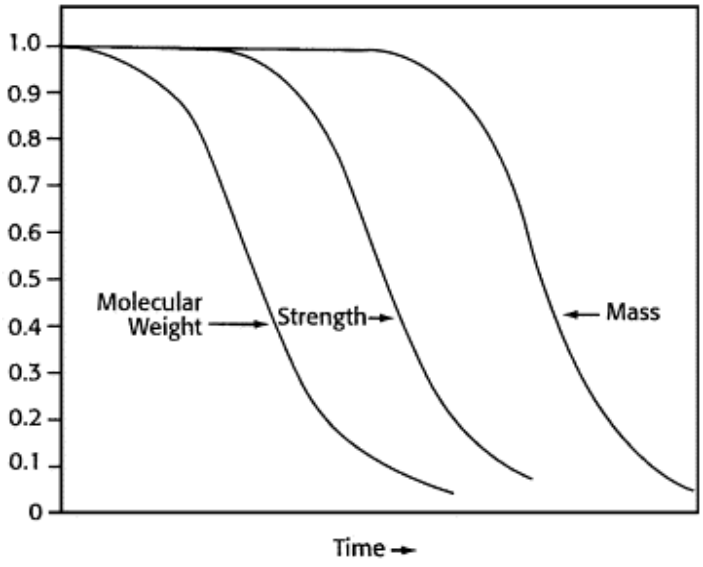


Figure 17 Physical process of degradation [40]

2.3.3 Factors affecting biodegradation of polymers

The most important factors that affect the rate of degradation are chemical composition of the polymer and the site of application in the body. Other important factors are: [41]

- water solubility and permeability of the polymer (hydrophilic/hydrophobic),
- mechanism of degradation (enzymatic vs. hydrolytic),
- diffusion coefficient,
- pH,
- molecular weight (M_w) and molecular weight distribution,
- glass transition temperature (T_g),
- morphology (amorphous or semicrystalline),
- physical factors of device (size, shape, surface vs. volume ratio),
- mechanical stresses and
- processing conditions.

All these factors affect the overall degradation rate and lifetime of the polymer. As was mentioned before, the water is the most important factor, so if the polymer is hydrophilic, degrades over the matrix and degradation mechanism is based on the bulk erosion. The hydrophilic polymers have degradation rate higher than hydrophobic polymers. Mechanism of hydrolysis depends also on the place in the body. For example, in the part of the stomach, the enzymatic degradation is more expected, than in other part of the body. [41]

The increase of molecular weight reduces the degradation rate, but the larges distribution of molecular weight usually increases degradation rate, because there are more free groups for the chain scission reaction. Polymers with higher T_g have less mobility and thus will require a longer time to degrade. The amorphous region in polymer degrades first than semicrystalline regions. [41]

The physical factors such as size and shape of the device also affect the degradation rate. The ticker samples require more time to complete degradation, but in some cases the autocatalytic reaction can accelerate degradation rate inside the matrix. The solubility of the degradation product is another important factor that governs their removal from the body. Same product can have the autocatalytic effect that result in higher degradation rate inside the polymeric matrix, than on the surface. This autocatalytic effect is usually because of change in pH, which cause acid or based catalysis. Processing condition as heat, pressure can also affect the degradation as well as a mechanical stresses during its uses. [41]

2.3.4 Methods of studying polymer degradation

Degradation of polymeric materials causes many significant changes in physical or chemical properties. These changes can be studied via numerous methods, and the choice always depends on which properties are important for the given application. [42]

The various changes and methods for their studying:

- Weight loss and rate of degradation
  - Analytical balance (measurement instrument)
- Structural changes (swelling, deformation, bubbling, disappearance etc.)
  - Optical (light) microscopy
  - Scanning electron microscopy (SEM)
- Thermal behavior changes
  - Differential scanning calorimetry (DSC)
- Molecular weight changes
  - Dilute solution viscosity (Rheometry)
  - Size exclusion chromatography (SEC)
  - Gel permeation chromatography (GPC)
  - MALDI mass spectroscopy
- Change in chemistry
  - Infrared spectroscopy (IR)
  - Nuclear magnetic resonance spectroscopy (NMR)
  - Time-of-Flight secondary ion mass spectrometry (TOF-SIMS)

There are many technical standards and technical methods available for testing polymer degradation, which can be used to evaluate specific products. Test methods usually require the full procedure used in a test.

International organizations for standards and test methods are:

- American Society for Testing and Materials (ASTM)
- European Standardization Committee (CEN)
- International Standards Organization (ISO)
- Institute for Standards Research (ISR)
- German Institute for Standardization (DIN)
- Organic Reclamation and Composting Association (ORCA)

3 POLYLACTIC ACID (PLA)

Poly(lactic acid) (PLA) is a synthetic biodegradable thermoplastic with structure given in given in Figure 18. It belongs to the family of aliphatic polyesters, which includes a wide range of biodegradable polymers. Among numerous polyesters, PLA is the most attractive and useful, due to a high strength, high modulus and excellent biocompatibility in contact with living tissue. This is why PLA it is the most promising biodegradable polymer for numbers of medical applications. [38] [43]

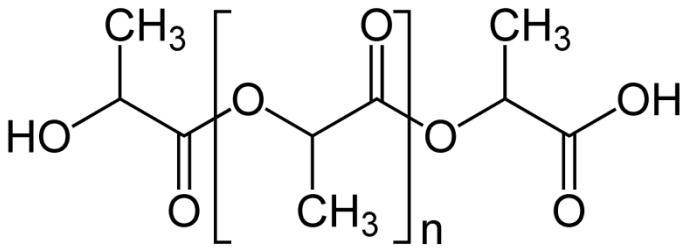


Figure 18 Structure of PLA, n – number of repeat units

PLA can be prepared as pure amorphous or partially crystalline. Both types have different properties, applications and mode of their production is also different. Practical utilizations of biodegradable polymers based on PLA include sutures, orthopedics, drug delivery and scaffold in tissue engineering. Some examples are shown in Figure 19, but the area of new potential applications is still growing. And last but not least, PLA could find utilization in otolaryngology as a temporary biodegradable ear tube. [43] [44]

Figure 19 Surgical fixation devices from PLA [45] [46]

Even if the use of biodegradable polymers has already been researched, so far there are very few papers dealing with ear tubes made of biodegradable materials. Some tests have been done on animals, where the tube form PLA was inserted into ears of guinea pigs. After some period of time, in vivo reaction between the ear tube and tympanic membrane was studied. Neither infection nor an inflammatory reaction to the tube within the middle ear was found in any animal, but the tubes were disintegrated in a short time. [47]

3.1 Lactic acid

The basic building blog of PLA, monomer lactic acid (2-hydroxypropionic acid) is the simplest hydroxycarboxylic acid with an asymmetrical carbon atom. Lactic acid may be produced by fermentation (usually anaerobic) of natural sugars in agricultural crops or by chemical synthesis. If lactic acid is produced by fermentation, it is optically active and exists in two different isomeric forms as a L(-) and D(+) lactic acid. D-form is simply a mirror image of the L-form, shown in Figure 20. Lactic acid naturally occurs and is most commonly found in the L(-) form. In case, lactic acid is prepared chemically, the product is a racemic mixture, having amorphous properties. In addition, PLA can be easily modified by polymerizing a controlled mixture of L(-) or D(+) isomers. [48] [49]

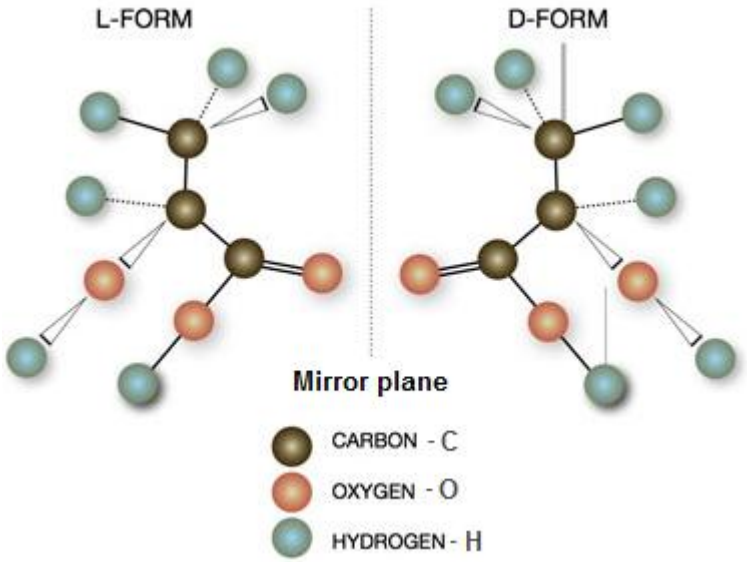


Figure 20 Two different isomeric forms of lactic acid [50]

3.2 Synthesis of PLA

PLA can be produced by many routes, differences are mainly in polymerization conditions or methods that result in various molecular weights, which affects polymer properties. Polymerization of PLA requires the monomer of high purity, since the impurities interferes with the course of reaction and reduces the quality of polymer. [43]

Generally, the lactic acid can be converted into polymer PLA by two different pathways: by polycondensation in a single step (1) or by ring opening polymerization in a multi-step process (2), both methods are schematically described in Figure 21. [36] [44] [49]

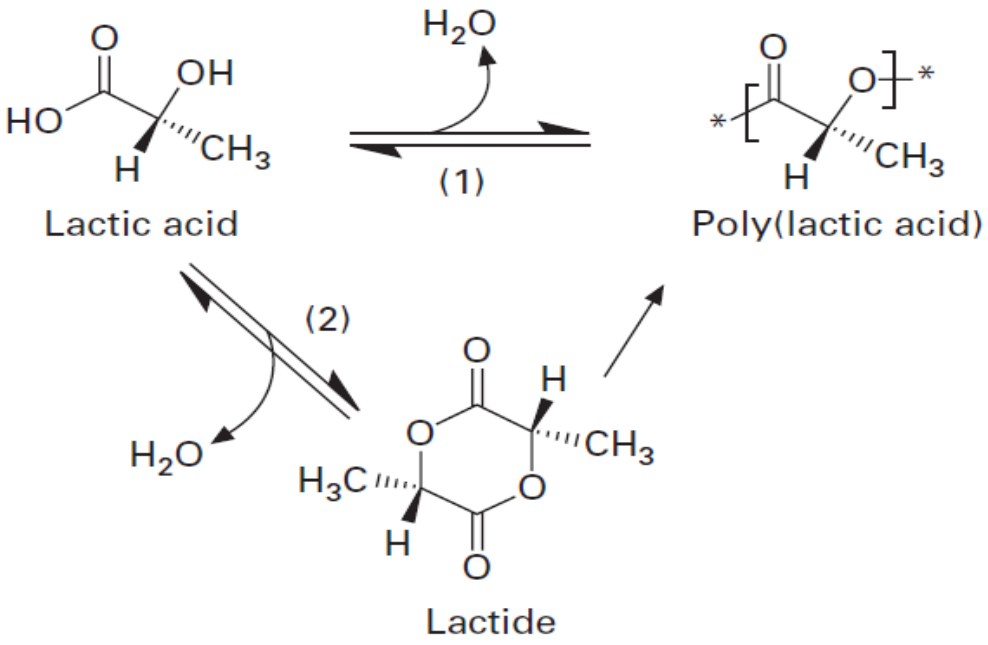


Figure 21 Polymerization routes to PLA

Direct polycondensation of lactic acid

Direct polymerization of lactic acid by polycondensation is used to produce PLA with low-molecular weight. Water produced during the polymerization process has to be removed, but there are some difficulties in doing this in the late stages of polymerization. This limits the ultimate molecular weight achievable by this method. Using azeotropic distillation (with the addition of another component) to remove the water of condensation, it is possible to achieve high-molecular weight PLA. This process is relatively simple, but molecular weight distribution and the end groups are fairly difficult to control. [36]

Ring opening polymerization

The more commercially used and efficient is the conversion of lactide (cyclic dimer of lactic acid) into poly(lactide) via ring opening polymerization in multi-step process. Ring opening polymerization of lactide provides a direct and easy access to the high-molecular weight PLA. The process starts with polycondensation of lactic acid into low molecular weight prepolymer followed by a controlled depolymerization into cyclic dimer called lactide. The molten lactide mixture is then purified by vacuum distillation. In the final step, catalytic ring-opening polymerization is used to convert the lactide into PLA. This process allows the production of high-molecular weight PLA in a controlled manner, but the cost of manufacturing and purification steps increase the cost of production. [36] [49]

3.3 Physical properties of PLA

Physical properties of PLA are strongly depended on its structure, morphology, crystallinity degree, molecular characteristics and particularly on molecular weight. Increasing of polymer chain length tends to decrease chain mobility, which result in increase of viscosity. On the other hand, as can be seen in Figure 22, the physical properties improve with increase of molecular weight. Physical properties are very important because they influence also mechanical properties as strength and toughness as well as physical changes during degradation.

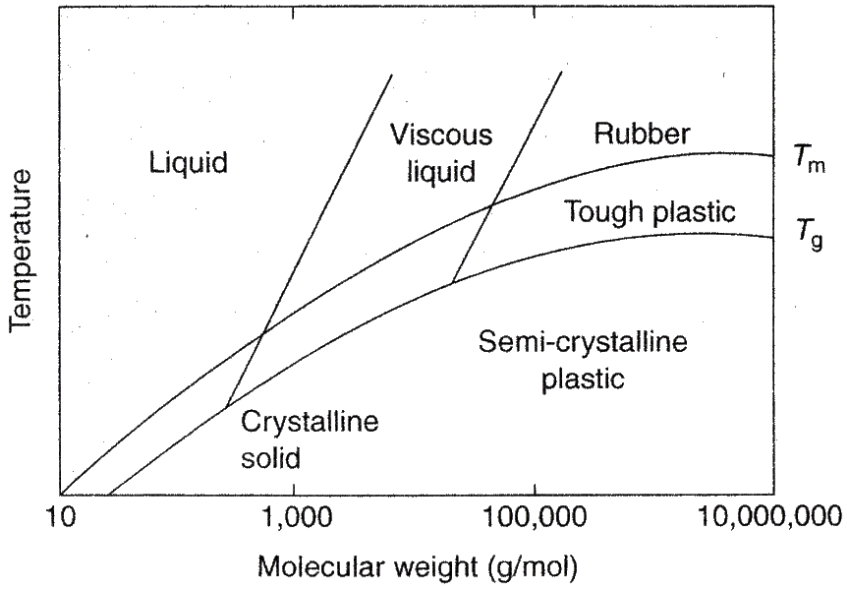


Figure 22 Effect of molecular weight on physical properties

Different behaviors are also related to the stereoregularity of the polymer chains. PLA obtained from the optically active L or D-form isomers is semicrystalline with higher physical properties than PLA obtained from DL-form, which is amorphous. Distribution of the D- and L- isomers in the polymer chains influence the degradation rate, which can vary from few months to more than two years.

PLA usually has a density circa 1.24 g/cm^3. Glass transition temperature for amorphous PLA is 55–60°C and melts temperature from 130 to 180°C. In general, the elastic modulus is about 3-4GPa and tensile strength approximately 50-70MPa. Mechanical properties of high molecular weight PLA are comparable to other commodity thermoplastics like polystyrene (PS) or polyethylene terephthalate (PET). The limitation of this polymer is low elongation at break, which results in brittle material at room temperature. To improve the properties, PLA can be copolymerized or blended with other biodegradable polymers. [44]

3.4 Degradation of PLA

In the body, PLA degrades mainly by simple chemical hydrolysis of the ester bond, which does not require the presence of enzymes to catalyze this reaction. This leads to a chain scission process during which polymer chains are cleaved to form oligomers and finally lactic acid, which can be absorbed by the body without leaving a trace. The degradation rate is influenced by the polymer's initial molecular weight and degree of crystallinity. Different physical properties as well as possibility of regulating the degradation time can be achieved also by copolymerization with other biodegradable polymers. The widely utilized is copolymer PLGA. [52] [53]

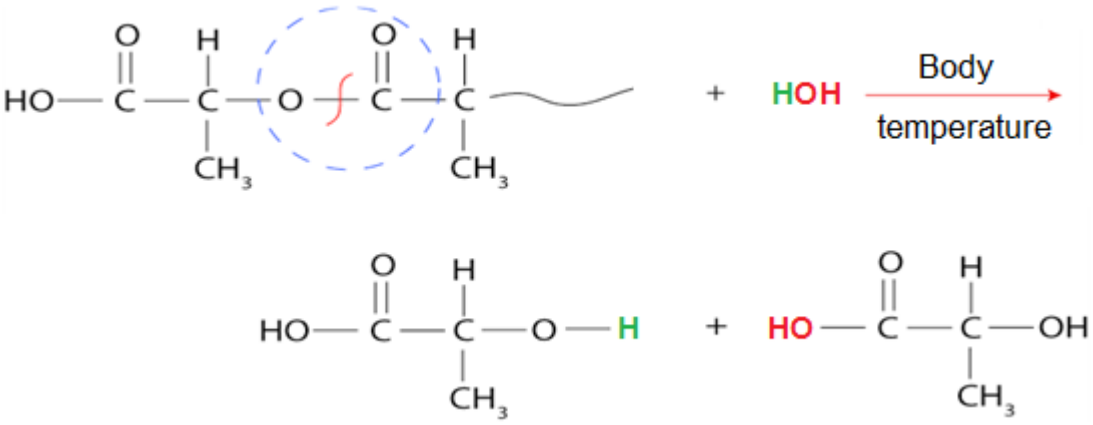


Figure 23 Degradation of PLA

3.5 Processing

PLA can be processed to a final product by conventional processing technologies used for thermoplastics such as injection, transfer or blow molding, extrusion, thermoforming, and fiber spinning from melt or solution. [48] Usually, PLA is supplied as amorphous or crystalline pellets, and before processing it is necessary to remove moisture from the polymer by drying. [54]

It is important to mention that PLA is sensitive to high temperature and exhibits rapid loss of molecular weight during processing, if PLA is dried sufficiently, the optimal process should exhibit molecular weight loss of 10% or less. The temperature for injection molding and extrusion is about 200°C, but above this temperature PLA can undergo thermal degradation. To facilitate processing of PLA, various additives are added during processing to enhance the material properties. PLA for medical applications can possess additives such as hydroxyketones, which increase biocompatibility. [52]

4 PRODUCTION OF EAR TUBE

There are three main techniques how to produce ear tubes. One of the major factors for the selection of the method is the quantity and quality of products. The technology tympanostomy tube production together with properties of materials will affect the qualities of the medical device, surface and particularly the lumen. For large-scale production, the polymer must possess adequate thermal stability to prevent degradation and maintain molecular weight and properties.

4.1 Injection molding

Injection molding is one of the most important technologies in the processing of polymers. It is characterized by complicated physical processes in which molten polymer is forced under high pressure into a tempered mold cavity through a sprue. This technology is convenient for production of a large number of identical parts, from high precision components to classical consumer goods. The equipment required for injection molding is a mold and injection molding machine, which consists of plasticating/injection unit, clamping unit, control system and tempering devices

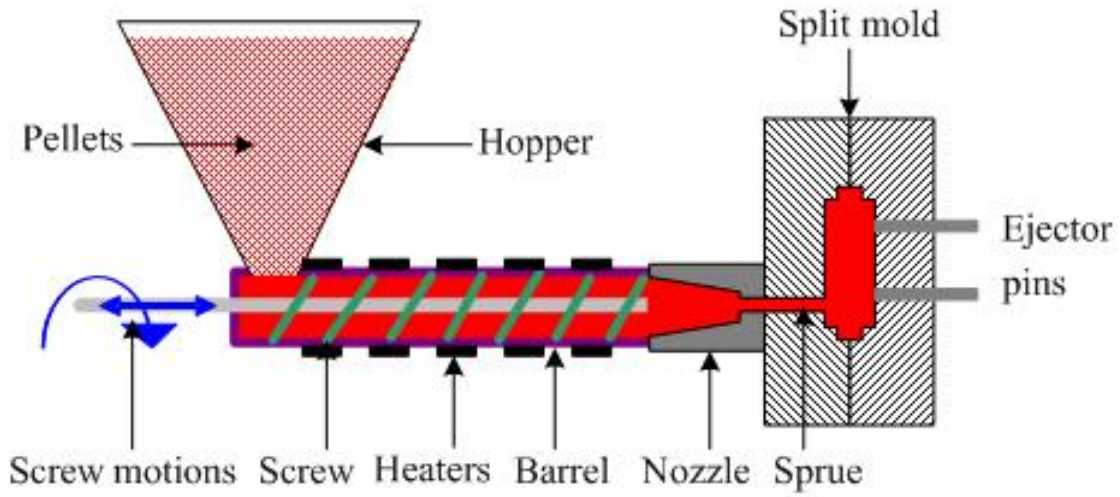


Figure 24 Injection molding [55]

For a production of very small polymeric parts, there is a technology called micromolding. Micromolding process is similar to conventional injection molding, but there is different process control of some areas. Micromolding is a thermally dominated process containing very high thermal gradient, rapid heat flows and large product surface area to volume area. Therefore, tool temperature will have a high influence on the mechanical and morphological properties. Some application can require raised temperature of the mold cavity before

injection to ensure that the cavity is filled adequately and then cooled rapidly. A typical screw diameter in micro-machines ranges from 12 to 16 mm and can contain up to 6 g of material, so there is also a demand for micro-pellets as a raw material. Computer modeling of this process is desirable, instead of fabrication and testing expensive molds. [56]

4.2 Transfer molding

Transfer molding is a simpler process in comparison with injection molding. In this process a pre-weighed amount of polymer is placed into a separate chamber (transfer pot), heated by hot mold and then transferred by a hydraulic plunger into the cavity through the sprue. The parts are then removed, usually manually. Transfer molding is preferable in the processing of shear sensitive materials, which cause problems in the screw plastication unit. This technology is used primarily for molding thermosets and rubbers, but can be useful for producing a medium number of thermoplastic items. The advantage is a low cost of machines, and molds with a large number of cavities are allowed to reduce the manufacturing cost. The disadvantage is the large amount of unused material that remains in the transfer pot. Therefore, other processes combining transfer molding have been developed. For example, injection transfer molding is a process that combines advantages of injection and transfer molding. This method is very suitable for production of very small parts. Although most parts are limited in shape, there are numerous applications and one of them can be medical devices. [57]

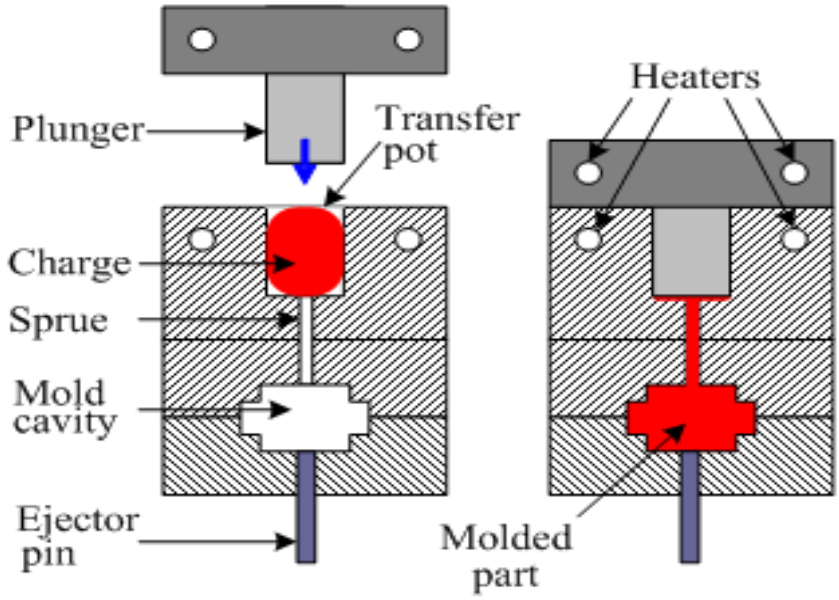


Figure 25 Transfer molding [51]

4.3 Casting

Casting is a standard method of making small quantities of polymer parts with a complex shape, which would be otherwise difficult or uneconomical to make by other methods. In this manufacturing process the material is first liquefied by proper heating. Then the liquid material is poured into the mold, which contains a hollow cavity of the desired shape, where the material solidifies. The solidified part is also known as a casting, which is ejected or broken out of the mold to complete the process. Silicone room temperature vulcanizing rubbers are most often used for making the mold. Silicone rubber tooling is not expensive and offers good accuracy and finish surface. The parts produced by casting are often adequate for prototypes or small production runs. At beginning of casting, the replica of the part is created. This replica is also called pattern can be generated for example by rapid prototyping and is used in developing the mold cavity. The process of preparation the silicone mold and the subsequent casting of polymeric material are shown in Figure 26. [58]

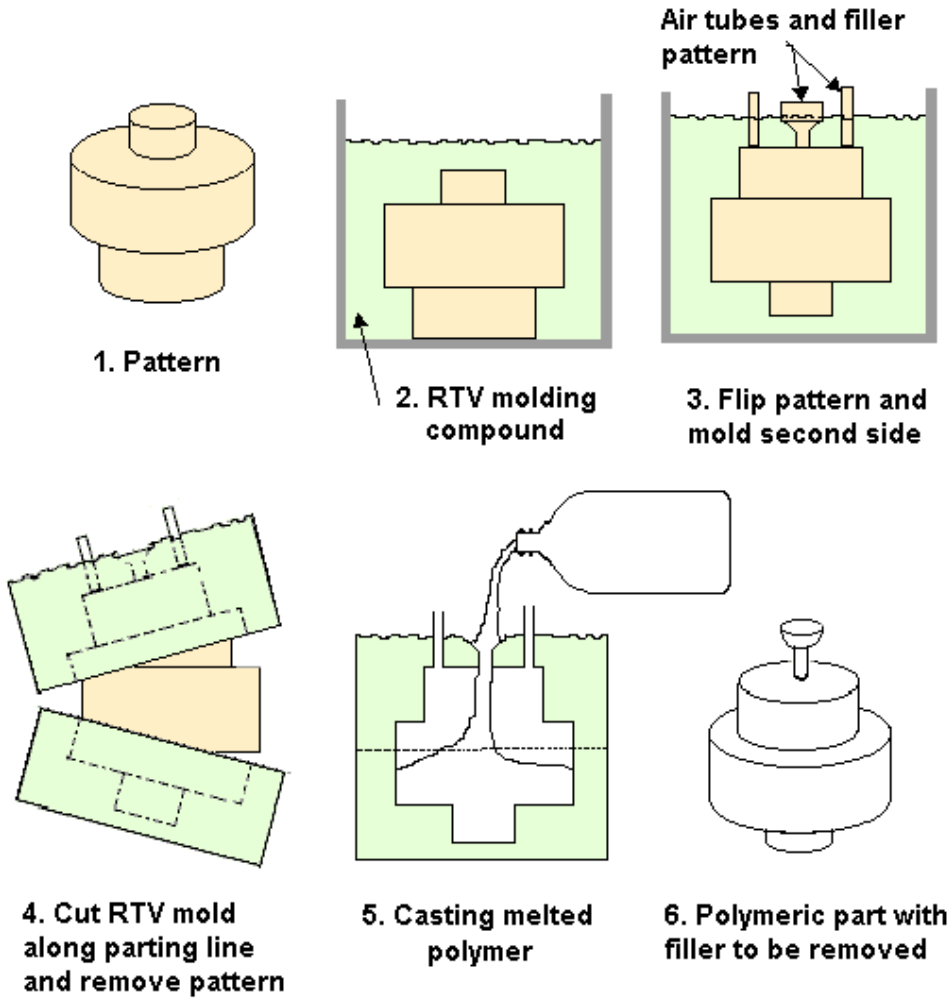


Figure 26 Preparation of silicone mold and polymer casting [58]

II. EXPERIMENTAL PART

5 AIMS OF THE MASTER THESIS

On the basis of assigned tasks and literature review, the aims of this master thesis are defined in the following items:

1) Preparation of PLA samples and characterization of basic material properties
- Thermal analysis
- Rheological properties
- Mechanical properties
2) Studying of PLA changes during in vitro degradation (100% relative humidity versus buffer solution)
- Weight loss, rate of degradation
- Structural changes (SEM)
- Thermal behavior changes (DSC)
- Molecular weight changes (GPC)
3) Development of the tool for a production of biodegradable tympanostomy tubes
- Design of the tubes
- Construction of a mold to produce designed tubes
- Production of the mold
4) Discussion of the results and their presentation in experimental and practical part of this thesis

6 MATERIAL AND SAMPLES PREPARATION

6.1 Material

The material utilized throughout this experimental part was pure poly(L-lactic acid) (PLLA) produced from L(-) lactic acid ($C_3H_6O_3$), without additives. This material was synthesized at the laboratory of Polymer Center on Faculty of Technology at Tomas Bata University in Zlín. The raw material was in form of a powder shown in Figure 27.

Figure 27 PLLA in form of powder

6.2 Samples preparation

There were many different types of samples and each type involved specific preparation techniques. For the mechanical properties testing there had to be prepared a silicon form for casting the samples. This procedure was chosen because this material was too brittle for pressing or injection molding.

6.2.1 Samples for degradation testing

For degradation testing were prepared small cylinders (4 mm diameter, 8 mm in length and weight about 125 mg).

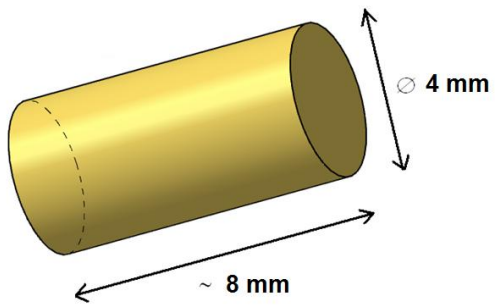


Figure 28 Sample for degradation testing

Sample preparation

The synthesized powder of PLLA was firstly poured into a glass vial and then was heated in silicone oil at 175°C until the material was melted. Because there was air into a powder, it was necessary to use vacuum extraction. After the exhaustion of all air from the glass vial, the melted material was casted into a small vial and the silicone tube was inserted. In this way cylinders for testing were created. Finally they were abraded to the desired length.

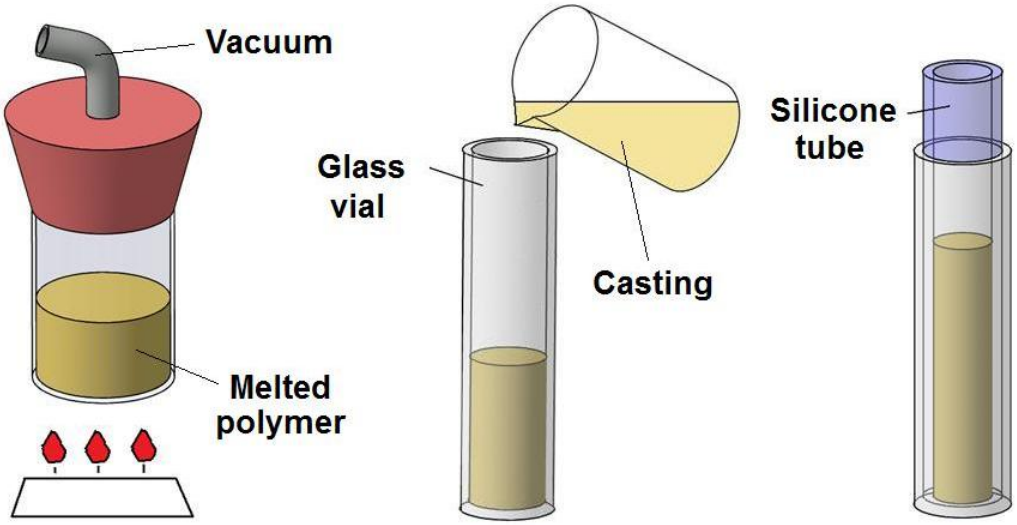


Figure 29 Principles of cylinders preparation

6.2.2 Samples for mechanical testing

There were prepared two types of specimens for mechanical testing. The first type of specimens has enlarged ends for gripping. The second type of specimens is a stick without enlarged ends. Both of specimens are standardized and they have a fixed length and depth, however with is permitted to vary between limits.

Silicone mold for samples

It was necessary to make the silicone mold for the production of samples intended to mechanical testing. The silicone mold was prepared from Rhodorsil RTV 3512. It is a two component silicone elastomer, which cures at room temperature by polyaddition reaction. The two components (A&B) were mixed 1:1 by hand and then degassed under a vacuum of 30 mbar. The prototype specimens were placed into a mold and then poured with prepared silicon mixture. The silicone mold cured at room temperature and after 24 hours was ready to use. Characteristics of the cross linked Rhodorsil from material list: hardness 12 (Shore A), tensile strength 5 MPa and linear shrinkage < 0.1 %.

Figure 30 Silicone mold for casting samples

The preparation of samples for mechanical testing had the same procedure as the preparation of cylinders. Firstly, PLLA powder was melted in the chemical glass and then the air was sucked to remove air gaps. Finally, the melted polymer was casted into a preheated silicone form (at 150°C).

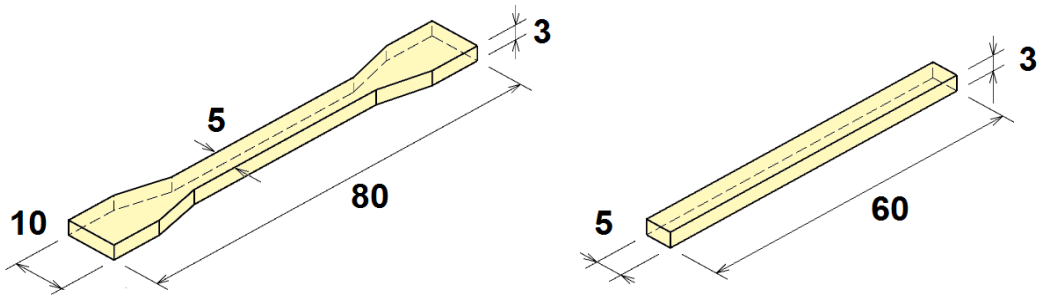


Figure 31 Shape and size of casting samples

6.2.3 Samples for microhardness testing

The sample for micro-hardness was prepared from a long cylinder, which was produced in silicone tube. This cylinder was put between two plates of a certain distance and loaded with a force in the furnace at temperature of 120°C for 10 minutes. The final product was a straight smooth plate. Finally, the smooth plate was glutted to the rectangular metal prism.

Figure 32 Sample for microhardness

7 CHARACTERIZATION METHODS

This chapter describes basic methods for characterization polymer properties used in this experiment.

7.1 Basic material properties testing

7.1.1 Density determination

Immersion method is a simple method to determine density of small solid samples. The sample is balanced firstly in one pan in air and then in the other pan immerged in the fluid of known density. This method uses the principle of Archimedes and the set for weighting is shown in Figure 33.

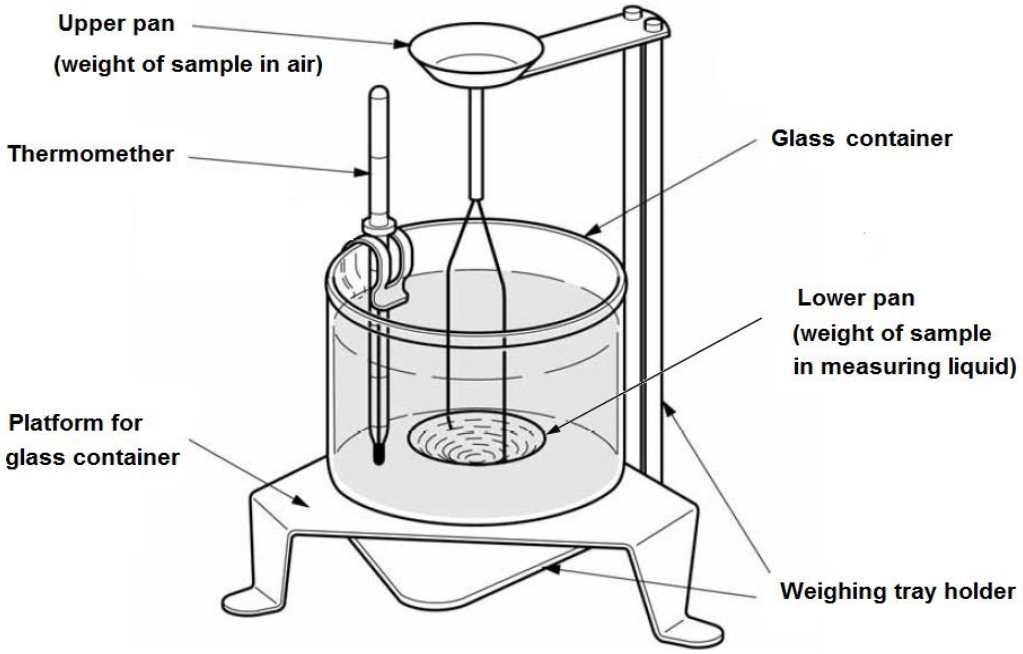


Figure 33 Density determination set

The weight of 10 samples were balanced using density determination set Kern ABS –A02 and analytical balance SI-64A from Denver Instruments according to the EN ISO 1183-1. The measuring fluid was water, which had temperature 24°C and density 0.997295 g/cm^3. Density of samples was calculated according to the formulae below:

$$\rho = \frac{m}{m - m_{im}} \cdot \rho_{im} \ [g/cm^3] \quad (1)$$

m – weight of the sample in air (g)

m_{ik} – weight of the sample in measuring fluid (g)

ρ_{im} – density of measuring fluid (g/cm^3)

7.1.2 Microhardness test

Microhardness testing is very useful for testing very small object or thin materials. The procedure of testing is similar to the standard Vickers hardness test, shown in Figure 34. The differences are only in applied load and high precision of instruments. The measurement is realized by penetration of the Vickers diamond pyramid or the Knoop elongated diamond pyramid into sample. Then precision microscopes are used to measure the indentations. The applied load is from 1 g to 1 kg during a predefined time. The smaller the load is used, the higher surface finish is required.

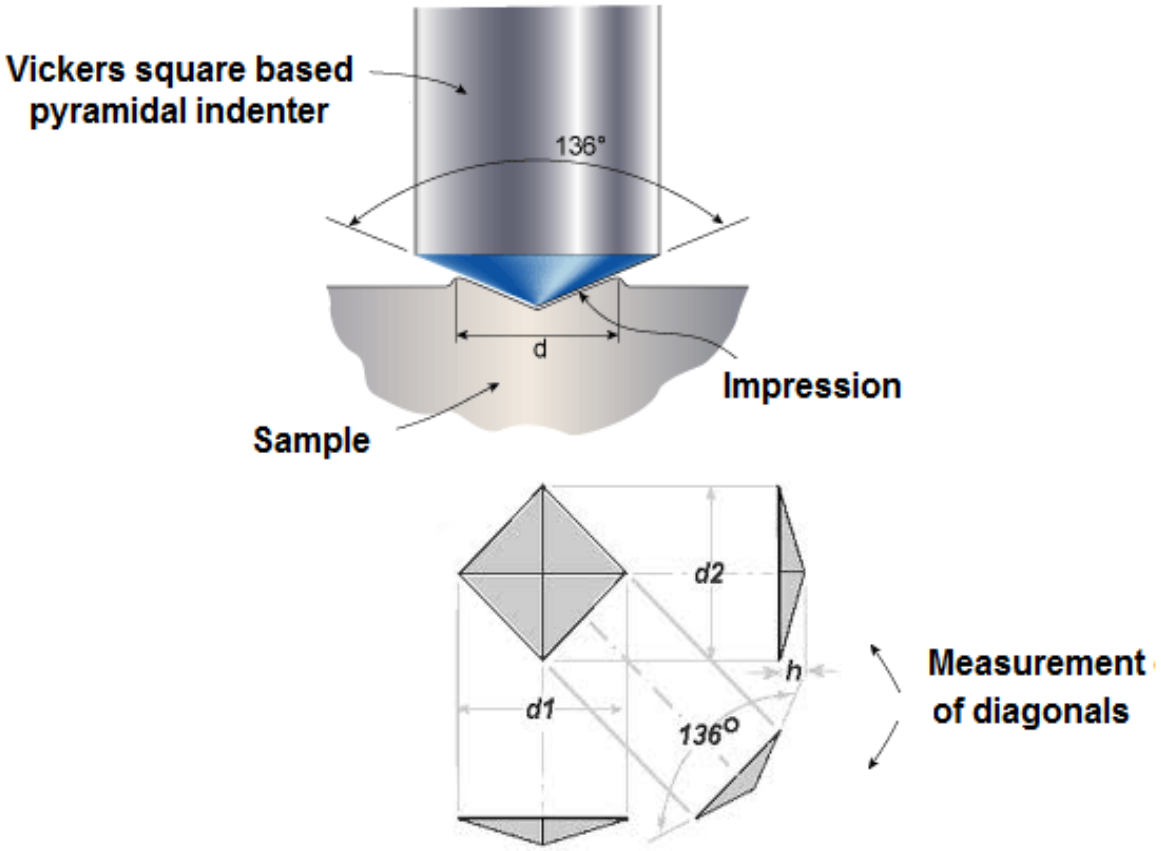


Figure 34 Vickers hardness test with square based pyramidal indenter [59]

The CSM Micro Combi Tester (Figure 35) with Vickers diamond intender with form of squared pyramid with an angle of 136° degrees between faces, was used to determine the mechanical characteristics according to the CSN EN ISO 6507-1. The maximum applied force was 1N and it was holding for 90s. The loading and the unloading rate was 2N/min. The measurement was carried out at room temperature and on each sample were done 9 indentations. The elastic modulus was calculated from unloading curve by Oliver and Pharr method. [60]

Figure 35 CSM Micro-hardness Tester

Table 2 Specification of CSM Micro-harness tester

Load range	0.01 to 30 N (load resolution of 0.3mN)
Load rate	up to 300N/min
Maximum depth	200µm (Depth resolution 0.3 nm)
Intenders	Vickers, Berkovich, Knoop, Cube-corner, Spherical
Microscope magnification	200x, 2000x
Displacement tables (x, y)	120 x 20 mm (resolution 0.25µm)
Maximum sample size (x, y, z)	250 x 480 x 120 mm
Usable area of analysis (x, y)	195 x 120 mm

Indentation hardness (H_{IT}) a measure of the resistance to permanent deformation or damage. H_{IT} was calculated according to formula:

$$H_{IT} = \frac{F_{max}}{A_p} \; [MPa] \tag{2}$$

F_{max} – is the maximum force (N)

A_p – is the projected contact area (mm)

Indentation modulus (E_{IT}) was calculated from the plane strain modulus (E^*) using an estimated sample Poisson's ratio (ν_s).

$$E_{IT} = E^* \cdot (1 - \nu_s^2) \; [GPa] \tag{3}$$

Poisson's ratio of 0.3 was used for modulus calculations.

Vickers indentation hardness (HV_{IT}) is the applied load divided by the surface area of indentation.

$$HV_{IT} = \frac{2 \cdot F \cdot \sin\left(\frac{136^\circ}{2}\right)}{d^2} \approx 1.8544 \cdot \frac{F_{max}}{d^2} \; [Vickers] \tag{4}$$

F – is the load (in kgf)

d – is the arithmetic mean of both diagonals, d_1 and d_2 (mm)

Indentation creep (C_{IT}) is defined as the relative change of the indentation depth whilst the applied load remains constant.

$$C_{IT} = \frac{h_2 - h_1}{h_1} \cdot 100\% \; [\%] \tag{5}$$

h_1 – is the indentation depth at time of reaching the test force, which is kept constant (nm)

h_2 – is the indentation depth at the end of holding constant test force (nm)

7.1.3 Bending test

The bend test is used to determine the ductility or the strength of a material. This test is often used for brittle materials, which do not hold up well under a normal test for tensile strength. Often, just a placing of brittle material in the grips of the tensile testing machine can cause cracking. The most common bending tests are the three-point and the four-point tests. In these tests a sample is supported by two pins and loaded with either the central ram (three-point test) or symmetrically positioned rams (four-point test). The thickness of the ram and distance between pins dependent on the sample thickness. The central three-point bending load causes bending moment in the beam, which varies linearly from zero to the maximum value at extreme fibers. Most materials fail under tensile stress, instead of compressive stress. The flexural strength is commonly higher than tensile strength.

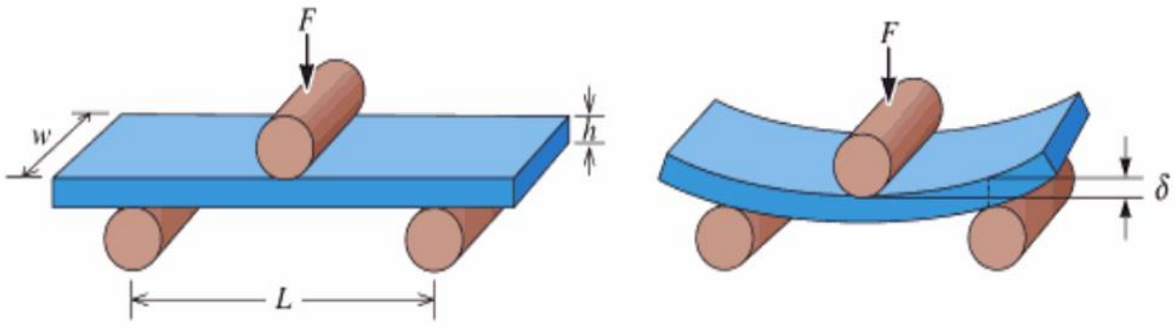


Figure 36 Three-point bending test

The static bending test was carried out using the universal testing machine ZWICK ROELL 1456 (Figure 37), according to CSN EN ISO 178. The flexural strength and modulus was obtained from three-point bending test. The supporting pins have a diameter of 4 mm and the distance between them was 20 mm. Load rate was 1mm/min and measurements were done on 9 samples at room temperature.

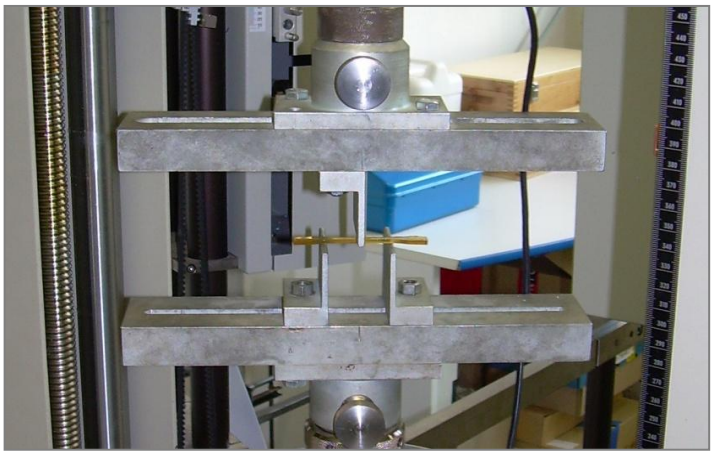


Figure 37 Example of bend testing (Zwick Roell 1456)

Table 3 Specification of the universal testing machine Zwick Roell 1456

High and with of machine	1284 x 630 mm
With of working place	420 mm
Maximum applied load	20 kN
Maximum load rate	750 mm/min
Extensimeter	macro, optional
Testing	tensile, compression, bending and cycling testing

Flexural strength for free-point bend test

$$R_{max} = \frac{M_{Omax}}{W_O} = \frac{3 \cdot F \cdot L}{2 \cdot w \cdot h^2} \ [MPa] \tag{6}$$

F – is the maximum fracture load (N)

L – is the distance between the two outer points (mm)

w – is the width of the specimen (mm)

h – is the height of the specimen (mm)

Flexural modulus

$$E_B = \frac{L^3 \cdot F}{4 \cdot w \cdot h^3 \cdot \delta} \ [MPa] \tag{7}$$

δ - is the deflection of the beam when a maximum force F is applied (mm)

7.1.4 Melt-flow index test

Melt flow index (MFI) or melt flow rate (MFR) is a measure of the flow rate of melted polymer. MFI is defined as the mass of polymer in grams, extruded in 10 minutes through a capillary of a specific diameter and length under prescribed conditions of temperature, load and piston position. The obtained value represents one point on the flow curve. MFI measurement is important for processing, and generally higher values of MFI are indicated for injection molding. Polymers with a lower MFI are indicated for extrusion. The methods are described by ASTM D1238 and ISO 1133 standards.

The instrument to determine MFI is the extrusion plastometer consisting of a heated barrel, piston, load, and capillary. The standard capillary has an inner diameter of 2.095 mm and 8 mm in length. The small amount of polymer sample is preheated in the barrel at constant temperature for specified time. After the preheating time, the prescribed load is introduced onto the piston and polymer melt is extruded by the pressure through the capillary. The extruded polymer is cut after desired period of time and weighted. The basic principle of the testing apparatus is shown in Figure 38.

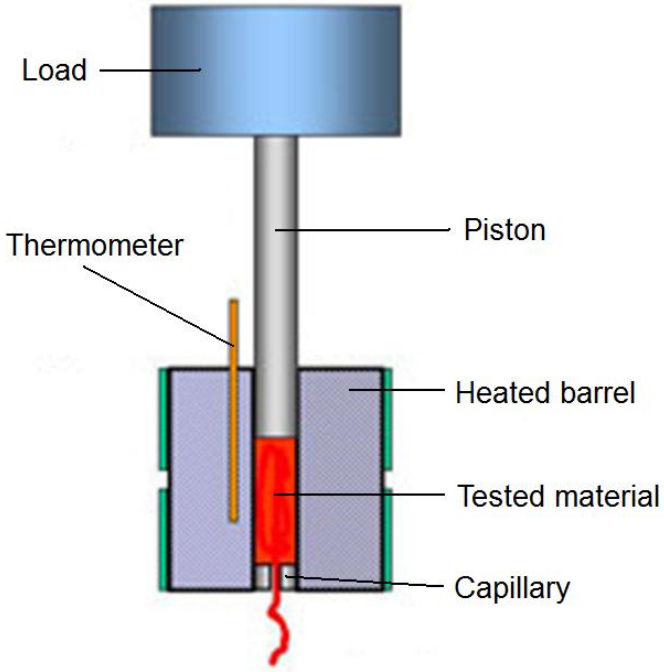


Figure 38 Apparatus to measure MFI [61]

The melt flow is affected by many factors. The results must always contain the test conditions, because the final value strongly depends on them. The MFI is an indirect measure of molecular weight, the high MFI corresponds to the low molecular weight.

Rheological examination of the MFI was performed by Dynisco Kayness LMI 4003 extrusion plastometer shown in Figure 39, according to ISO 1133. The test was conducted by method B that measures the time of the polymer flow of a fixed volume. The flow rate of PLLA was measured at 125°C using a 100 g piston as a load.

Figure 39 Extrusion plastometer Dynisco Kayeness LMI 4003

The MFI was calculated by the following formula:

$$MFI = \frac{426 \cdot L \cdot \rho}{t} \ [\mathrm{g/10\ min}] \tag{8}$$

L – is the length of calibrated piston travel (cm)

ρ- is the density of molten polymer at test temperature (g/cm^3)

t – is stands for time of extrusion (10 min)

Density of the molten polymer was calculated as:

$$\rho = \frac{m}{0.711 \cdot l} \ [\mathrm{g/cm^3}] \tag{9}$$

m – is the weight of molten material extruded on defined length (g)

l – is defined length of piston (cm)

7.2 Methods for studying degradation changes

7.2.1 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is thermal analysis method based on measurement of enthalpy changes (ΔH) in samples due to changes in their physical and chemical properties as a function of temperature or time. Sample and thermally inert reference material are heated (or cooled) by controlled heating rate and both of them are maintained at the same temperature. The differences in heat flow arise when a sample absorbs or releases heat due to thermal effects. Processes that increase enthalpy such as melting, evaporation or glass transition are endothermic while crystallization and decomposition are called exothermic reactions. The result of DSC measurement is a curve of heat flow versus temperature which can be used to determine glass transition (T_g), melting temperature (T_m), crystallization temperature (T_c), heat capacity (Δc_p), percentage of crystallinity (χ_c), and etc. [62]

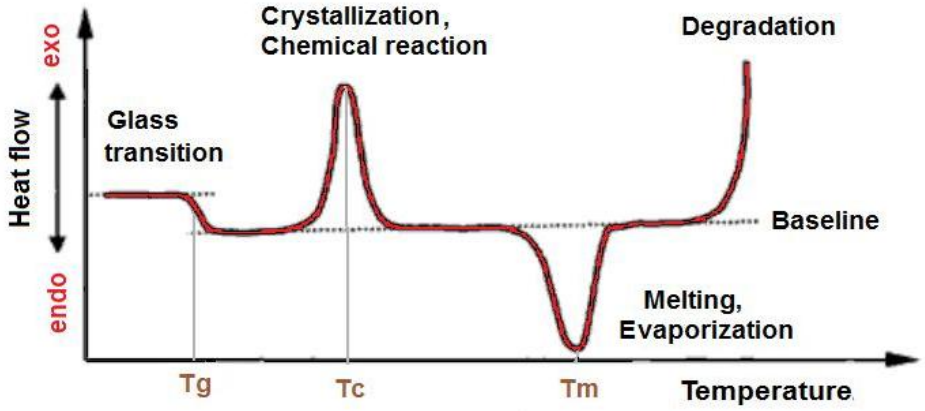


Figure 40 Schematic diagram of a DSC scan [62]

Thermal behavior changes during in vitro degradation were examined by Metteler Toledo DSC1 START system. Preparation of samples for DSC required only a small amount of material, approximately 5 - 10 mg. Samples were taken from entire cylinders, placed into an aluminum pan and subsequently sealed by a special sealing press. Initial temperature was set up to 5°C and samples were heated to 180°C by heating rate 10°C/min. Then the samples was cooled and subsequently again heated to180°C. This cycle was applied to all samples and the measurements were performed under nitrogen atmosphere (60 ml/min). The melting temperature (T_m) and percentage of crystallinity was measured from the first heating using the Metteler analysis software. Crystallinity (χ_c) was calculated based on a heat of fusion of 93.6 J/g. The glass transition temperature (T_g) was observed from second heating scan.

7.2.2 Gel permeation chromatography

Gel permeation chromatography (GPC) is a separation technique widely used to determine the molecular weight and molecular weight distribution known as the polydispersity. Using GPC method the polymers molecules are separated by their size involving the transport of a liquid mobile phase through a column containing the separation medium, a porous material. The Column separates samples by size of the molecules in solution. Molecules larger than pores of the gel cannot enter into the pores and pass through the column at the same speed as the mobile phase. The smaller molecules can enter into the pores easily and therefore spend more time there, which increase their retention time into the column. Hence, the large molecules are eluted firstly from column, followed by smaller and smaller molecules. As molecules elute from the column, the detector measures their relative concentration.

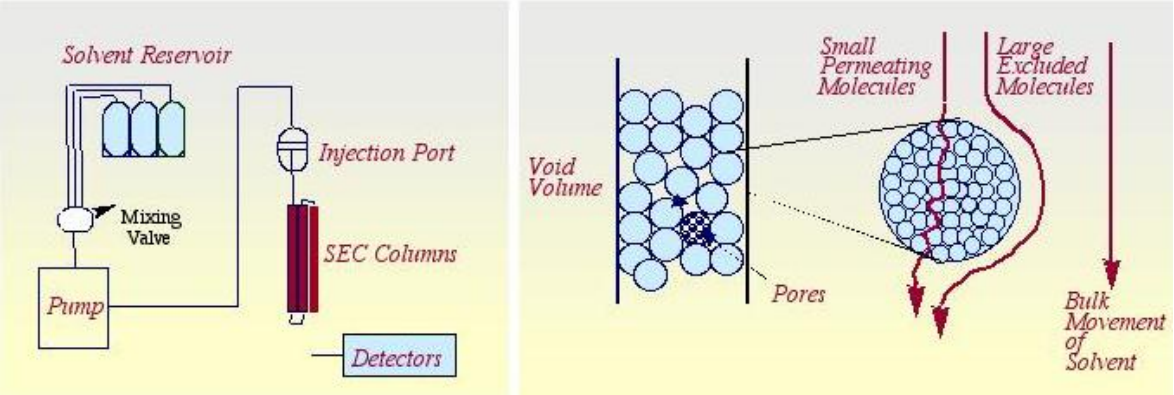


Figure 41 Principle of gel permeation (or size extrusion) chromatography [63]

Weight average molar mass (M_w), number average molar mass (M_n) and molecular weight distribution (M_w /(M_n) of PLLA during in vitro degradation were determined by GPC using a Breeze Waters system. Samples were prepared by adding 1 ml of chloroform to approximately 1.5 mg of polymer taken from core and cortex of cylinders. The system was calibrated with narrow PS standard. The eluent was chloroform with a flow rate of 1ml/min. The experiment was performed at room temperature. Data processing was carried out using the Waters Breeze GPC Software.

7.2.3 Mass loss measurements

The influence of temperature and humidity cause the degradation and subsequently the mass loss of PLLA. The weight of cylinders was measured every week using analytical balance. Before each measurement cylinders were dried under vacuum over night.

7.2.4 Scanning electron microscopy

Scanning electron microscopy (SEM) is the technique used for investigations of surface structural changes or morphology of samples. The electron microscope uses a beam of high energy electrons generated by an electron gun. The beam is processed by magnetic lenses and then is focused at the sample surface, which is systematically scanned (rastered). Signals generated from the sample are collected by an electron detector and converted to electrical signals, which are used to modulate the intensity of the image on the screen. The SEM forms the electronic image, not the real image. This yields a good contrast of SEM images with a very good spatial visibility of details and a high depth of focus (a factor of about 100 greater than in light optical imaging). Increased magnification is produced by decreasing the size of the area scanned. [64]

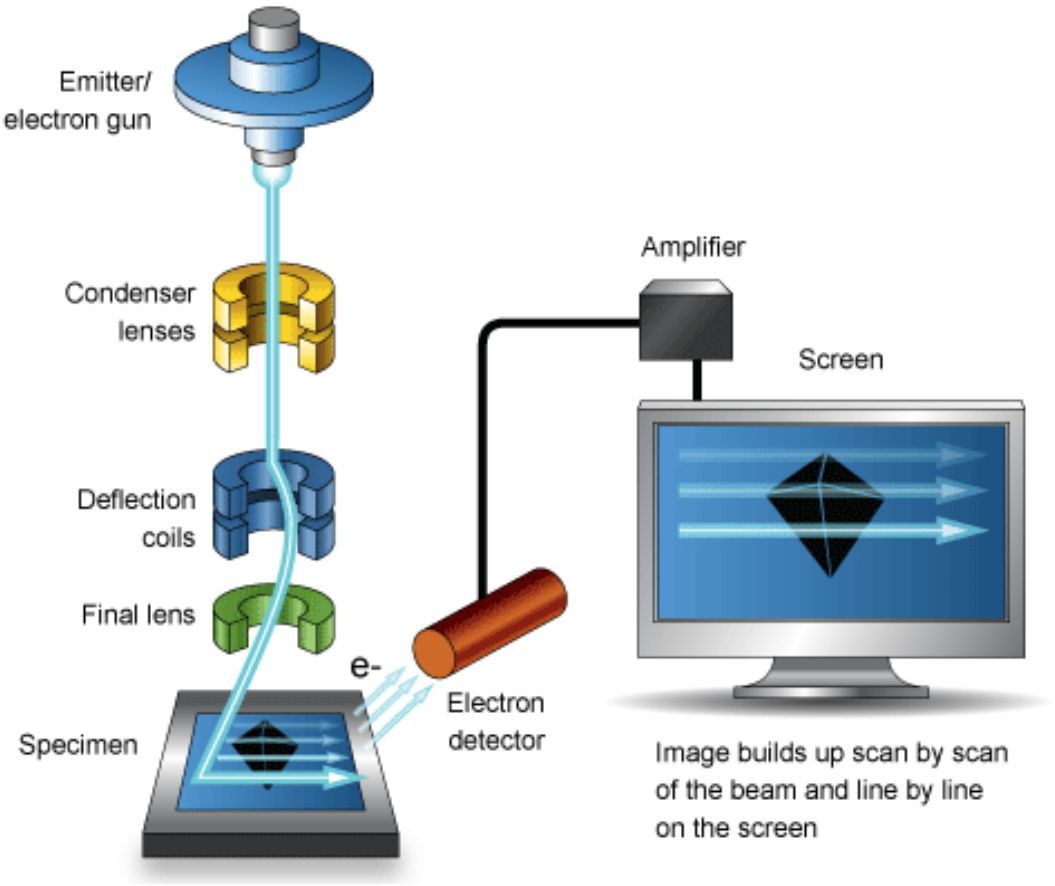


Figure 42 Schematic diagram of SEM [64]

The structural changes of PLLA cylinders during in vitro degradation were observed by simple light-optical microscopy and the inner morphology of samples was investigated by SEM using Vega II LMU, Tescan electron microscope. Samples for SEM were cut and sputter-coated with the gold layer before characterization.

8 RESULTS AND DISCUSSIONS

This chapter is divided in two main parts. In the first part there are results from the basic measurements of material properties of PLLA, such as the density, mechanical properties and melt flow index. The second part is dedicated to monitoring in vitro degradation process of PLLA. The cylinder samples were exposed for 12 weeks under two types of external conditions: in chamber with 100% relative humidity (RH) and in phosphate buffered solution (PBS, pH 7.4), both of them at 37°C. Over this time, changes of molecular weight, thermal properties and structural characteristics were investigated.

8.1 Material properties

8.1.1 Density determination

Density, the elementary physical properties of matter was determined by simply immersion method. The measurement of PLLA samples weights in air and in water as well as calculated density are shown in Table 4. Density was calculated by formula (1).

Table 4 Measurement of density

Sample	Weight in air	Weight in water	Density
	g	g	g/cm^3
1	0.1034	0.0204	1.242
2	0.1260	0.0246	1.239
3	0.1388	0.0282	1.252
4	0.1784	0.0354	1.244
5	0.1218	0.0244	1.247
6	0.1046	0.0207	1.243
7	0.0897	0.0181	1.249
8	0.1092	0.0218	1.246
9	0.1129	0.0223	1.243
10	0.1234	0.0244	1.243
Mean			**1.2449**
Standard deviation			0.00365
Coefficient of variation			0.29

As can be seen in Table 4, the mean density of PLLA is 1.24 g/cm^3. This value is typical for PLA, which is indicated in the literature. This value can be little influenced by air pressure, air bubbles, immersion depth or by porosity of the solid samples as well as deviance of the analytical balance.

8.1.2 Microhardness

Microhardness test was done twice on the same sample. The sample was straight smooth plate and from the first measurement was obtained initial mechanical properties of PLLA. Then the sample was put into chamber with 100% relative humidity at 37°C and the measurement was performed again after two weeks. The progress of loading force and the average penetration depths of both measurements are shown in Figure 43 and in Figure 44.

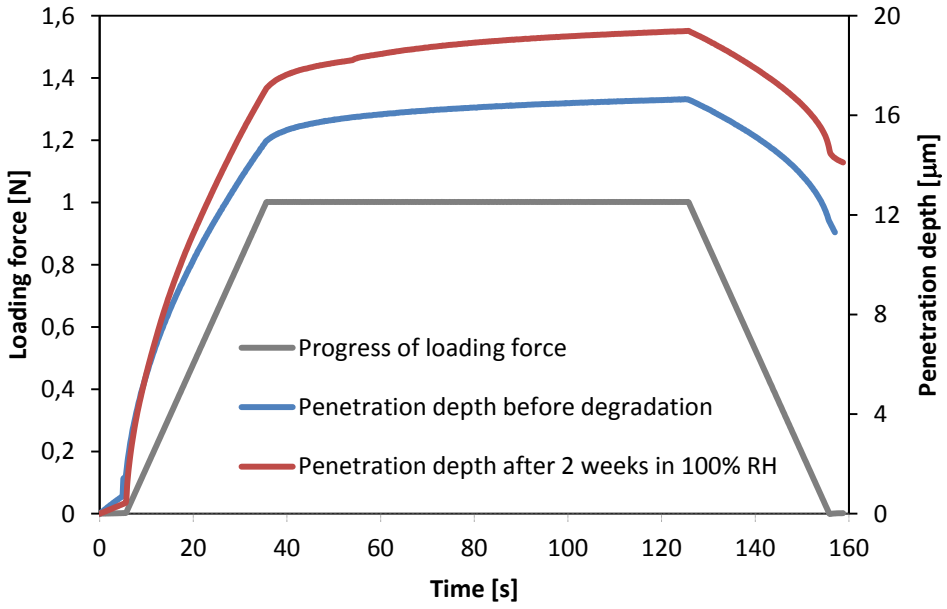


Figure 43 Load vs. penetration depth as a function of time

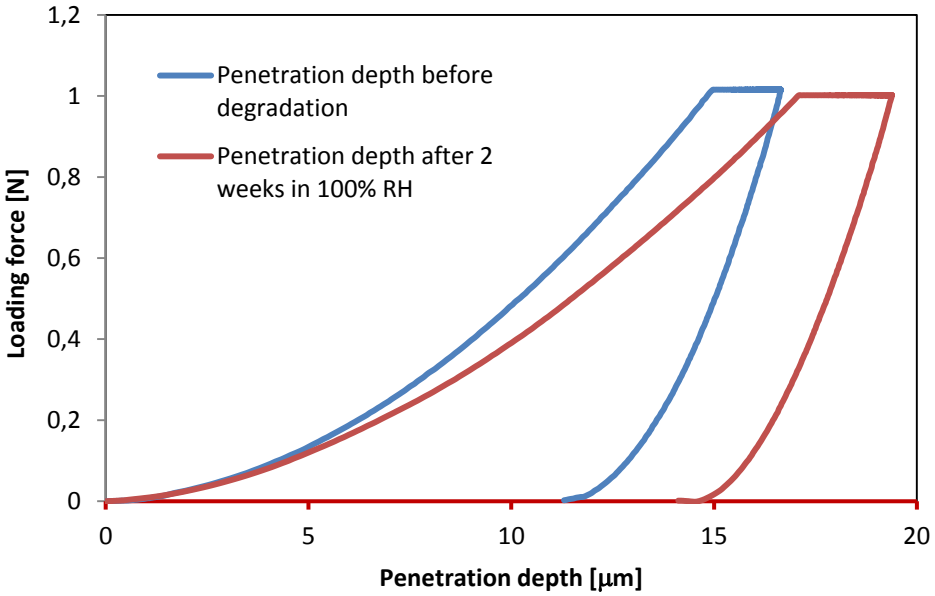


Figure 44 Correlation between the force and the depth of the indentation

Figure 45 shows the micrograph of indentation, in which the two diagonals are measured using a microscope.

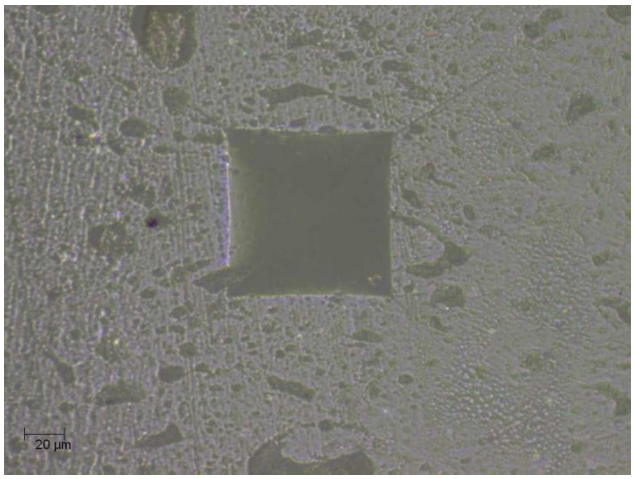


Figure 45 Micrograph of indentation

The obtained values from microhardness testing are shown in Table 5 and Table 6. Indentation hardness (H_{IT}) was calculated by formula (2), indentation modulus (E_{IT}) by (3), Vickers indentation hardness (HV_{IT}) by (4) and indentation creep (C_{IT}) by (5).

Table 5 Initial mechanical properties of PLLA before degradation

Sample	H_{IT}	E_{IT}	E*	C_{IT}	HV_{IT}	F_{max}	h_{max}	A_p
	MPa	GPa	GPa	%	Vickers	N	nm	nm^2
1	202.72	4.11	4.51	10.39	19.13	1.02	16335.81	5.03E+09
2	200.36	4.14	4.54	11.03	18.91	1.02	16371.93	5.08E+09
3	189.28	4.05	4.45	11.15	17.87	1.02	16773.28	5.38E+09
4	191.64	4.06	4.47	11.25	18.09	1.01	16656.29	5.29E+09
5	190.87	4.09	4.50	11.23	18.02	1.02	16710.52	5.34E+09
6	187.23	4.13	4.53	11.01	17.67	1.02	16795.27	5.43E+09
7	194.77	4.15	4.56	11.03	18.38	1.02	16552.63	5.23E+09
8	179.41	3.97	4.36	10.67	16.93	1.01	17090.74	5.63E+09
9	194.22	4.02	4.41	11.00	18.33	1.02	16612.20	5.23E+09
Mean	**192.28**	**4.0785**	**4.4818**	**10.973**	**18.148**	**1.018**	**16655**	**5.29E+09**
StDev	6.95	0.0594	0.0653	0.277	0.656	0.004	229	1.82E+08
CoefVar	3.62	1.46	1.46	2.53	3.62	0.43	1.38	3.43

Table 5 shows the initial values of mechanical properties obtained from microhardness test before their degradation. As can be seen, the indentation hardness and Vickers hardness have quite high values in comparison with other polymers (HV of PP – 7, PC – 14, PVC – 16, PS - 21 Vickers). The elastic modulus was calculated from unloading curve and its mean value 4.1 GPa is high too.

Table 6 Mechanical properties of PLLA after 2 weeks in 100% RH

Sample	H_{IT}	E_{IT}	E*	C_{IT}	HV_{IT}	F_{max}	h_{max}	A_p
	MPa	GPa	GPa	%	Vickers	N	nm	nm^2
1	155.12	3.81	4.18	10.16	14.64	1.00	18110.61	6.45E+09
2	134.79	3.35	3.68	13.36	12.72	1.00	19392.80	7.42E+09
3	160.22	3.30	3.62	10.39	15.12	1.00	18208.97	6.26E+09
4	151.65	3.60	3.95	10.07	14.31	1.00	18402.39	6.60E+09
5	164.10	3.59	3.95	9.68	15.49	1.00	17831.61	6.08E+09
6	113.41	2.86	3.15	10.12	10.71	1.01	21238.30	8.93E+09
7	135.10	3.25	3.57	11.06	12.75	1.00	19467.09	7.41E+09
8	141.31	3.23	3.55	11.12	13.34	1.01	19206.51	7.15E+09
9	112.19	3.20	3.51	9.18	10.59	1.01	21057.96	8.97E+09
Mean	**140.88**	**3.3538**	**3.686**	**10.571**	**13.297**	**1.003**	**19213**	**7.25E+09**
StDev	18.97	0.2778	0.305	1.21	1.791	0.005	1245	1.08E+09
CoefVar	13.47	8.28	8.28	11.44	13.47	0.5	6.48	14.83

Table 6 shows the values of mechanical properties obtained from microhardness test after 2 weeks in chamber with 100% relative humidity (RH) at 37°C. As can be seen, the mechanical properties decrease on average around 17%, only the creep maintains the same values.

8.1.3 Bending test

The results from three-point bending test are shown in Table 7. Flexural strength (R_{max}) was calculated by formula (6) and flexural modulus (E_{Bend}) by formula (7).

Table 7 Experimental data for bend testing of materials

Sample	h	w	$R_{max} = R_{Break}$	$\delta_{Fmax} = \delta_{Break}$	$\delta_{Fmax} = \delta_{Break}$	E_{Bend}
	mm	mm	MPa	%	mm	GPa
1	2.83	5.49	11.27	0.54	0.51	2.98
2	2.72	5.24	8.15	0.81	0.79	2.54
3	3.38	5.61	9.81	0.56	0.44	2.70
4	2.82	5.22	10.53	0.92	0.87	3.63
5	2.60	5.38	7.77	1.06	1.08	2.59
6	3.00	5.38	5.77	0.78	0.70	3.25
7	3.04	5.28	8.18	1.17	1.02	2.56
8	2.71	5.30	6.66	1.61	1.58	2.59
9	3.18	5.29	7.21	1.16	0.97	3.19
Mean	**2.92**	**5.3544**	**8.372**	**0.957**	**0.884**	**2.891**
StDev	0.2511	0.1269	1.824	0.337	0.341	0.39
CoefVar	8.6	2.37	21.79	35.24	38.52	13.5

Results from bending tests demonstrated that PLLA is very brittle at room temperature. Samples were broken without significant deformation and the maximal flexural strength has the same values as the stress at break. The flexural modulus is lower than the modulus obtained from indentation. And as can be seen in Figure 46, there is no evidence of plastic deformation.

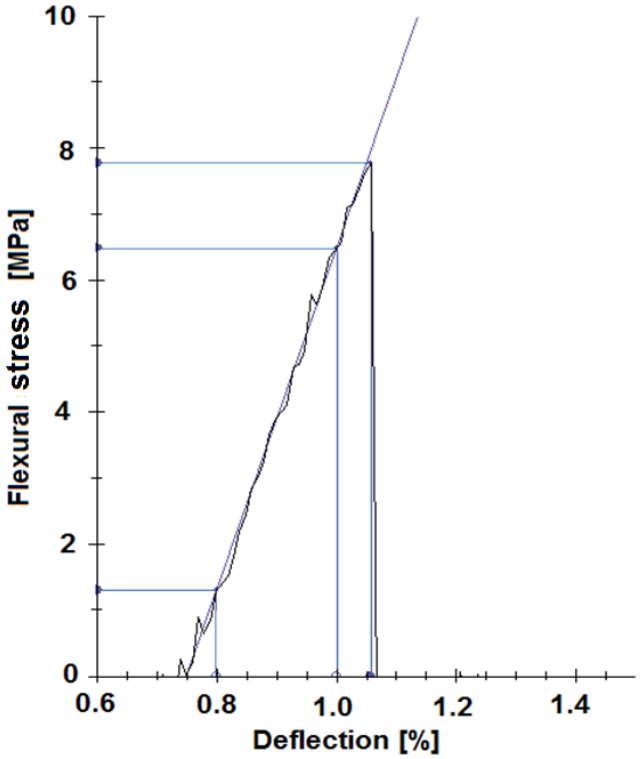


Figure 46 Three-point bend test of PLLA

8.1.4 Melt flow index

The melt flow index (MFI) of PLLA was measured at 125°C, load 100 g and calculated by formula (8). As can be seen in Table 8, the MFI of PLLA is very high. This indicates good flow properties. The high fluidity may be caused by low molecular weight of polymer.

Table 8 Melt flow index measurement of PLLA

PLLA	Time	Weight	ρ_{melt}	MFI
125°C/0.100g	s	g	g/cm^3	g/10min
1	61.8	1.783		
2	60.9	1.762		
3	61.5	1.759	0.9645	106.83
Mean	61.4	1.768		
StDev	0.46	0.0131		

8.2 Degradation process

Degradation process, as was mentioned before, was performed under two different conditions: in a chamber with 100% relative humidity (RH) and in a phosphate buffered solution (PBS) at pH 7.4, both of them at temperature 37°C. Cylindrical samples were exposed for 12 weeks and degradation kinetics was monitored over time.

8.2.1 Mass loss and molecular weight changes

The degradation in abiotic conditions under body temperature and humidity or aqueous medium leads to cleavage molecular chains. This results in rapid molecular weight and when degradation products are able to migrate out to the surrounding environment, there is a decrease in mass loss.

In Table 9 and in Table 10 are shown results from GPC for PLLA during the degradation process. As can be seen, the initial number average molecular weight (M_n) is 20 071 g/mol, while the weight average molecular weight (M_w) is 28 730 g/mol and the molecular weight distribution, called polydispersity index is 1.43 (PDI = M_w/M_n).

Table 9 M_n, M_w and polydispersity of PLLA during degradation in 100% RH at 37°C

100% RH, 37°C	Core			Surface		
	Mn	Mw	PDI	Mn	Mw	PDI
Week	g/mol	g/mol	-	g/mol	g/mol	-
0	20071	28730	1.43	20071	28730	1.43
2	5806	14886	2.56	5367	12277	2.29
4	2458	5808	2.36	3510	6713	1.91
6	1439	2820	1.96	1361	2961	2.17
8	936	1853	1.98	1207	2152	1.78
10	761	1384	1.82	894	1528	1.71
12	884	1435	1.62	679	1207	1.78

Table 10 M_n, M_w of PLLA during degradation in PBS, pH 7.4 at 37°C

PBS (pH 7.4), 37°C	Core			Surface		
	Mn	Mw	PDI	Mn	Mw	PDI
Week	g/mol	g/mol	-	g/mol	g/mol	-
0	20071	28730	1.43	20071	28730	1.43
2	5851	12784	2.18	5243	12516	2.39
4	3771	7620	2.02	2790	6386	2.29
6	1563	3104	1.99	2442	4977	2.04
8	1134	2271	2.00	1996	4548	2.28
10	927	1837	1.98	2024	4477	2.21
12	762	1448	1.90	2325	4645	2.00

The changes in mass retention of the PLLA samples versus decreases weight average molecular mass (M_w) during the in vitro degradation under two different conditions are shown in Figure 47 and Figure 48.

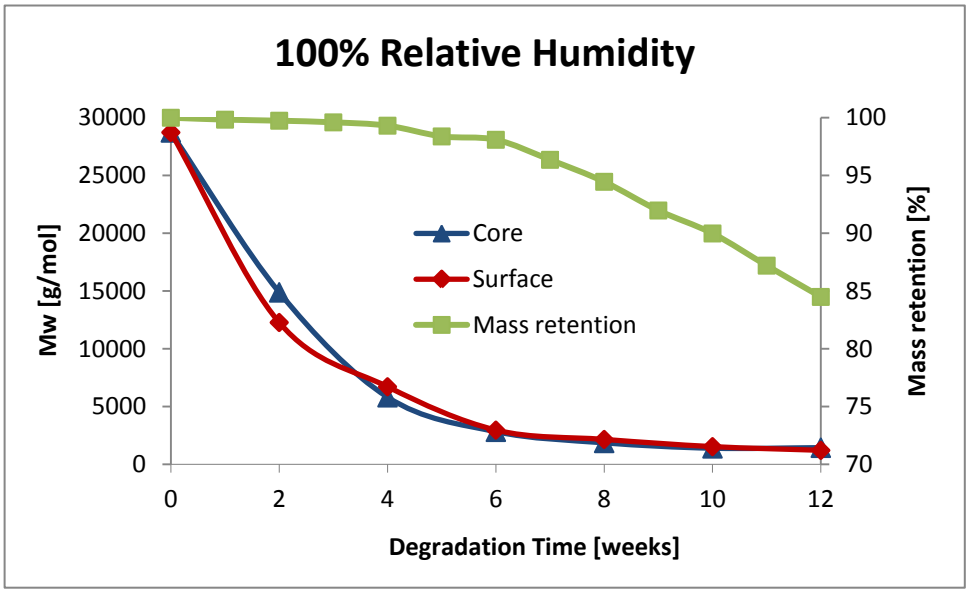


Figure 47 Mw and mass retention of PLLA in 100% RH during degradation

Figure 47 shows that the changes in weight of the samples in 100% RH started after 6 weeks, when the degradation products have circa M_w 5000 g/mol. In the 12th week, there is about 15% mass loss. The decrease of M_w is homogenously in the whole sample, only in the 1st week, the M_w decreases is a quite faster on the surface of samples.

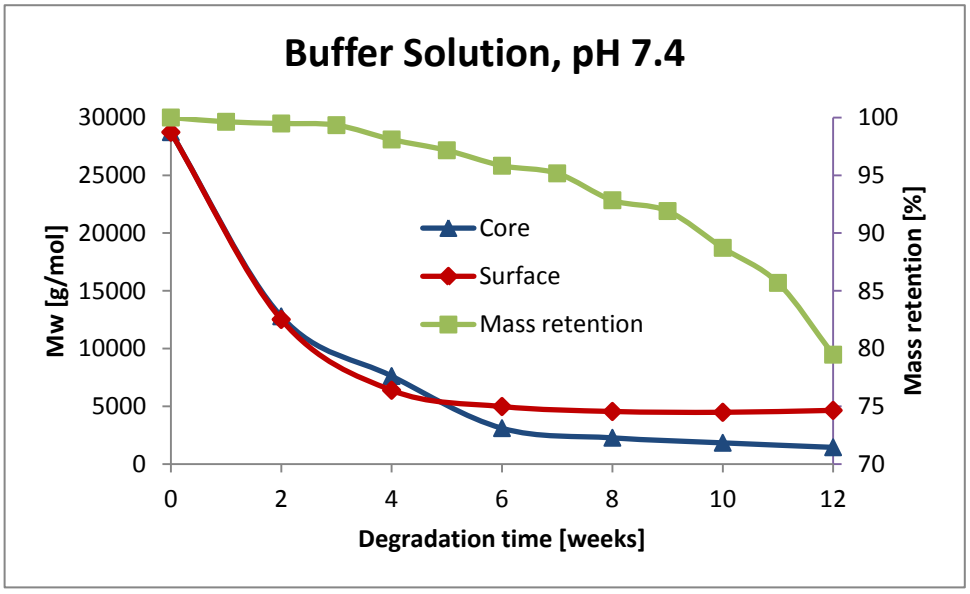


Figure 48 Mw and mass retention of PLLA in PBS during degradation

Figure 48 shows the changes in weight of the samples in PBS, which started after 5 weeks, when degradation products have circa M_w 7500 g/mol. In the 12th week, there is about 20% mass loss. The decrease of Mw is homogenous in the whole sample into 5th week, and then the decrease of Mw is slower at the surface. This effect may be caused by easy migration of degradation products from the surface of sample in aqueous medium. So there is no autocatalytic effect on the surface, which accelerates the hydrolysis process.

In the Table 11, there are initial values of PLLA in comparison with commercially available PLA produced from NaturesWorks. As can be seen the Mw of commercial PLA are four to five times higher than PLLA. This means that commercial PLA will have better physical properties. The commercial PLA have slightly higher values of polydispersity.

Table 11 Comparison of M_n, M_w and PDI of PLLA and commercial PLA

Sample	M_n	M_w	PDI
	g/mol	g/mol	-
PLLA (Laboratory CPM)	**20071**	**28730**	**1.43**
PLA 2002D (NatureWorks)	92884	144755	1.56
PLA 4042D (NatureWorks)	79278	122402	1.54
PLA 4060D (NatureWorks)	71515	119584	1.67

In Figure 49 and Figure 50 are shown chromatograms of PLLA and commercial PLA, where is notable molecular weight distribution and retention time of polymeric chains.

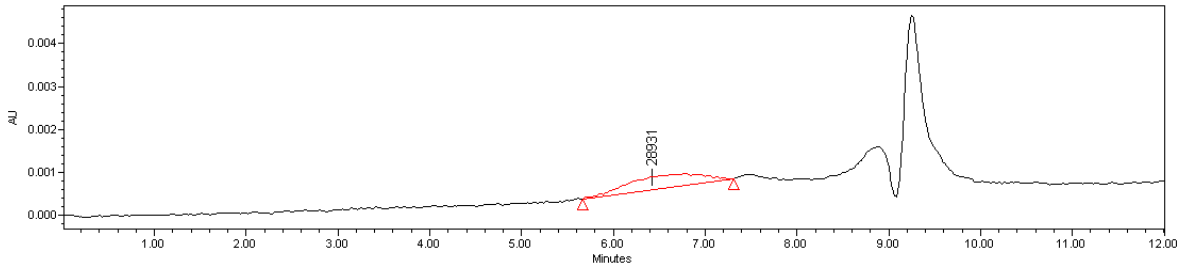


Figure 49 Molecular weight distribution of PLLA before degradation

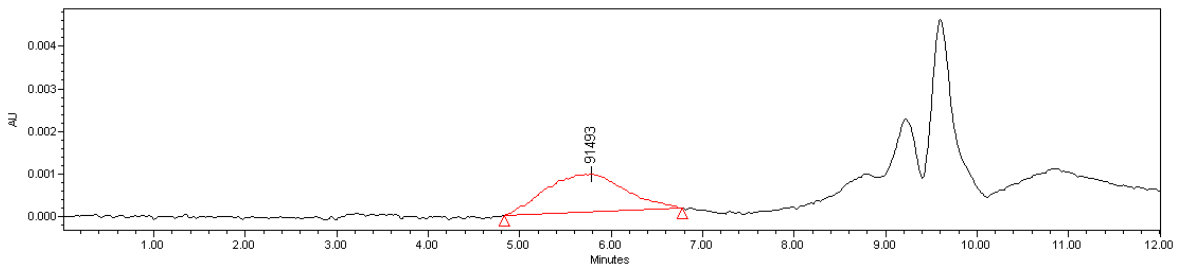


Figure 50 Molecular weight distribution of PLA 4042D (NatureWork)

8.2.2 Thermal behavior changes

The obtained results from DSC are summarized in Table 12 table and Table 13. DSC curves from 1st heating scans are shown in Figure 51 and Figure 52.

Table 12 DSC results for PLLA during degradation in 100% RH

100% Relative humidity at 37°C										
Week	1st Heating Scan									
	Tg [°C]	cp [J/g.K]	Tc [°C]	dHc [J/g]	Tm_0 [°C]	dHm_0 [J/g]	Tm_1 [°C]	Tm_2 [°C]	dHm [J/g]	Xc [%]
0	53.4	0.48	113.6	0.27				134.1	-0.84	0.61
2	47	0.434	98	10.84				132.2	-25.12	15.24
4							110.9	128.9	-30.07	32.09
6							108.4	123.4	-38.80	41.41
8							106.0	120.1	-41.60	44.40
10							107.1	119.8	-41.33	44.11
12							106.3	118.7	-45.14	48.18
Week	Cooling		2nd Heating scan							
	Tg [°C]	cp [J/g.K]	Tg [°C]	cp [J/g.K]	Tc [°C]	dHc [J/g]	Tm_1 [°C]	Tm_2 [°C]	dHm [J/g]	Xc [%]
0	44.2	-0.651	45.8	0.483				130.8	-0.27	0.29
2	35.1	-0.600	36.4	0.463	106.6	0.61		132.1	-0.7	0.10
4	29.3	-0.638	30.3	0.542	103.7	0.68	122.3	128.6	-1.37	0.74
6	18.7	-0.599	21.3	0.412	93.3	5.49	112.0	119.9	-4.97	-0.55
8	22.4	-0.489	21.2	0.496	93.2	4.36		119.1	-4.90	0.58
10	18.6	-0.490	20.2	0.350	92.2	5.85	113.5	117.5	-7.09	1.32
12	16.3	-0.520	18.6	0.430						

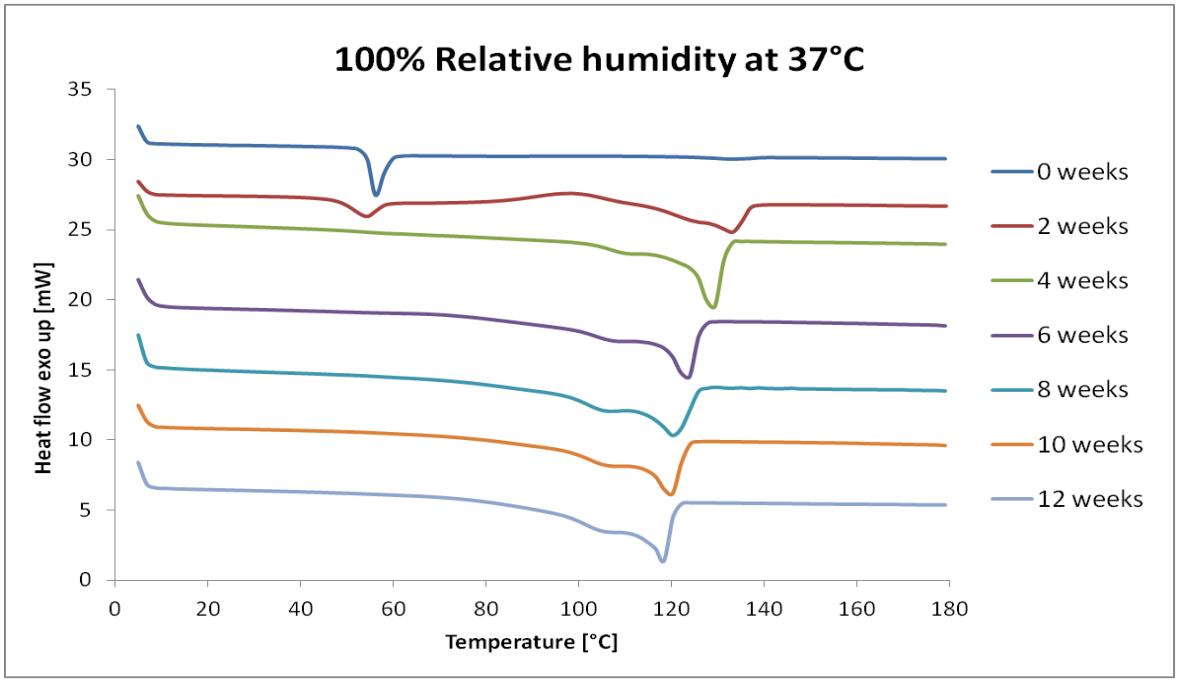


Figure 51 DSC curves (1st scans) of PLLA samples during degradation in 100% RH

The obtained curves from DSC demonstrated the differences in heat flow between the samples and reference. The absorbed or released heat during transition or phase changes of PLLA samples are then monitored in dependence on temperature.

Table 13 DSC results for PLLA during degradation in PBS

Phosphate buffer solution, ph 7.4 at 37°C										
Week	1st Heating Scan									
	Tg [°C]	cp [J/g.K]	Tc [°C]	dHc [J/g]	Tm_0 [°C]	dHm_0 [J/g]	Tm_1 [°C]	Tm_2 [°C]	dHm [J/g]	Xc [%]
0	53.4	0.480	130.4	0.27				134.1	-0.84	0.61
2	44.0	0.525	87.8	20.81			121.0	133.0	-25.19	4.67
4	46.5	0.198	80.0	1.39	56.3	-0.98	116.0	131.9	-28.38	29.85
6					58.5	-1.03	119.5	131.7	-43.90	47.95
8					56.3	-0.86	115.8	128.3	-40.69	44.34
10					59.6	-1.68	121.6	131.7	-42.20	46.83
12					60.2	-1.29	122.5	130.9	-52.1	56.98
Week	Cooling		2nd Heating scan							
	Tg [°C]	cp [J/g.K]	Tg [°C]	cp [J/g.K]	Tc [°C]	dHc [J/g]	Tm_1 [°C]	Tm_2 [°C]	dHm [J/g]	Xc [%]
0	44.2	-0.651	45.8	0.483				130.8	-0.27	0.29
2	37.1	-0.565	38.4	0.484	111.3	0.97	127.4	132.9	-1.70	0.78
4	36.7	-0.652	36.8	0.460	109.3	0.42	125.9	131.6	-0.59	0.18
6	39.7	-0.606	40.4	0.479	107.4	0.22	125.6	131.1	-0.59	0.39
8	35.1	-0.578	35.4	0.517	107.7	0.48	123.1	126.8	-0.88	0.43
10	43.6	-0.607	42.7	0.496	88.4	8.47	116.1		-9.63	1.24
12	37.7	-0.646	40.0	0.598						

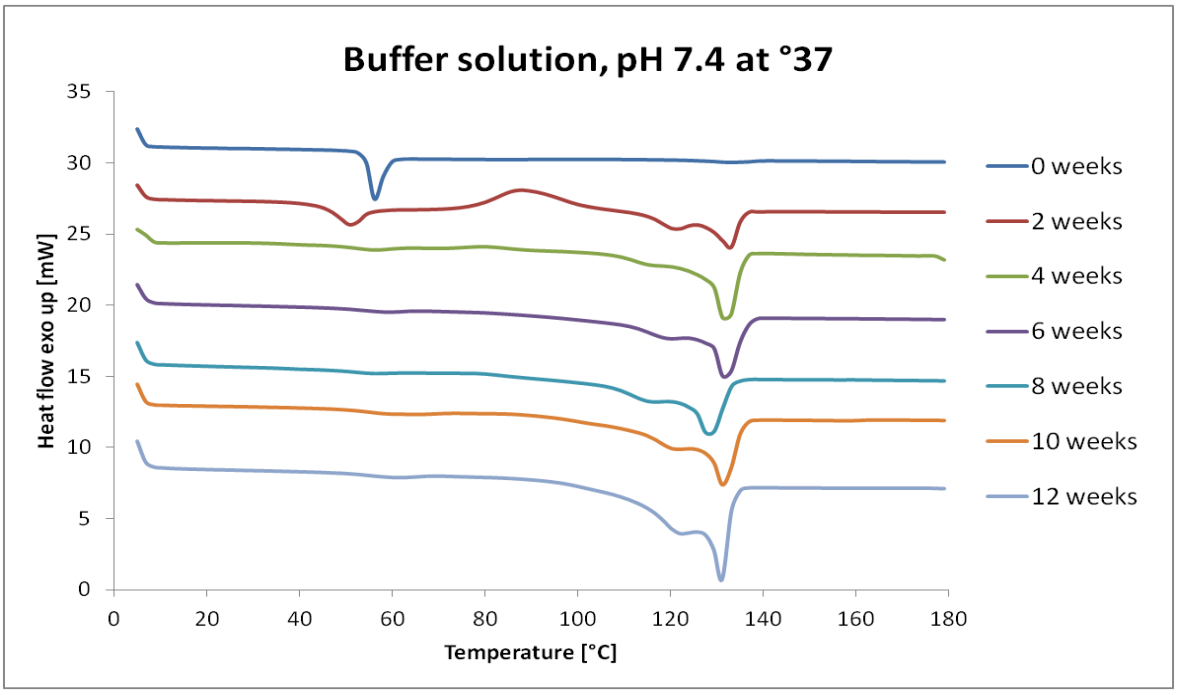


Figure 52 DSC curves (1st scans) of PLLA samples during degradation in PBS

The percentage of crystallinity (χ_c) was calculated by comparing the melting enthalpy of the partially crystalline PLLA sample with the theoretical melting enthalpy of fully crystalline PLA according to formula:

$$\chi_c = \frac{(\Delta H_{m0} + \Delta H_m) - \Delta H_c}{\Delta H_m^*} \cdot 100\ [\%] \qquad (10)$$

$_{\Delta}H_m$ – melting enthalpy (J/g)

$_{\Delta}H_c$ - crystallization enthalpy (J/g)

$_{\Delta}H_m$* - enthalpy of 100% crystalline PLA (93.7 J/g)

Differences between results from 1st and 2nd scan are caused due to the fact that the 1st scan eliminated thermal history of samples.

As can be seen in Figure 53, the glass transition (T_g) is observed from the 2nd heating scan, because in the 1st scan it was not possible to capture T_g from the 4th week of degradation. The initial T_g of PLLA is 46°C and during the degradation in 100% RH, there is a rapid fall up to 6th week, then the decline is moderate. On the other hand T_g in PBS decreased rapidly in first 2 weeks and then the values alternate up and down.

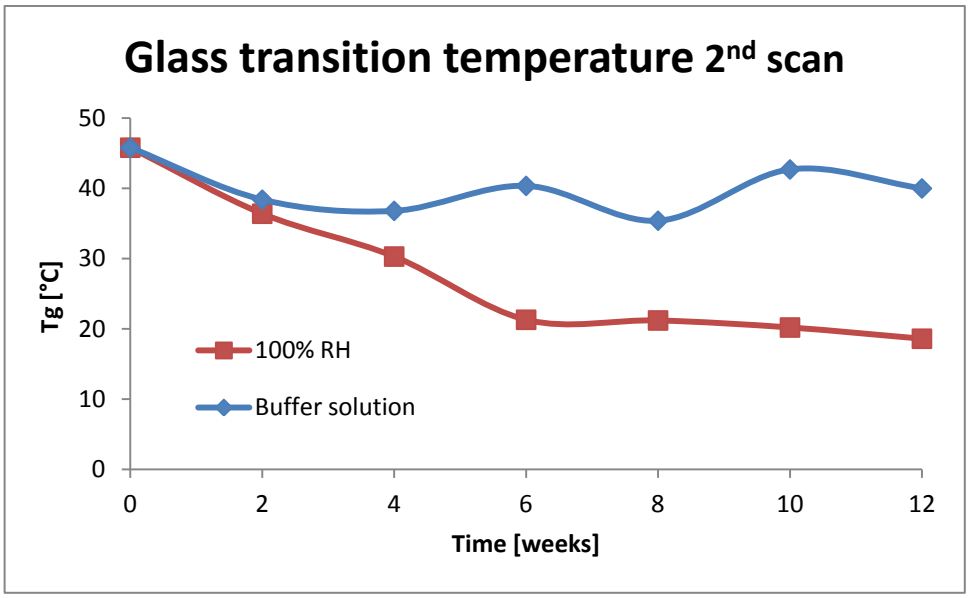


Figure 53 Changes of glass transition temperature

The Figure 54 shows the changes of melting point (T_{m2}), which was observed from the 1st heating scan. In both degradation conditions, the two endothermic peeks with some small differences are visible at range of 110 – 140°C. These peeks represent the melting temperature of PLLA. The T_m in 100% RH declines almost linearly, while in PBS the declination is notable up to 6th week and then the values jump up and down.

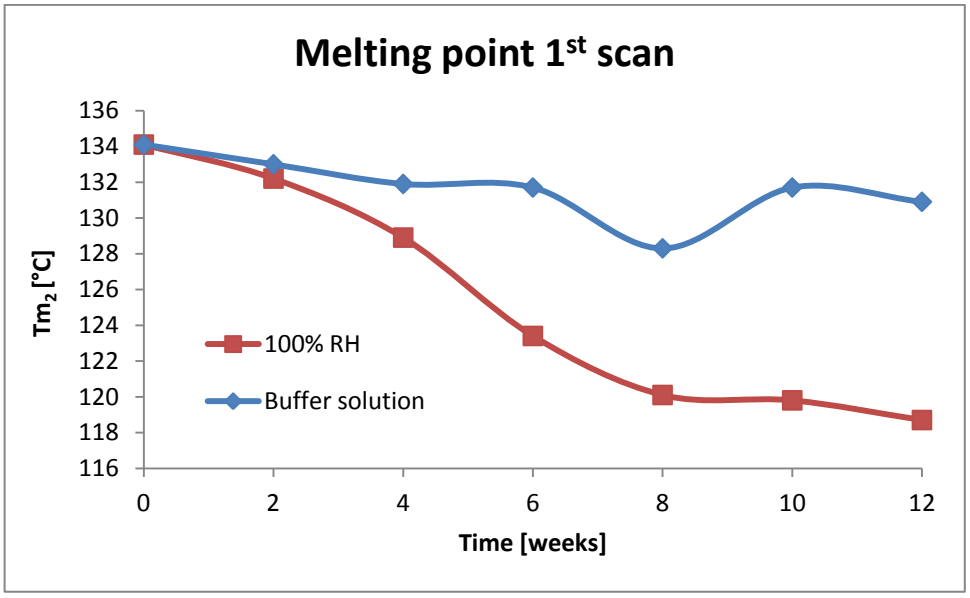


Figure 54 Changes of melting point

As can be seen in Figure 55, the PLLA samples before degradation were amorphous with only 0.61% of crystalline phase. This value was detected from the 1st heating scan and it is confirmed by the fact that there is not endothermic peak, which could represent the melting point. But during degradation, the crystalline phase grows and in 100% RH reaches a value of 48% while in PBS the crystalline phase achieves the value of 60%.

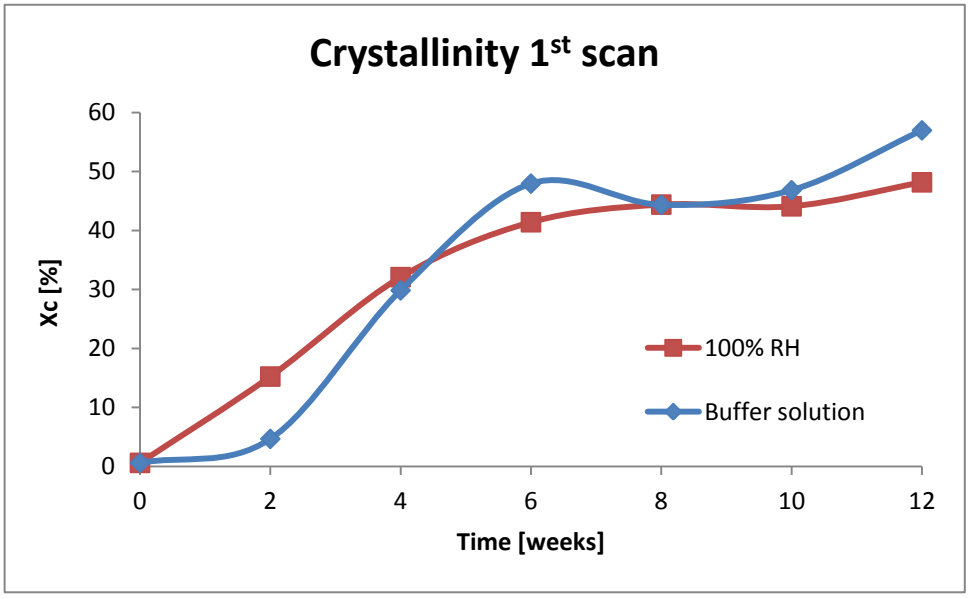


Figure 55 Changes in the percentage of crystallinity

Finally, it should also be noted that in the 1st scan after 2 weeks there are exothermal peaks in temperature range of 88 – 100°C in both condition. These peaks represent the additional crystallization, also called cold crystallization.

8.2.3 Structural changes

Initially, all PLLA cylindrical samples were transparent with a pale yellow color. The Figure 56 shows the micrographs of surface and cross-section as well as image of inner morphology of PLLA before degradation. The surface and cross-section was taken by light-optical microscopy, while the inner structure was observed by scanning electron microscopy (SEM). As can be seen, the surface of prepared samples is not smooth, because there are some pores. These pores were probably formed during processing technology, when the melt PLLA was casted into a small vial and silicone tube was inserted.

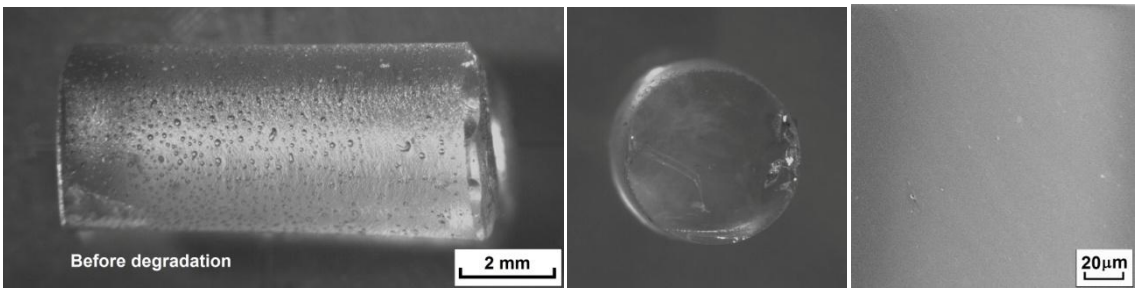


Figure 56 Micrographs and SEM image of PLLA sample before degradation

During in vitro degradation, the structural changes are different in both degradation conditions, as can be seen in Figure 57. The initial transparent sample after 2 weeks in 100% RH begins to be fractionated on clear parts and partially crystalline. However, the SEM indicates the amorphous phase with some defects in the inner structure of sample. After 6 weeks, the sample seems to be more affected by the crystalline phase in whole cross-section. The inner structure becomes slightly rough and after 12 weeks, the sample is homogenously crystalline and inner structure is greatly rough. This means that the degradation of the samples took place homogenously in the bulk sample. On the other hand, degradation in PBS, core cortex structure shows on different degradation mechanism through cross-section. As can be seen, after 2 weeks, there is crystalline cortex, while the core of the sample is amorphous. This also confirm SEM image that shows amorphous inner structure and roughed surface. After 6 weeks, the core of the sample becomes crystalline, but the cortex remained there. And after 12 weeks, the micrographs show that the core of sample is separated by cortex and there is also a kind of gap. The SEM image shows pores in the inner structure of the core. This means that degradation rate there is faster than in the cortex and water soluble fragments are created firstly inside the sample. This effect may be caused by easy migration of degradation products from the surface of sample in aqueous medium, so there is no autocatalytic effect, which accelerates the hydrolysis process.

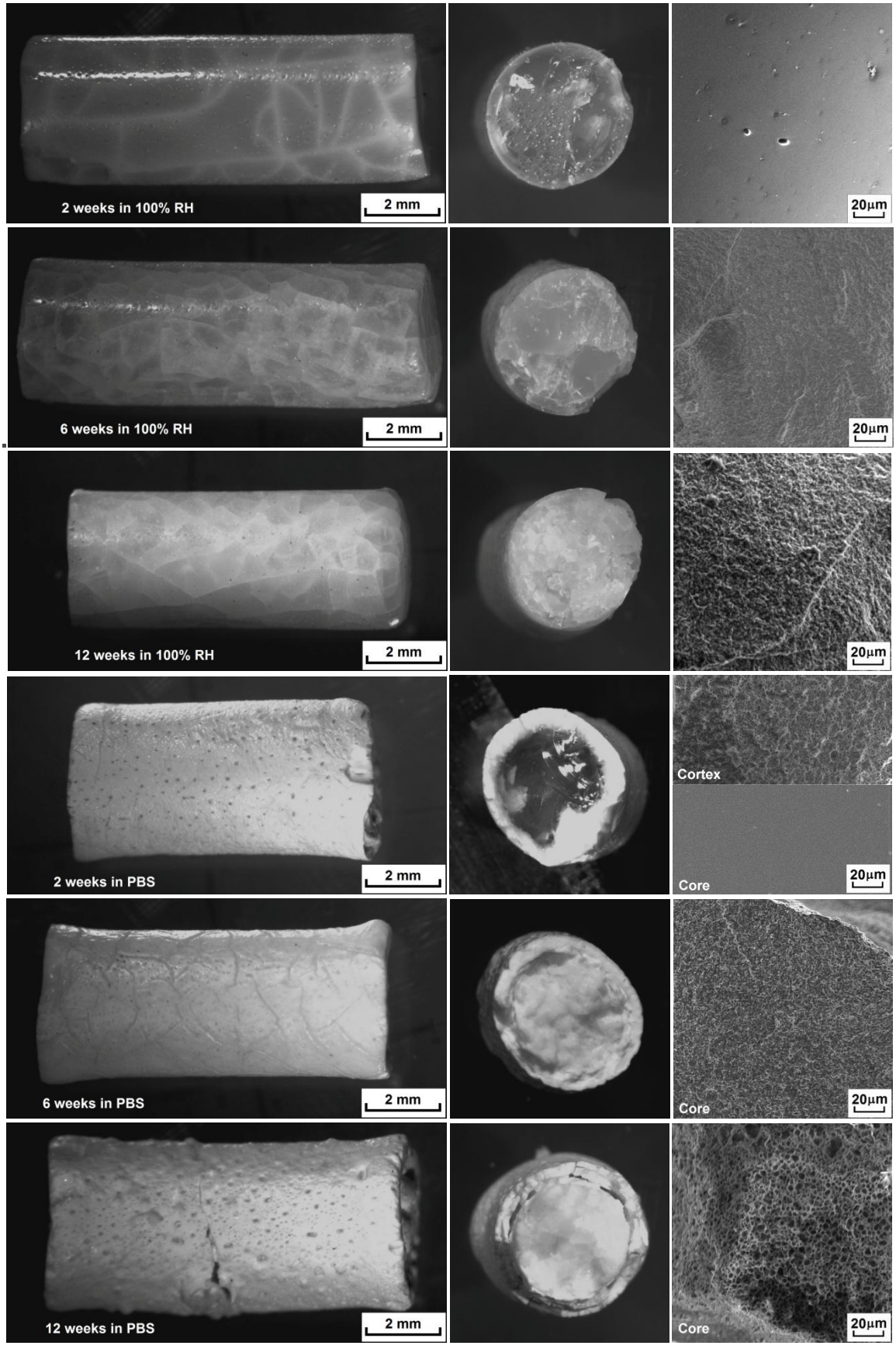


Figure 57 Micrographs and SEM image of PLLA sample during degradation

III. PRACTICAL PART

9 DESIGN OF THE EAR VENTILATION TUBES

This part is focused on the design and construction of biodegradable tubes based on PLA, which will be used for the future in vitro testing. The tubes must be designed with respect to their function, degradation and also production. The selection of the suitable technology for the production of ear tubes is based on the required quality and quantity. In our case, it is a small series production and the transfer molding technology seemed to be the most suitable.

Figure 58 demonstrates two types of proposed biodegradable tympanostomy tubes modeled in 3D CAD software Catia V5R18. The shapes of the tubes were consulted with an otologist and their dimensions were designed on the basis of commercially available ear tubes. As can be seen in Figure 59, the size of the tubes is very small, so the manufacturing of the tool for their production will be a little complicated.

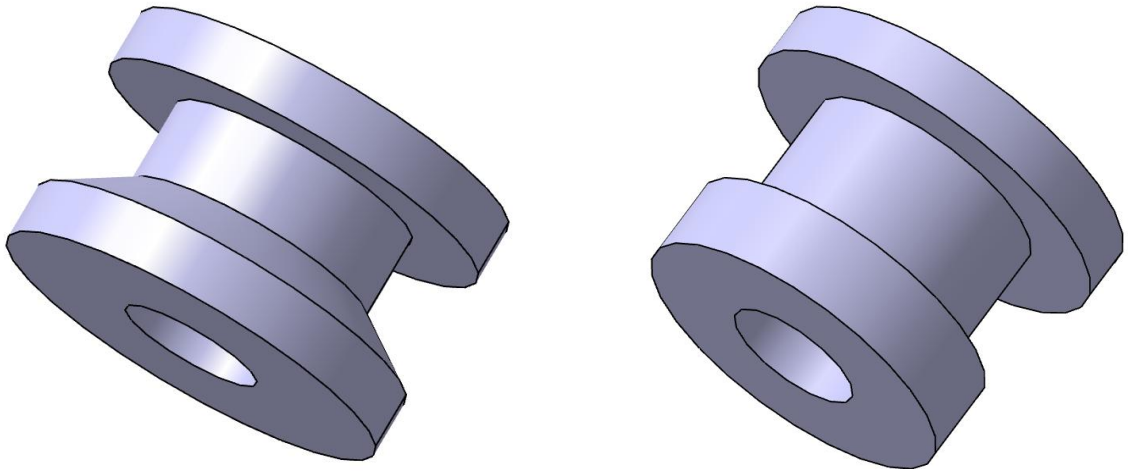


Figure 58 Two types of proposed tympanostomy tubes based on PLA

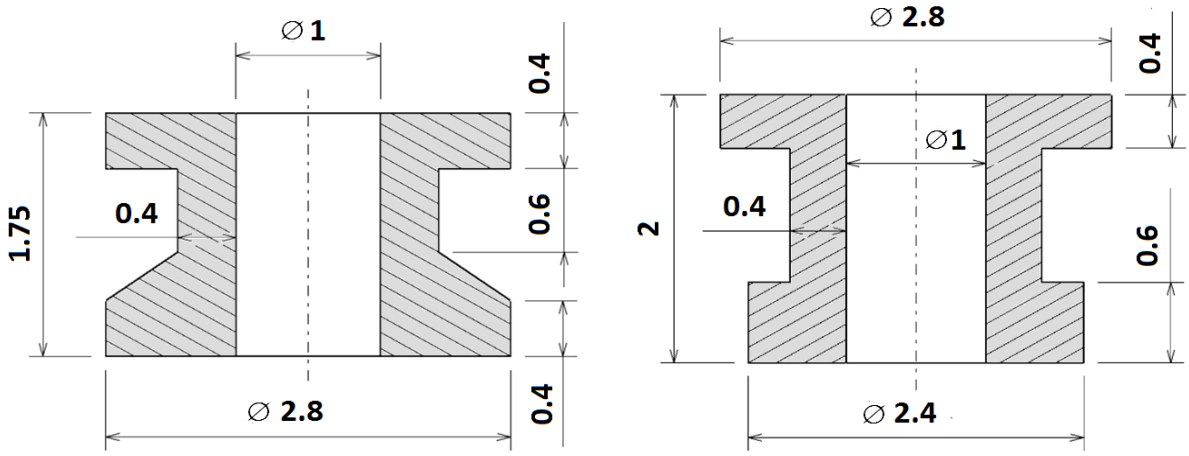


Figure 59 Dimensions of the proposed tubes

10 DESIGN OF THE MOLD FOR PRODUCTION OF EAR TUBES

Figure 60 shows the construction of complete transfer mold for production of proposed tubes. The mold was designed in 3D CAD software Catia V5R18 and consists of 4 basic parts: the piston, transfer pot, top half of the mold and bottom half of the mold. The transfer mold will be heated from upper part by hot plate placed in the press and cooling will be ensured by external cooling device. In the bottom part of the mold are shown tempering tubes. The high of the mold is 63 mm and maximum diameter is 60 mm.

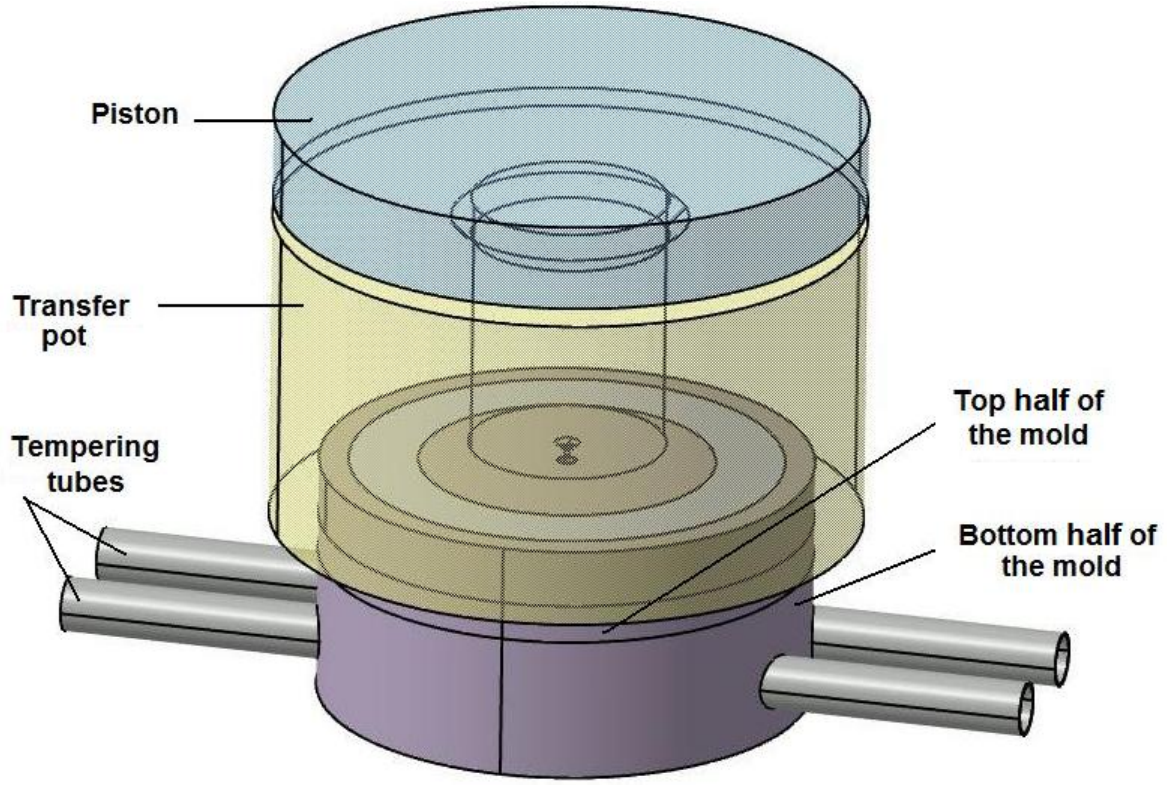


Figure 60 Construction of complete transfer mold for production of ear tubes

Figure 61 demonstrates the schematic cross-section of designed transfer mold. And as can be seen, the top and the bottom half of the mold is then constructed from other components. The top half of the mold is composed of the top ring, upper cavity plate and sprue while the bottom part consists of bottom ring, lower cavity plate and two tempering tubes. All these components in both halfs are casted by epoxy resin with aluminium powder. The top and the bottom half of the mold was not constructed as a whole part, because the cavity plates will be manufactured using a special technology explained in following chapter. The surface area of the pot is larger than the total projected area of polymer between top and bottom plates, so the top cavity plate will not tend to float up and material and do not allow to polymer to leak out between open parting line.

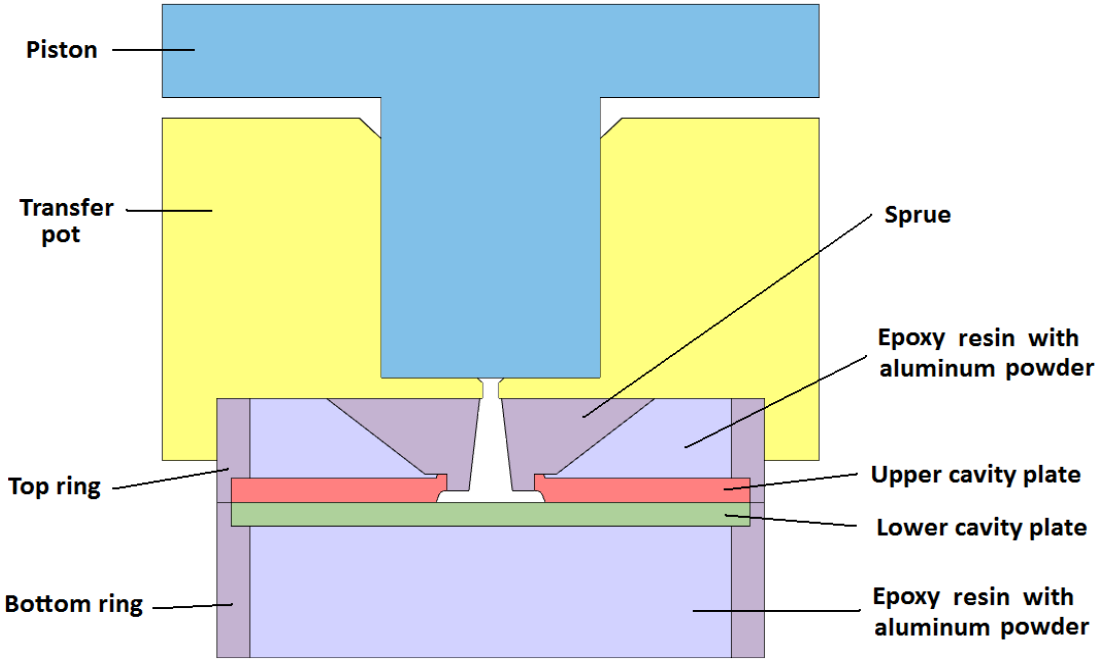


Figure 61 Schematic cross-section of designed transfer mold

The transfer mold allows manufacturing of four tubes at once (two and two of each types), due to multiple cavity. As can be seen in Figure 62 and Figure 63, the multiple cavity also includes two cores, which serve to make the inner lumens into tubes. The holes under cores serve to take the cores with produced tubes out of mold. Tubes are shown there for illustration. The top and bottom cavity plate are protected against rotation by centering elements.

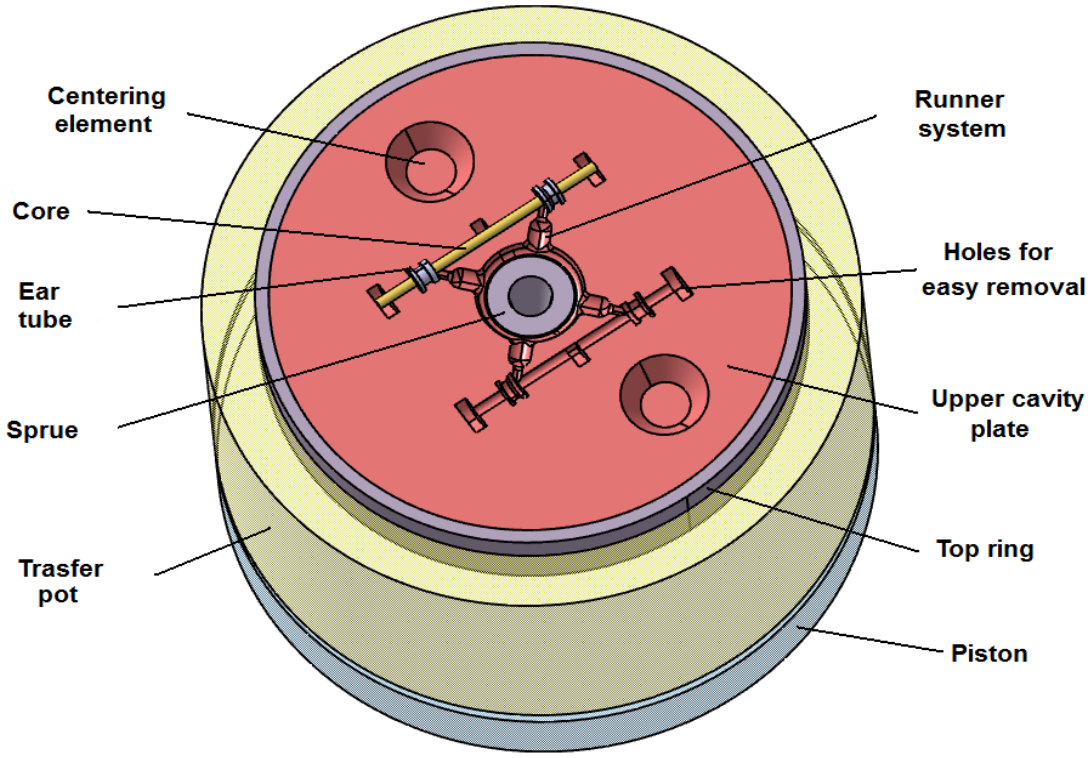


Figure 62 Detailed view into the upper cavity plate

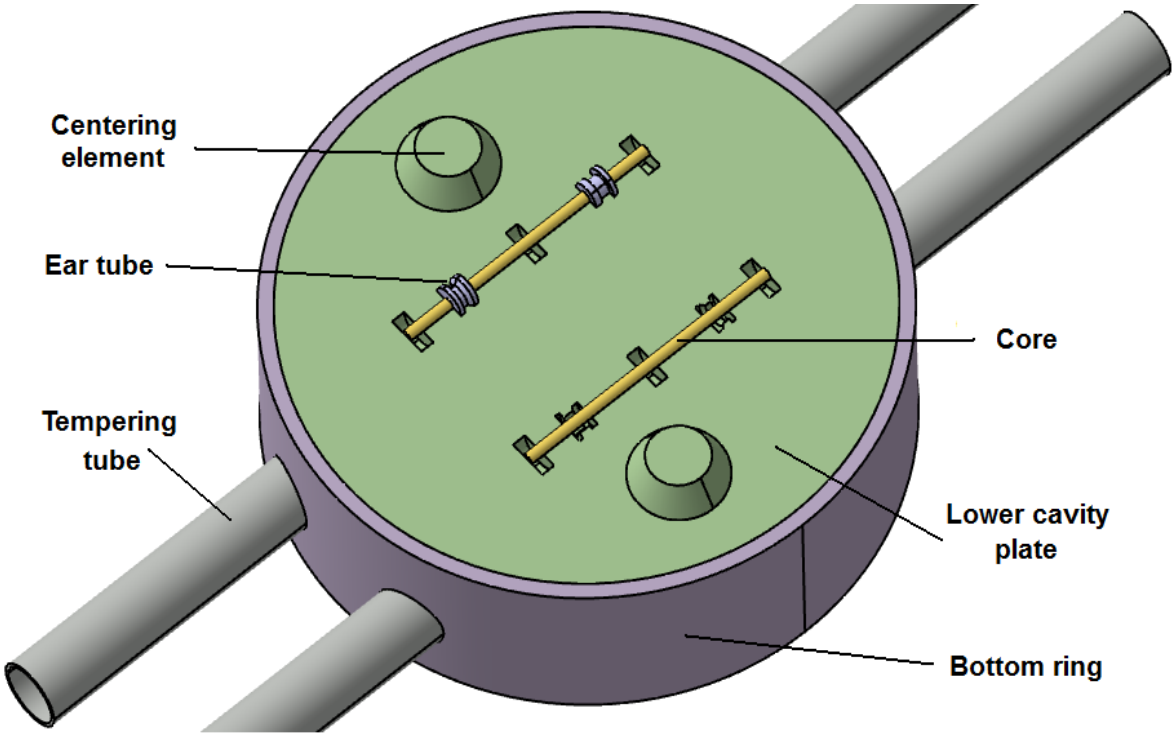


Figure 63 Detailed view into the lower cavity plate

The principle of the production of tubes is very easy. First of all, it is necessary to preheat the press, transfer pot and piston at processing temperature. The heating will be assured by plates placed in the press. Both, the top and the bottom half of the mold with two cores must be preheated too, but the temperature would be lower than the process temperature. When the parts reach the desired temperature, the proper amount of polymer is fed in the transfer pot and the complete transfer mold is inserted into the preheated press. Under the mold is also placed isolating plate, which avoids the heat conduction from the lower part of press. The polymer is heated in the pot at processing temperature and then the molten polymer is pushed by the piston through the runners and gates into the cavities. The rapid cooling is ensured by the water circling in tempering tubes, which are connected to the cooling device. The runner system is solved using circular and semi-circular canals, which are designed as short as possible. The gates were designed on the available space in the thicker part of the tubes and their size is chosen on the basis of the dimensions of ear tubes. Escaping of the air from the cavity is expected though parting line. The easy opening of the mold is provided by the construction gab between piston and the transfer pot. The manufactured tubes together with runner system and gates will be taken out of the mold by hand using simple tools. At the end of cycle the mold must be cleaned. It is important to mention, that polymers based on PLA is necessary to dry before processing.

11 MANUFACTURING OF THE TRANSFER MOLD

The components of designed transfer mold are made of metals as Dural, varies steels and nickel. Most of the parts of this tool were machined by classical technology as on a lathe or by milling machine and by grinding machine. The exception was the upper and lower cavity plates, which was produced using an unconventional method.

11.1 Production of cavity plates

Due to the size of the ear tubes, it is not possible to manufacture the mold cavities by conventional methods. The mold cavity, in our case the whole cavity plates will be done by electroforming (electroplating). This technology can be used for production of metal molds or its parts.

11.1.1 Principe of electroforming

Electroforming is used to create exact metal replicas of various shapes and textures using the electrolysis. As can be seen in Figure 65, the principle is in electrodeposition of nickel or other metal onto a conductive model also called mandrel in electrolytic bath. Due to a direct current between anode and cathode, the metal at the anode is oxidized and forms metal ions with positive charge, which are attached to the negative cathode. This causes a deposition of the metal layer on the model surface and amount of deposited material is governed by Faraday's laws. After the electroforming process, the plated metal is separated from the model to obtain the exact replica of the underlying surface. [65]

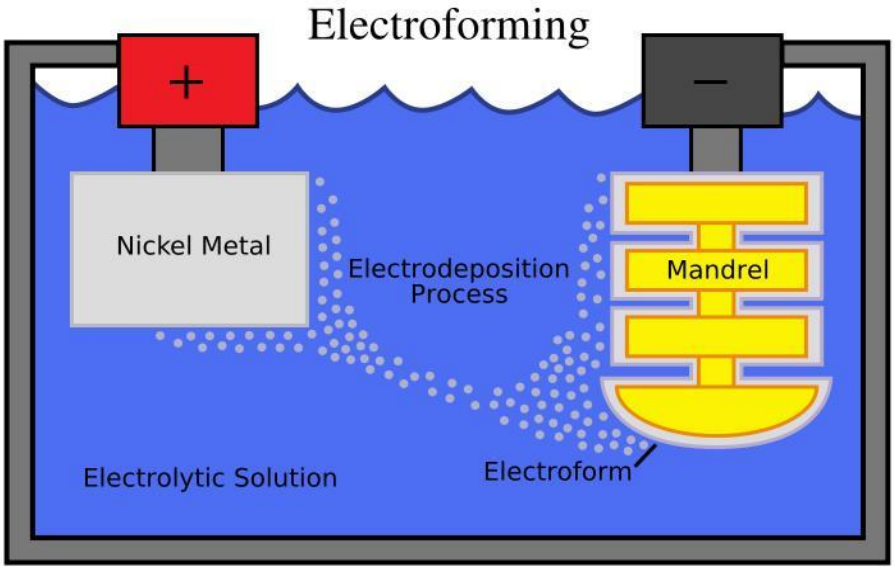


Figure 64 Schematic principle of electroforming [66]

In order to take advantage of electroforming, it is necessary to create firstly the negative models of the mold cavity plates. These models will be served to electrochemical deposition of metal for creation the proper cavity plates of the mold. The most suitable technology for a quickly fabrication of models is a rapid prototyping, which allow the production of the real object from 3D CAD data.

11.1.2 The 3D models of negative cavity plates

Figure 65 demonstrates the 3D modes of negative cavity plates with a platform for clamping designed in Catia V518. The diameter of platforms is 47 mm and their high is12 mm. There are also two blind holes on the bottom part of each platform, which are not visible. These holes will serve to insertion of clamping device during electroforming. The 3D data were converted into *.stl file supported by rapid prototyping and they are given also in appendices.

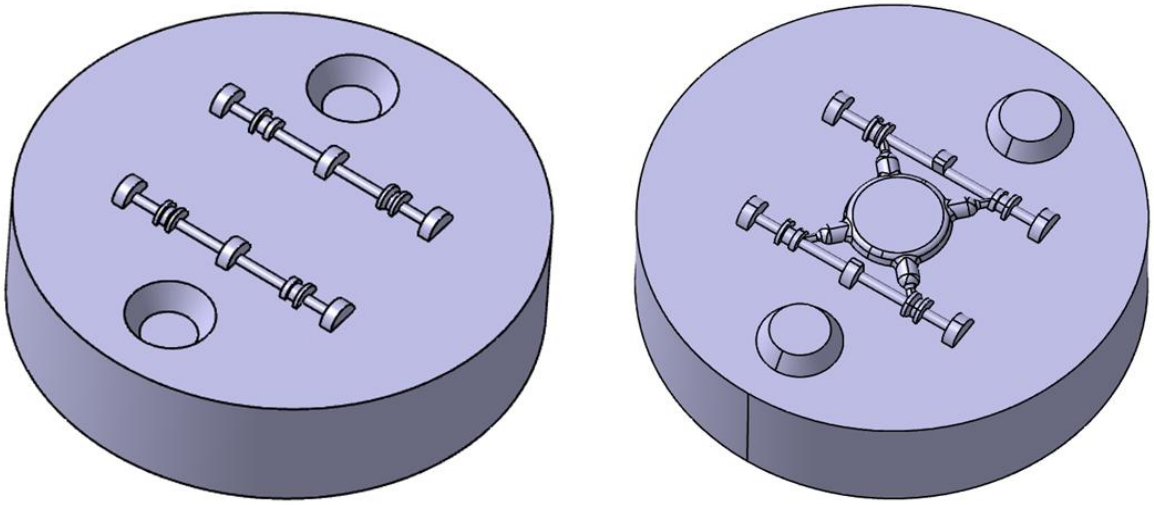


Figure 65 Models representing the negatives of cavity plates in 3D

11.1.3 Printing of real negative models using rapid prototyping

For the production of real models was used the 3D printer from Objet (model Eden250), which use the PolyJet method. As can be seen in Figure 66, the PolyJet method is basis on the jetting photo-sensitive polymer also called photopolymer. The photopolymer is applied from the print head with 96 jets and creates the ultra thin layer onto the build tray. Immediately after the building of a layer, the photopolymer is cured by UV lights. The thickness of the layer is 0.016 mm so the final model has a smooth surface with accuracy ranging from 0.05 to 0.1 mm. The support material is based on the gel and it is easy to remove it from the part.

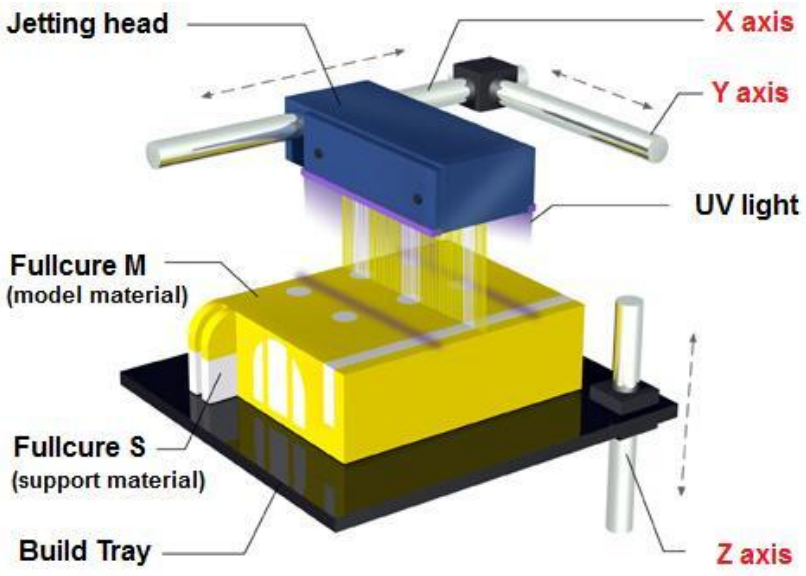


Figure 66 PolyJet technology [67]

Figure 67 shows the real models created by 3D printing (PolyJet technology). The photopolymer used for their production has the trade name VeroWhite. Production of both models took about 2 hours.

Figure 67 Real models created by PolyJet technology

The basic physical properties of model material together with technical specifications of 3D printer Eden 250 (Objet) are shown in Table 14.

Table 14 Physical properties of material and technical specifications of 3D printer

Model material VeroWhite			**3D printer Eden 250 (Objet)**		
Tensile strength	MPa	50	**Machine dimensions (w, d, h)**	mm	870x735x1200
Modulus of Elasticity	GPa	2.5	**Tray size (x, y, z)**	mm	250x250x200
Shore	D	83	**Thickness of layer**	mm	0.016 or 0.32
T_g	°C	58	**Input format**	file	STL/SLC

11.1.4 Final fabrication of cavity plates

The fabrication of the cavity plates was carried out in collaboration with company Kapa Zlín s.r.o., which is engaged in the production of galvanoplatic (electroplating) molds. We arranged the proper negative models and they manufactured the cavity plates from nickel. Figure 68 shows electrolytic baths for electroforming in company Kapa Zlín s.r.o.

Figure 68 Department for manufacturing galvanic molds from nickel

The negative models are made of photopolymer, which is not a conductive material. This means that, the models must be firstly degreased and then covered with a conductive coating. In our case on the surface of model was applied the argent powder while the lateral sides of platforms were shaded by tube from PVC. Such prepared models were put for one week into electrolytic bath with nickel and as can be seen in Figure 69, the results were the cavity plates with thickness about 2 mm.

Figure 69 Nickel cavity plates produced by electroforming

11.2 Production and assembly of other components

As was mention before, the other components were produced by conventional technology as the machining, milling, cutting and grinding. The drawing documentation is given in appendices.

11.2.1 Supporting rings

The supporting rings were done from steel tube (11 373), which has the outside diameter of 50 mm. The high of top ring is 10 mm, while the high of bottom ring is 15 mm. There are differences because in the bottom part will be passed tempering tubes. One edge of each ring is also graduated, which ensure the placement of the cavity plates. Figure 70 shows the front and back side of supporting rings with cavity plates and tempering tubes. The tempering tubes are simple stainless steel tubes with inner diameter of 5 mm.

Figure 70 Upper and bottom supporting rings with cavity plates and tempering tubes

11.2.2 Sprue

The sprue is the component from Dural through which the melted polymer will be introduced into a mold. The inside diameter is 2 mm at the upper side while in bottom side spreads to 4 mm in diameter, which allow an easy removal of the solidified material from the mold. The sprue has cone shape to ensure greater stability and on the bottom side is created a cylindrical graduation for easy insertion into the upper cavity plate. Figure 71 shows the machining of the sprue on the lathe.

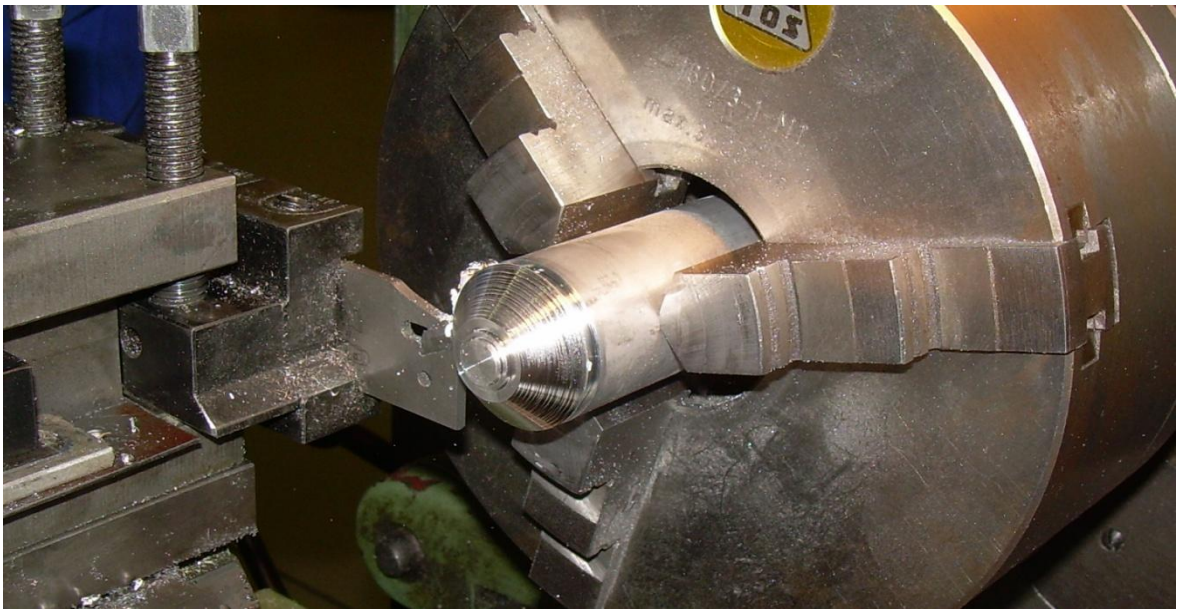


Figure 71 Machining of the sprue on the lathe

As can be seen in Figure 72, the gap between sprue and ring as well as the space between tempering tubes and bottom ring is filled up with epoxy resin and aluminum powder.

Figure 72 Sprue and supporting ring filled by epoxy resin with aluminum powder

Figure 73 shows the front and back side of the transfer pot. The pot was made from steel cylinder (12 050) and on the lateral side is the small hole, which serve to insertion of thermostat to control the inside temperature. On the back side is the graduation, which allows the precise placement on the mold.

Figure 73 Front and back side of the transfer pot

11.2.3 Piston

Figure 74 show the piston, which was made from steel 11 600. At the bottom is the small cylindrical hole, which serves to catch the air that does not manage to escape from transfer pot. The length of the thinner cylindrical part is longer than the depth of the hole in transfer pot to ensure the easier opening after processing.

Figure 74 Piston

11.3 Complete construction of transfer mold

Figure 75 shows the final transfer mold that is ready for use. Inside the mold are also 2 cores, which are made from steel wire with 1 mm in diameter.

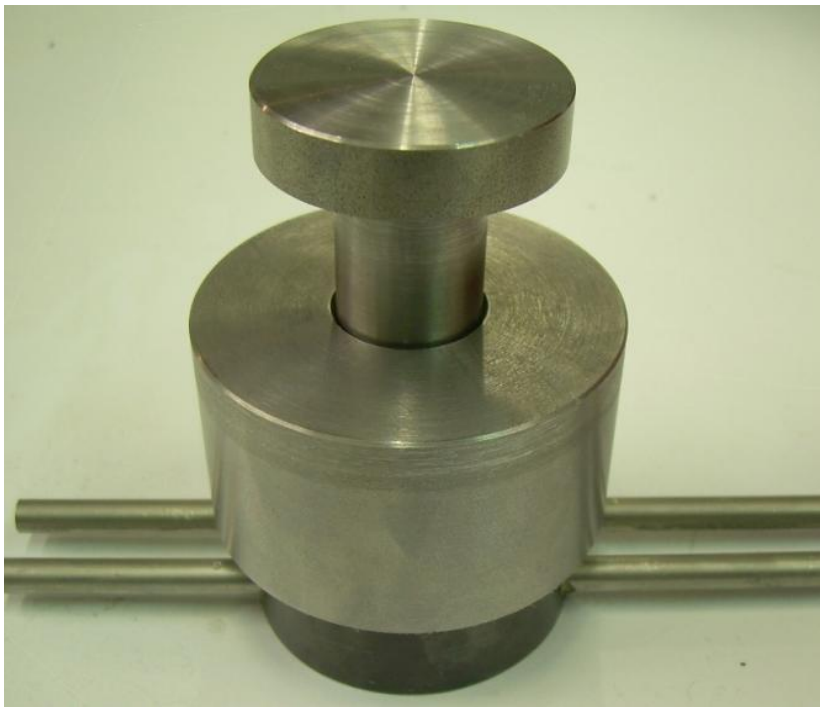


Figure 75 Complete final transfer mold

Figure 76 shows the required equipment for the production of ear ventilation tubes. The hydraulic press ensures press and heating while the thermostat provides the cooling. Connection between thermostat and transfer mold will done by silicone tubes.

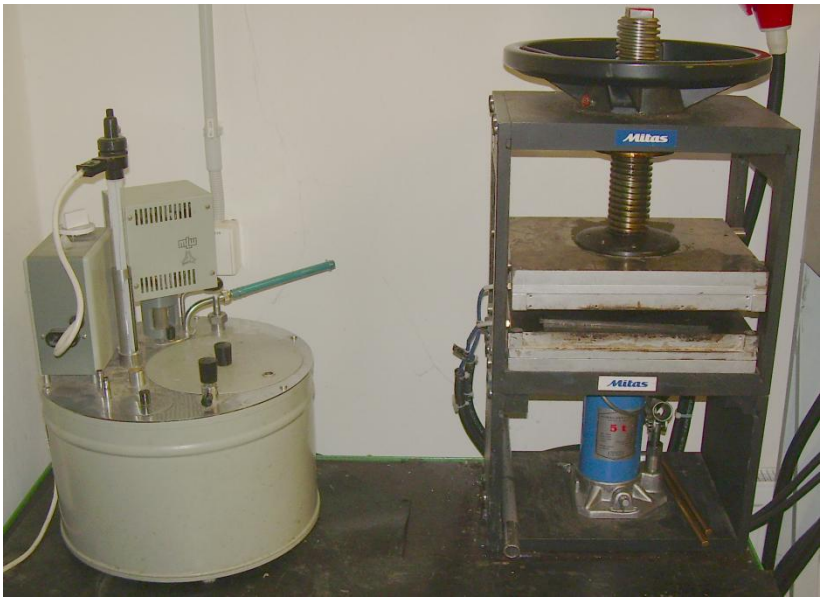


Figure 76 Thermostat for cooling and hydraulic press

CONCLUSION

In recent years, there has been a steadily growing interest in development of biodegradable polymers for application in medicine. Especially aliphatic polymers based on PLA and PGA or their copolymers are very promising. Today, these polymers are used as absorbable sutures, orthopedic implants, drug delivery systems and scaffolds in tissue engineering. The tympanostomy tubes, which are used in otolaryngology in case of persistent Eustachian tube dysfunction, to treat repeatable otitis media could be another field for their potential applications.

Therefore, the theoretical part of this work is concerned with surgical treatment of the middle ear diseases using tympanostomy tubes. Nowadays, ear ventilation tubes are made from metals or non-biodegradable polymers and after their role in eardrum they must be surgically removed, if they do not fall out themselves. The removing can cause certain problems, so the tubes based on biodegradable polymers could replace currently used materials. The material for medical devices must meet specific criteria, particularly in terms of their biocompatibility, sterilization, processability and mechanical properties. In this way, PLA could be the most attractive polymer for this application due to excellent biocompatibility, easy processing and degradation rate, which varies from 12 to 24 months.

In the experimental part, the poly (L-lactic acid) (PLLA) produced by polycondensation at laboratory of Polymer Center was examined and the basic physical properties as well as degradation behavior were obtained. In vitro degradation of PLA was investigated in many research papers, but usually only in phosphate buffered solution. If we applied PLA tubes into the eardrum, the conditions would be rather similar to the 100% relative humidity. So the degradation experiments were done in these two different environments (in PBS and in 100% RH).

The basic characterization of PLLA properties were done at room temperature before degradation. The results demonstrated that the density was 1.24 g/cm^3. The results from microhardness were: indentation harness 192 MPa, indentation modulus 4.1 GPa and Vickers indentation hardness 18.2 Vickers. While the modulus from bending test was 2.9 GPa and flexural strength 8.4 MPa. These results demonstrate that material is very brittle at room temperature. Melt flow was measured at 125°C with load of 100 g and melt flow index was 107 g in 10 min. This means that material is very liquid, which is caused by low molecular weight.

Initially, in this experiment, we wanted to investigate the mechanical properties as tensile strength and mechanical changes particularly compressive strength during degradation process by compression test. Finally, the tensile strength could not be measured on prepared specimens, because material was so brittle and has crashed just by clamping into holding grips. And there was no possibility to monitor mechanical changes during degradation, because samples after 12 weeks under degradation conditions were disintegrated into small pieces without any grater load. We managed to measure only mechanical properties by microhardness after 2 weeks in 100% RH and these values decreased on average 17%.

Monitoring of degradation process by GPC demonstrate the differences of molecular weight decreasing through a cross section of the samples in PBS condition. This indicates different hydrolysis mechanism. Degradation rate was marginally slower in 100% RH. The thermal changes obtained by DSC seem to be more affected by hydrolysis in 100% RH conditions while the values in PBS are more scattered. Optical microscopy and SEM provide interesting insight into the morphology of samples during in vitro degradation. In PBS is visible core-cortex structure while in 100% RH is shown the homogenous degradation entire the whole cross-section of samples.

Conclusion of experimental part is that PLLA prepared from polycondensation at laboratory of Polymer Center has low molecular weight and due to it has poor mechanical properties particularly the flexural strength and brittleness. The degradation rate is also very fast and therefore the proposed PLLA is not suitable for construction of ear ventilation tubes. On the other hand the modulus and indentation hardness have quite high values in comparison with other standard commodity polymers. This means that PLA with higher molecular weight or in copolymerization with other biodegradable polymer could be a very promising candidate for this application.

The practical part deals with the design of biodegradable ear ventilation tubes, development of the tool for their production and tool manufacturing. There were designed two types of tubes and the tool for its production is simply transfer mold allowing to produce two and two tubes of each type. The mold consists of piston, transfer pot, top and bottom part of the mold as well as two cores. All parts of the mold were manufactured using conventional technologies, the exception were the upper and lower cavity plates due to the dimension of tympanostomy tubes. These cavity plates were done by electroplating and it was necessary to prepare real negatives of the plates, which served to metal deposition in

electrolytic bath. This method of production of cavity plates was proven as rapid and inexpensive way for production of mold components with such small cavities.

The results, which were obtained in experimental and practical part of this master thesis, were also presented at Plastko Conference 2012 and at Student Scientific Conference 2012.

Conference presentations:

HNÁTKOVÁ, E., KUCHARCZYK, P., SEDLAŘÍK V., DVOŘÁK, Z. Investigation of poly(lactic acid) degradation process under various abiotic conditions. *Conference: Plastko 2012,* April 11 - 12, 2012, Zlín, Czech Republic, ISBN 978-80-7454-137-7, pp. 240-243

HNÁTKOVÁ, E., DVOŘÁK, Z. Development of the tool for a production of ear ventilation tubes. *Student Scientific Conference 2012 (*Faculty of technology, Tomas Bata University), May 10, 2012, Zlín, Czech Republic, ISBN 978-80-7454-152-0

Other conference presentation:

HNÁTKOVÁ, E., SEDLAŘÍK V. The effect of crystallinity on mechanical and thermal properties of biodegradable polylactide, *Conference: Plastko 2010*, April 13 - 14, 2010, Zlín, Czech Republic, ISBN 978-80-7318-909-9, pp. 299-301

BIBLIOGRAPHY

[1] KIRCH, W. *Encyclopedia of Public Health*. Dresden: Springer, 2008. ISBN 978-1-4020-5613-0

[2] ROTSCHILD, M. *Ear Infections, Hearing and Ear Tube Surgery* [online]. [cit. 2011-04-12]. Available on www: http://www.kids-ent.com/website/pediatric_ent/ear_infections/index.html#surgery

[3] NEWTON, V. E., VALLELY, P. J. *Infection and Hearing*. Chichester, England: Wiley, 2006. ISBN-10: 1-86156-507-0

[4] KUMAR, P., CLARK, M. *Clinical medicine*. 7th ed. London: Elsevier, 2009. ISBN 9780702029936

[5] HANAVAN, P. C. Virtual Tour of the Ear [online]. [cit. 2011-8-12]. Available on www: http://moon.ouhsc.edu/dthompso/balance/vestib.htm

[6] BALASUBRAMANIAN, T. *Foundation Otorhinolaryngology for Nursing*. Japan, New Delhi: Publications Pvt. Ltd., 2006. ISBN 81-7225-297-8

[7] JACSON, Ch. *Diseases of the Nose, Throat, and Ear*. Philadelphia: Saunders, 1946

[8] ANNIKO, M., BERNAL-SPREKELSEN, M., BRADLEY, P. *Otorhinolaryngology, Head and Neck Surgery*. Berlin: Springer, 2010. ISBN 978-3-540-42940-1

[9] ALAN, R., GREENE, M. D. *The Parent's Complete Guide to Ear Infections*. Allentown, PA: People's Medical Society, 1997. ISBN 1882606299

[10] BLUSTONE, CH. D. *Conquering otitis media*. Hamilton, Ontario: Decker, 1999. OSBN 1-896998-05-4

[11] Medline Plus: *Eustachian tube* [online]. [cit. 2011-15-11]. Available on www: http://www.nlm.nih.gov/medlineplus/ency/imagepages/19596.htm

[12] HANAVAN, P. C. *Virtual Tour of the Ear* [online]. [cit. 2011-15-11]. Available on WWW: http://moon.ouhsc.edu/dthompso/balance/vestib.htm

[13] RICE, J. *Medical Terminology with Human Anatomy*. 2st ed. Mishawaka, U.S.A.: Pearson/Prentice Hall, 2005. ISBN: 9780536919151

[14] ROLAND, P.S. Medscape Education Infectious Diseases: All Otitis Is Not Created Equal [online]. [cit. 2011-15-12]. Available on www:

http://www.medscape.org/viewarticle/730698

[15] CUNEYT, M. A., BLUESTONE, Ch. D. Cuneyt M. Alper, Charles D. *Advanced therapy of otitis media*. Hamilton, Ontario: Decker, 2004. ISBN 1-55009-201-4

[16] BLUSTONE, CH. D., KLEIN, J. O. Otitis media in infants and children. 4th ed. Hamilton, Ontario: Decker: 2007. ISBN 1-55009-335-5

[17] DEINER, P. L. *Inclusive Early Childhood Education: Development, Resources, and Practice*. 5th ed. California: Wadsworth, 2009. ISBN 1-4283-2086-5

[18] FDA. Medical devices: regulation guidance [online]. PDF [cit. 2011-20-11]. Available on www:
http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm080204.pdf

[19] The Medical City: Ear Tubes [online]. [cit. 2012-05-01]. Available on WWW:
http://www.designink.info/medicalcityent/images/eartubes.jpg

[20] GRAY, S.,LUSK, R. P. Tympanic Membrane - Tympanostomy Tubes. In: CUMMINGS, C. W., FREDRICKSON, J. M. HARNER, L. J., et al. Otolaryngology–*Head and Neck Surgery*. 2nd ed. St. Louis, MO: Mosby Year Book, 1993

[21] SHERMAN, E. G. *Ear Flow Chamber for Studying Tympanostomy Tube Occlusion*. Master thesis. University of Florida: 2005

[22] Tubes for Ear Infections [online]. [cit. 2012-02-01]. Available on www: http://www.health.com/health/library/mdp/0,,zm6208,00.html

[23] RICHARD, M. ROSENFELD, M.D. *Who Needs Ear Tubes* [online]. Hearing Health Magazine [cit. 2012-12-01]. Available on WWW:
http://www.drf.org/magazine/19/Summer+2007+Issue/article/36

[24] NADOL, J. B., KENNA, M. J, NADOL, J. B. *Surgery of the ear and temporal bone*. 2th ed. Philadelphia, Lippincott: Williams & Wilkins, 2005. ISBN 0-7817-2046-X

[25] SUYMA, T. *Tympanostomy tube and method for producing the same*. Patent number: 5645584, 1997

[26] SHUSTER, B. *Middle Ear Ventilation Tube* [online]. [cit. 2012-16-01]. Available on www: http://plastische-operationen-schuster.de/en/tympanic-drainage.php

[27] PITCHR, P. A. *Care of the Child with Tympanostomy Tubes: A Guide for the Primary Care Physician* [online]. [cit. 2012-18-01]. Dalhousie Medical Journal, December 1997. Available on www: http://edmj.medicine.dal.ca/archives/winter97/orig7.htm

[28] SHERMAN, E. G. *Development of biocompatible and dissolvable tympanostomy tube*. Dissertation thesis, University of Florida: 2009

[29] WONG, J, Y., BRONZINO J.D. *Biomaterials*. CRC press: Boca Raton 2007, ISBN 0-8493-4788-5

[30] WILLIAMS, D., F. On the nature of biomaterials. *Biomaterials*. vol. 30 (30) pp. 5897–5909 (2009)

[31] RATNER, B. D. *Biomaterials Science: An Introduction to Materials in Medicine*. 2nd ed. Amsterdam: Academic Press, 2004. ISBN 0-12-582463-7

[32] RAMAKRISHNA, S., HUANG, Z. M., KUMAR, G.V., BATCHELOR, A.W., MAYER, J. *An Introduction to Biocomposites*. London: Imperial College Press, 2004

[33] IKADA, Y. TSUJI, H. *Biodegradable polyesters for medical and ecological applications*, Macromolecular Rapid Communications. 21, 117–132 (2000)

[34] PORTMANN, M. BOUDARD, P., PORTMANN, D. *Transplants and Implants in Otology III*. Kugler Publications Amstrdam 1996

[35] BENDIX, D. Chemical synthesis of polylactide and its copolymers for medical applications. Publication: *Polymer Degradation and Stability*. Elsevier, 1998, vol. 59, Issues 1–3, p. 129–135

[36] SCOTT, G. *Degradable Polymer: Principles and Applications*. 2nd ed. Dordrecht: Kluwer 2002. ISBN 1-4020-0790-6

[37] GOPFERICH, A. Mechanisms of polymer degradation and erosion. *Biomaterials*, vol. 17 (2), pp 103 – 114, 1996

[38] ALBERTSSON, A-C. *Advances in polymer science: Degradable aliphatic polyesters*. Berlin: Springer, 2002

[39] Biodegradable Polymers in Controlled Drug Delivery [online]. [cit. 2012-01-22]. Available on www: http://www.pharmainfo.net/raghanaveen/biodegradable-polymers-controlled-drug-delivery

[40] MIDDLETON, J., TIPTON, A. Synthetic biodegradable polymers as orthopedic devices. *Biomaterials*, Vol. 21, No. 23, pp. 2335–2346, 2000

[41] DOMB, A. J., NEERAJ, K. *Biodegradable Polymers in Clinical Use and Clinical Development*. New Jersey: Wiley, 2011

[42] THAKKAR, N. T. Seminar on biodegradable polymers [online]. [cit. 2010-02-02]. Available on www: http://www.authorstream.com/Presentation/naitik33-1152427-polymer-science/

[43] GUPTA, A.P., KUMAR, V. New emerging trends in synthetic biodegradable polymers-Polylactide: A critique. *European Polymer Journal*. 2007, 43, 10, p. 4053-4074. ISSN 0014-3057

[44] AURAS, R., LIM, L-T., SELKE, S. E. M., et. al. *Poly(lactic acid): Synthesis, Structures, Properties, Processing, and Applications.* New Jersey: Wiley, 2010. ISBN 978-0-470-29366-9

[45] Medscape: *The Use of Bioabsorbable Implants in Spine Surgery: Bioabsorbable Implants as a Protective and Permissive Barrier* [online]. [cit. 2010-02-04]. Available on www: http://www.medscape.com/viewarticle/472962_7

[46] Medscape: *The Use of Bioabsorbable Implants in Spine Surgery: Recent Trials of Bioabsorbable Implants in Humans* [online]. [cit. 2010-02-04]. Available on www: http://www.medscape.com/viewarticle/472962_5

[47] EREDITA, R., MARSH, R. R., KAZAHAYA, K. *A new absorbable pressure-equalizing tube*. Otolaryngol Head Neck Surg. 2002, 127(1), p. 67-72

[48] HENTON, D. E., GRUBER, P., LUNT, J., et al. Polylactic acid technology, in: AK MOHANTY, M., DRZAL, L. Z. *Natural Fibers, Biopolymers, and Biocomposites*. Taylor & Francis, Boca Raton, FL, 2005, pp. 527–577

[49] WOLF, O., et al. *Techno-economic Feasibility of Largescale Production of Bio-based Polymers in Europe*. Technical Report EUR 22103 EN, 2005. ISBN 9279012304

[50] HUGH. A. J. *Modeling a polymer's properties: A biodegradable fiber's lightning-quick behaviors pose a challenge*. Resolve (magazine), vol. 2, 2010

[51] KOPELIOVICH, D. Transfer molding of polymers [online]. [cit. 2010-02-03]. Available on www:

http://www.substech.com/dokuwiki/doku.php?id=injection_molding_of_polymers

[52] GARLOTTA, J. A Literature Review of Poly(Lactic Acid). *Journal of Polymers and the Environment*, Vol. 9, No. 2, 2001

[53] BELGACEM, M. N., GANDINI, A. Monomers, polymers and composites from renewable resources. In AVEROUS, L. *Polylactic Acid: Synthesis, Properties and Applications*. Amstrdam: Elsevier, 2008

[54] JAMSHIDIAN, M., TEHRANY, E. A., IMRAN, M., et al. *Poly-Lactic Acid: Production, Applications,Nanocomposites, and Release Studies*. Comprehensive Reviews in Food Science and Food Safety, vol. 9, Issue 5, p. 552–571, 2010

[55] KOPELIOVICH, D. Injection molding [online]. [cit. 2010-02-03]. Available on www: http://www.substech.com/dokuwiki/doku.php?id=injection_molding_of_polymers

[56] GREENER, J., WIMBERG-FRIDEL, R. *Precision Injection Molding: Process, Materials, and Applications*. Munich: Hanser, 2006. ISBN 1-56990-400-6

[57] POTSCH, G., MICHAELI, W. *Injection molding: An introduction*. 2nd ed. Munich: Hanser, 2008. ISBN 1-56990-419-0

[58] Silicone Rubber Tooling [online]. [cit. 2012-29-02]. Available on www: http://www.additive3d.com/tl_20.htm

[59] Hardness testing [online]. [cit. 2012-29-04]. Available on www: http://www.twi.co.uk/services/technical-information/job-knowledge/job-knowledge-74-hardness-testing-part-1/

[60] OLIVER, W. C., PHARR, G. M. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation. *Journal of Materials Research*, vol. 7 (6), pp. 1564–1583, 1992

[61] Melt flow indexer [online]. [cit. 2012-29-04]. Available on www: http://www.lmsscientific.com/products.php?prod_name=melt-flow-indexer

[62] G. W. EHRENSTEIN, G., W., RIDEL, G., TRAWIEL, P. *Thermal analysis of plastics: theory and practice*. Munich: Hanser, 2004. ISBN 1-56990-362

[63] Gel Permeation Chromatography [online]. [cit. 2012-02-04]. Available on www: http://web.mit.edu/3.082/www/team1s/images/GPC2.jpg

[64] Training for advanced research – scanning electron microscopy [online]. [cit. 2012-022-04]. Available on www:

http://www.ammrf.org.au/myscope/sem/practice/principles/layout.php

[65] SHLESINGER, M., PAUNOVIC, M. *Modern Electroplating*, 5th edition. New Jersey: Wiley, 2010. ISBN 978-0-470-16778-6

[66] Electroforming [online]. [cit. 2012-10-05]. Available on www: http://www.absoluteastronomy.com/topics/Electroforming

[67] Polyjet technology [online]. [cit. 2012-12-3]. Available on www: http://www.engineershandbook.com/RapidPrototyping/polyjet.htm

LIST OF ABBREVIATIONS

ENT	Ear, nose, and throat
PLA	Poly(lactic acid)
PLLA	Poly(L-lactic acid)
PGA	Poly(glycolic acid)
PLGA	Poly(lactide-co-glycolide)
PCL	Poly(ε-caprolactone)
PP	Polypropylene
PE	Polyethylene
PS	Polystyrene
PU	Polyurethane
PA	Polyamide
PET	Polyethylene terephthalate
PVC	Polyvinyl chloride
PTFE	Polytetrafluoroethylene
PMMA	Poly(methyl methacrylate)
UHMWPE	Ultra-high-molecular-weight polyethylene
SEM	Scanning electron microscopy
GPC	Gel permeation chromatography
SEC	Size exclusion chromatography
DSC	Differential scanning calorimetry
IR	Infrared spectroscopy
MALDI	Matrix-assisted laser desorption/ionization
NMR	Nuclear magnetic resonance spectroscopy
TOF-SIMS	Time-of-Flight secondary ion mass spectrometry

FDA	Food and drug administration
CFR	Code of federal regulations
ISO	International organization for standardization
ASTM	American society for testing and materials
CEN	European standardization committee
IRS	Institute for standards research
DIN	German institute for standardization
ORCA	Organic reclamation and composting association
RH	Relative humidity
PBS	Phosphate buffered solution
EO (EtO)	Ethylene oxide
RTV	Room temperature vulcanizing rubber
MFR	Melt flow rate (g/10 min)
MFI	Melt flow index (g/10 min)
pH	Potential hydrogen
StDev	Standard deviation
CoefVar	Coefficient of variation
M_w	Weight average molecular mass (g/mol)
M_n	Number average molecular weight (g/mol)
PDI	Polydispersity index (M_w/M_n)
T_g	Glass transition temperature (°C)
T_c	Crystallization temperature (°C)
T_m	Melting temperature (°C)
H_{IT}	Indentation hardness (MPa)
E_{IT}	Indentation modulus (GPa)
E*	Plane strain modulus (GPa)

HV_{IT}	Vickers indentation hardness (Vickers)
C_{IT}	Indentation creep (%)
H	Enthalpy (J/g)
ρ	Density (g/cm^3)
δ	Deflection (mm)
c_p	Heat capacity (J/g.K)
χ_c	Percentage of crystallinity (%)
R_{max}	Flexural strength for free-point bend test (MPa)
E_B	Flexural modulus (GPa)
kgf	kilogram force

LIST OF FIGURES

LIST OF TABLES

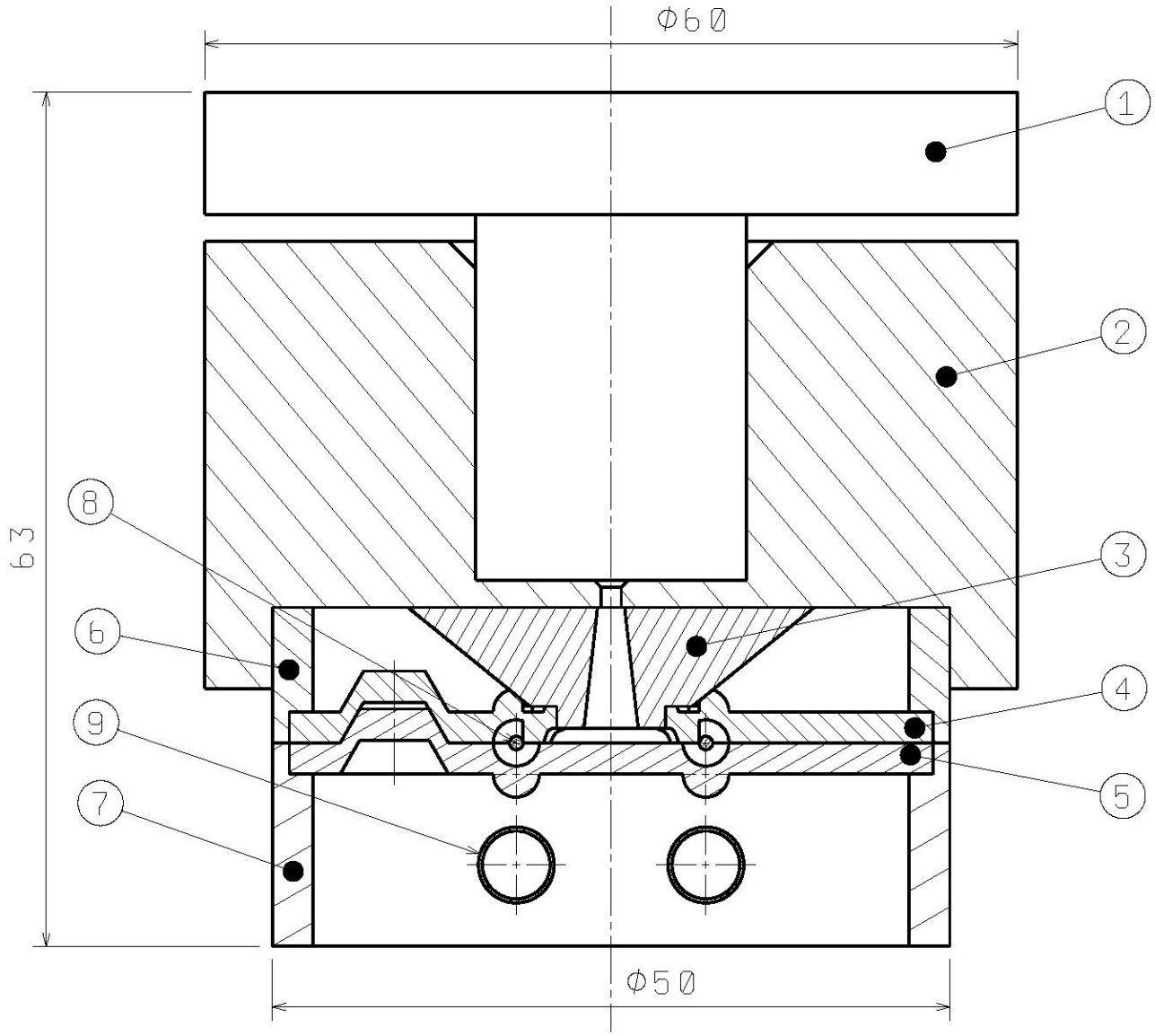
APPENDICES

PI **Drawing documentation**

- Transfer mold assembly
- Piston
- Transfer pot
- Sprue
- Upper cavity plate
- Lower cavity plate
- Top ring
- Bottom ring

PII **CD with Master thesis in pdf format**

APPENDIX P I: DRAWING DOCUMENTATION



NUMBER	NAME	QUANTITY	MATERIAL
1	Piston	1	Steel 11 600
2	Transfer pot	1	Steel 12 050
3	Sprue	1	Dural
4	Upper cavity plate	1	Nickel
5	Lower cavity plate	1	Nickel
6	Top ring	1	Steel 11 373
7	Bottom ring	1	Steel 11 373
8	Core	2	Steel wire
9	Tempering tube	2	Stainless steel

DESIGNED BY: Eva Hnátková
DATE: 13.5.2012
CHECKED BY: XXX
DATE: XXX

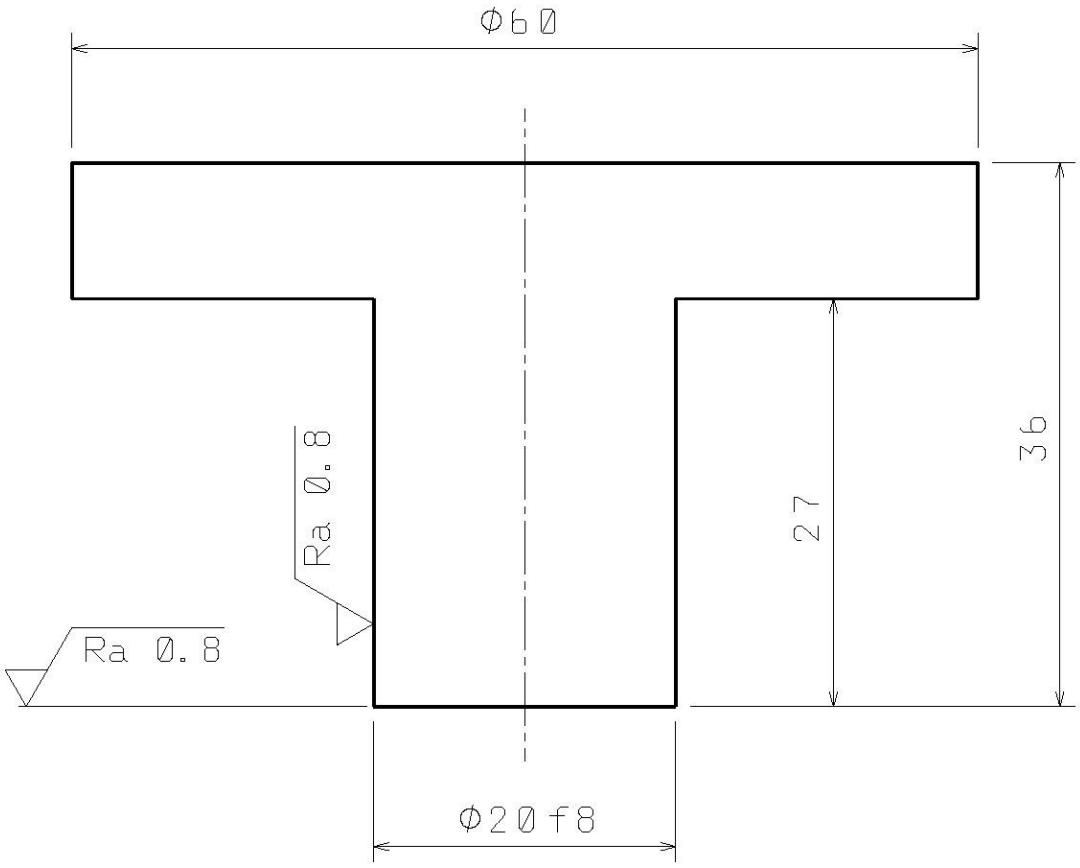
TRANSFER MOLD ASSEMBLY

SIZE: A4

TBU in Zlín, Faculty of Technology

SCALE: 2:1
WEIGHT (kg): 0,15
DRAWING NUMBER: Transfer mold
SHEET: 1/0

I	-
H	-
G	-
F	-
E	-
D	-
C	-
B	-
A	-

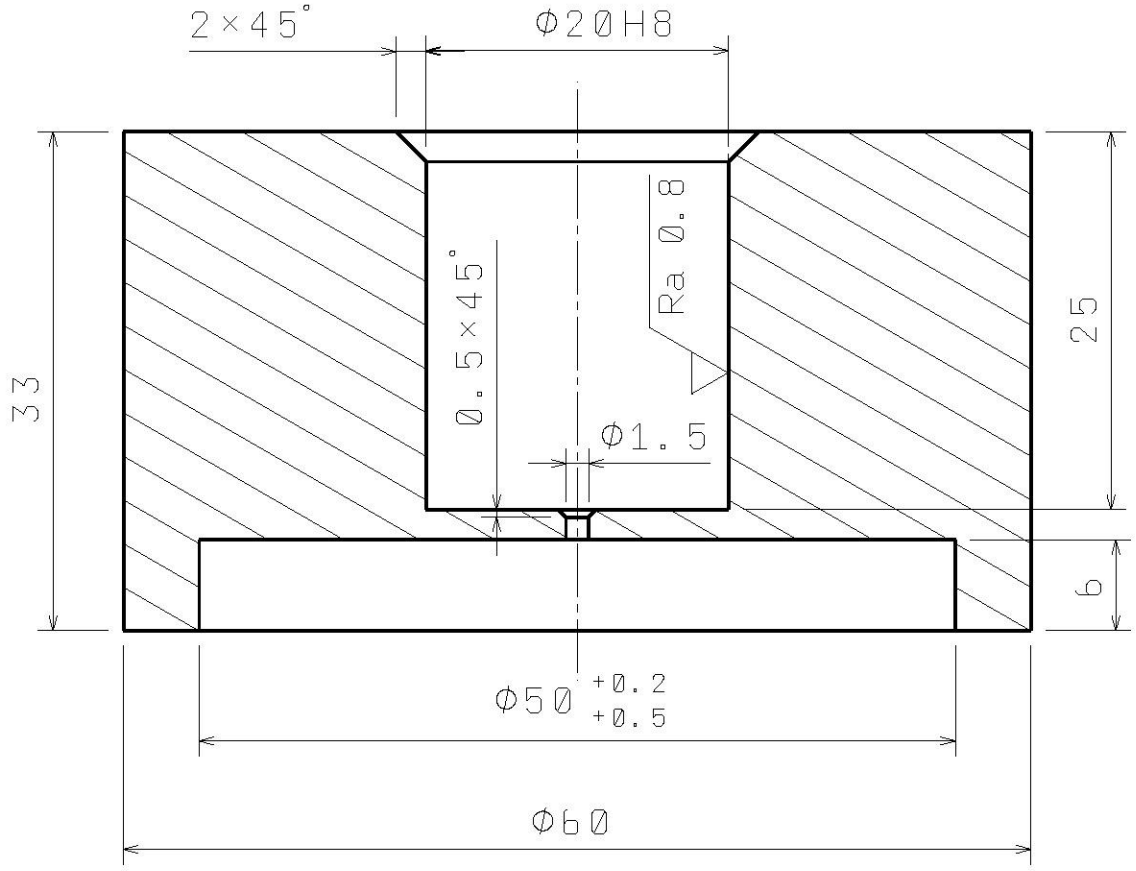


UNQUOTED RADIUS R 0.5

Ra 3.2 (Ra 0.8)

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	**PISTON**		
CHECKED BY: XXX DATE: XXX			
SIZE: A4	TBU in Zlín, Faculty of Technology		
SCALE: 2:1	WEIGHT (kg): 0,15	DRAWING NUMBER: **1**	SHEET: 1/1

I	–
H	–
G	–
F	–
E	–
D	–
C	–
B	–
A	–

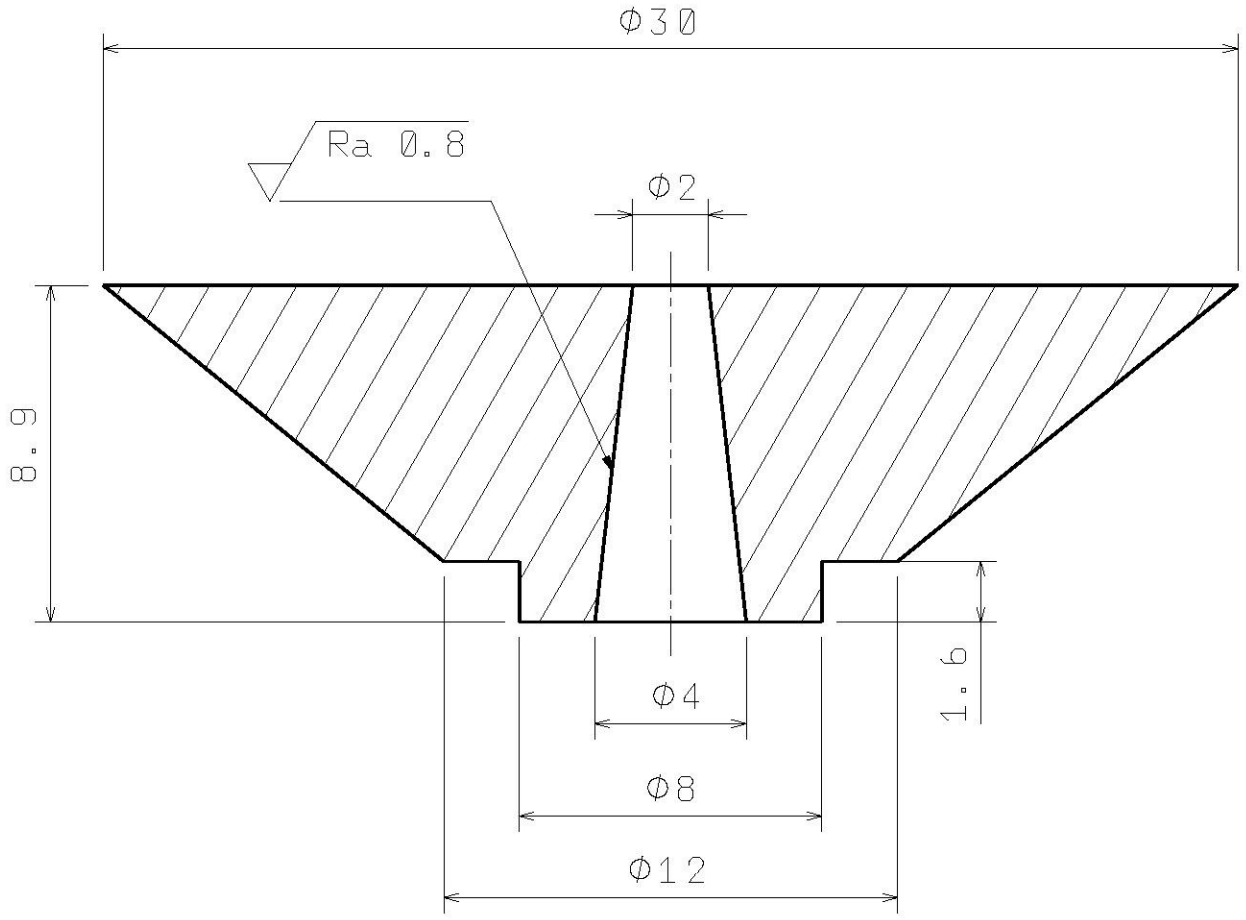


UNQUOTED RADIUS R 0.5

Ra 3.2 (Ra 0.8)

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	**TRANSFER POT**		
CHECKED BY: XXX DATE: XXX			
SIZE A4	TBU in Zlín, Faculty of Technology		
SCALE 2:1	WEIGHT (kg) 0,15	DRAWING NUMBER **2**	SHEET 1/2

I	–
H	–
G	–
F	–
E	–
D	–
C	–
B	–
A	–

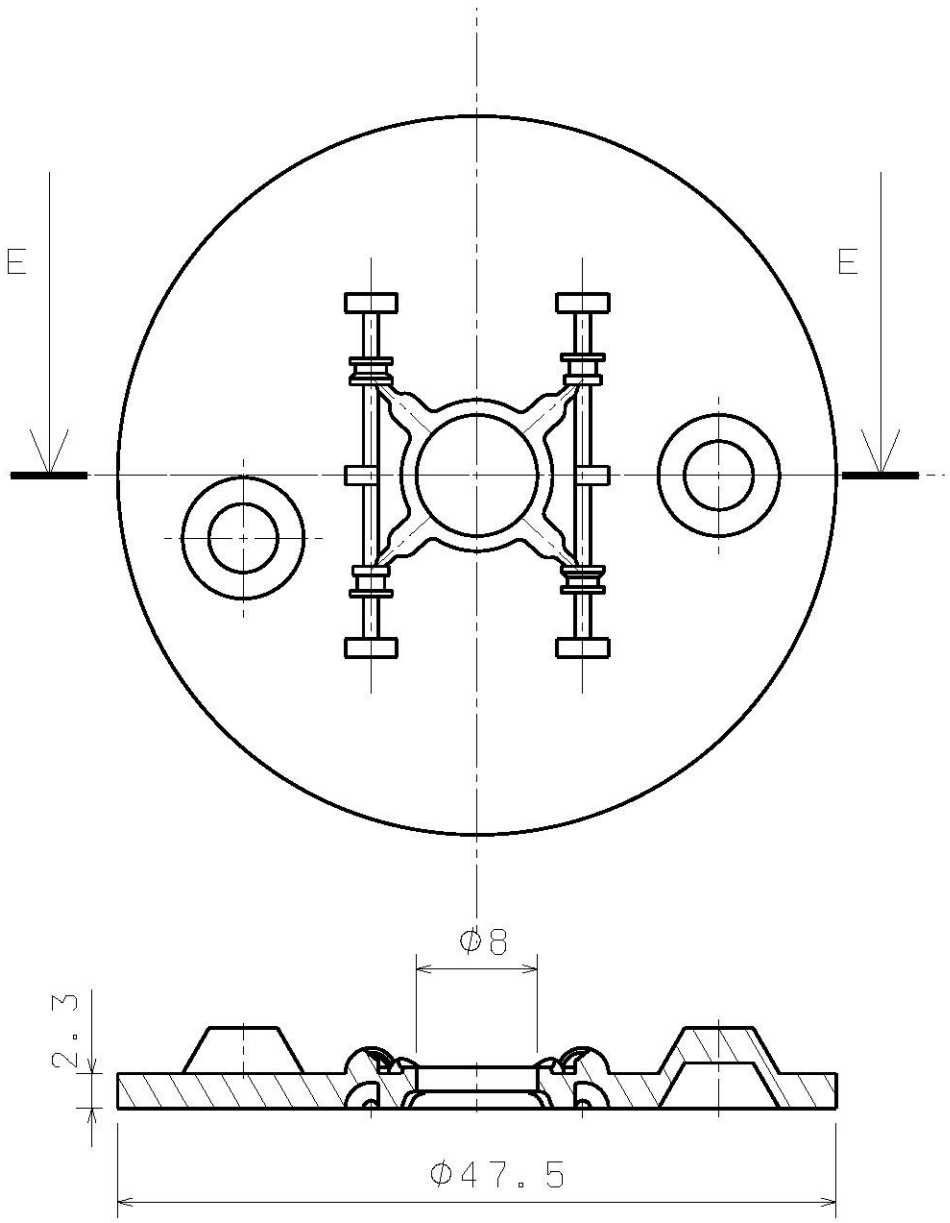


UNQUOTED RADIUS R 0.5

Ra 3.2 (Ra 0.8)

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	SPRUE		
CHECKED BY: XXX DATE: XXX			
SIZE A4	TBU in Zlín, Faculty of Technology		
SCALE 5:1	WEIGHT (kg) 0,15	DRAWING NUMBER 3	SHEET 1/3

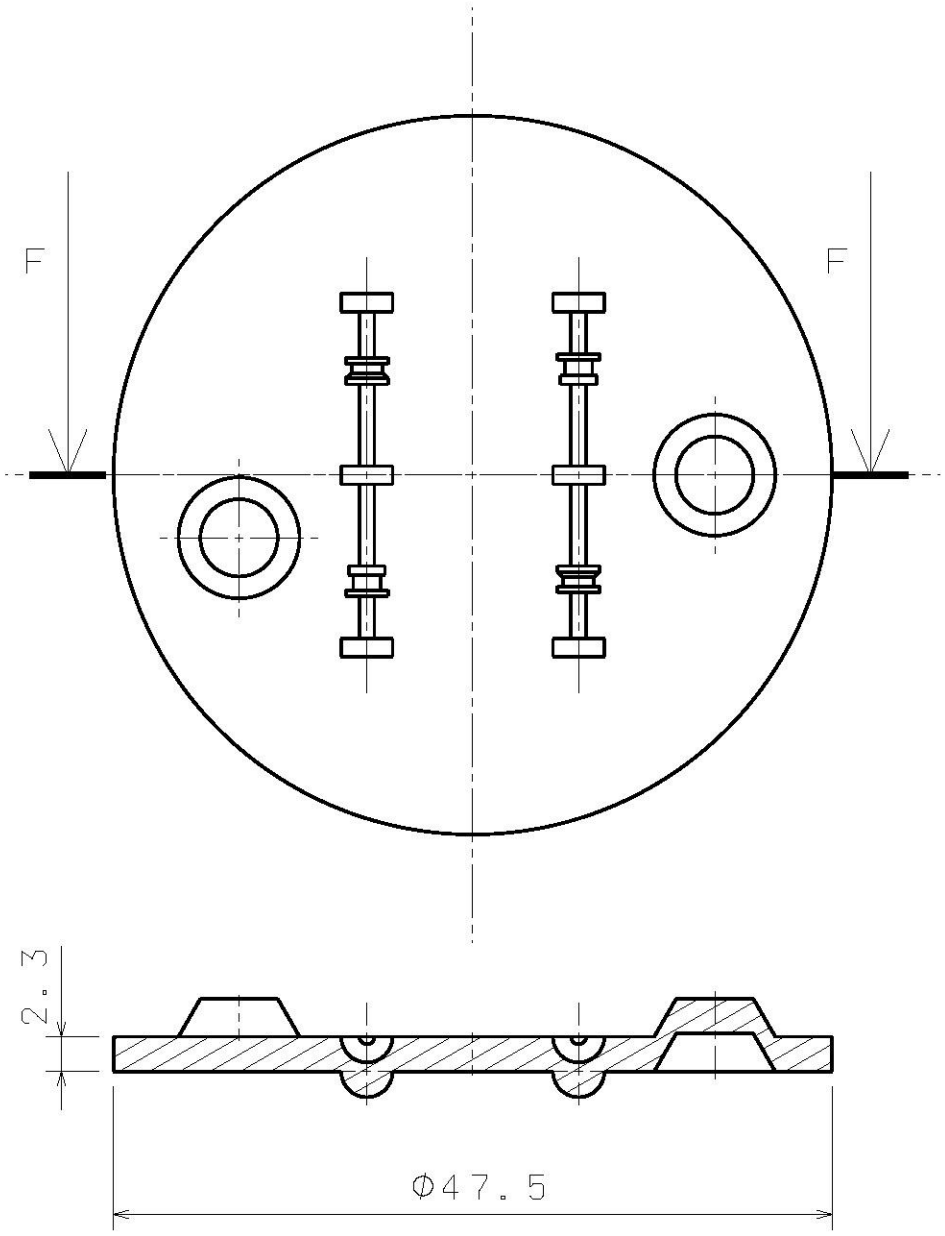
I	–
H	–
G	–
F	–
E	–
D	–
C	–
B	–
A	–



Section view E-E
Scale: 2:1

THIS PART IS DONE BY ELECTROPLATING

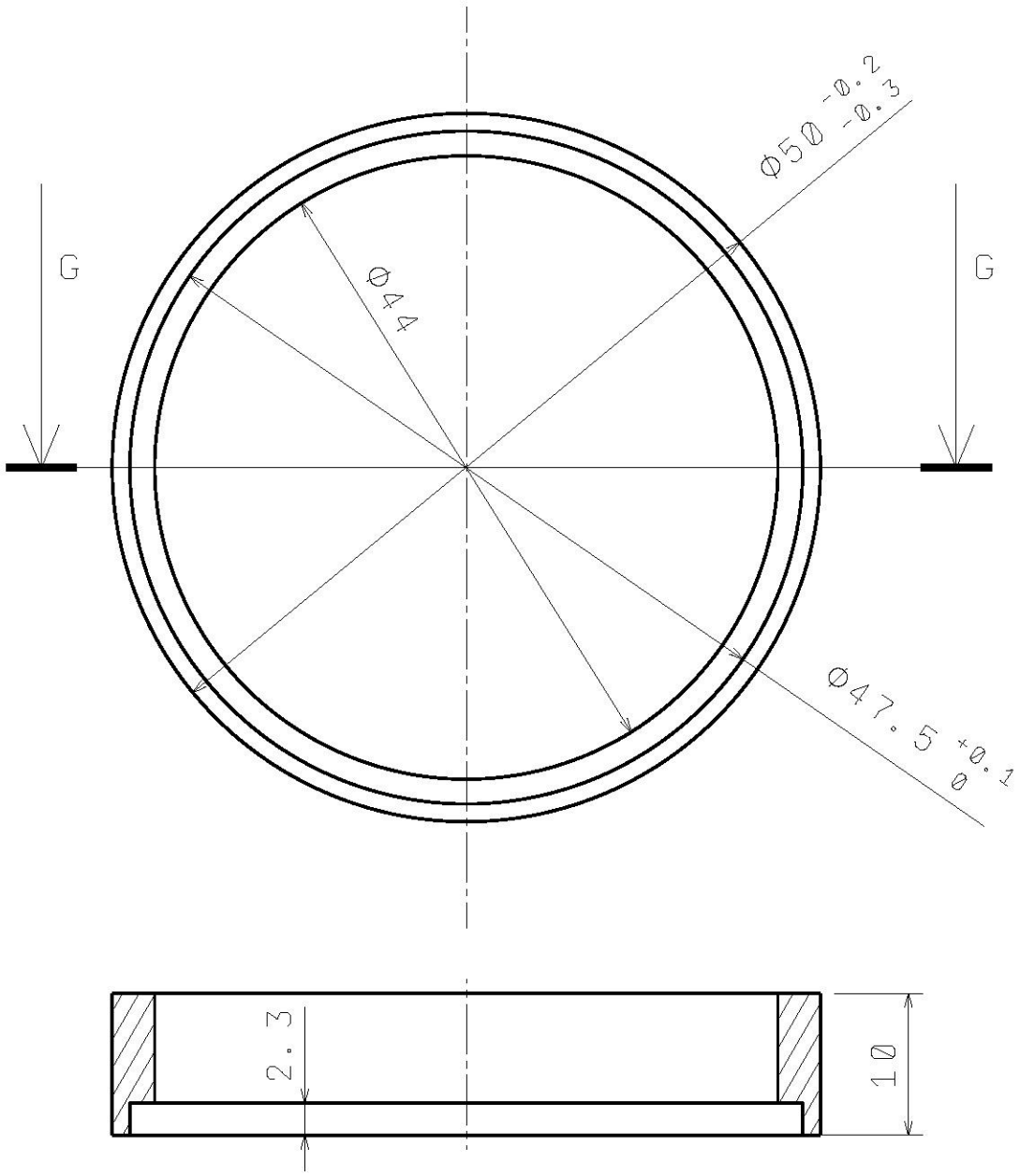
DESIGNED BY: Eva Hnátková DATE: 13.5.2012	UPPER CAVITY PLATE		I –
CHECKED BY: XXX DATE: XXX			H –
			G –
			F –
SIZE A4	TBU in Zlín, Faculty of Technology		E –
			D –
SCALE 2:1	WEIGHT (kg) 0,15	DRAWING NUMBER 4	SHEET 1/4
			C –
			B –
			A –



Section view F-F
Scale: 2:1

THIS PART IS DONE BY ELECTROPLATING

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	# LOWER CAVITY PLATE		I _ H _ G _ F _
CHECKED BY: XXX DATE: XXX			E _
SIZE A4	TBU in Zlín, Faculty of Technology		D _
SCALE 2:1 / WEIGHT (kg) 0,15	DRAWING NUMBER 5	SHEET 1/5	C _ B _ A _

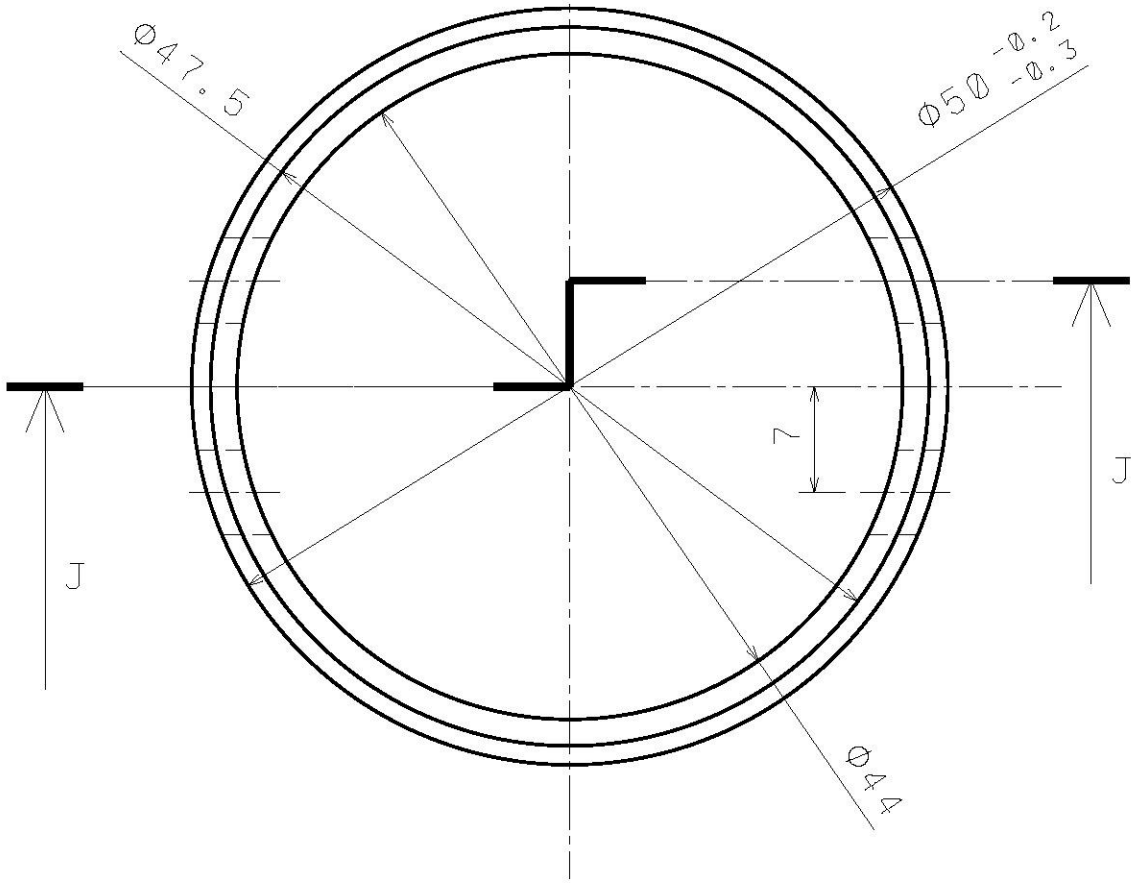


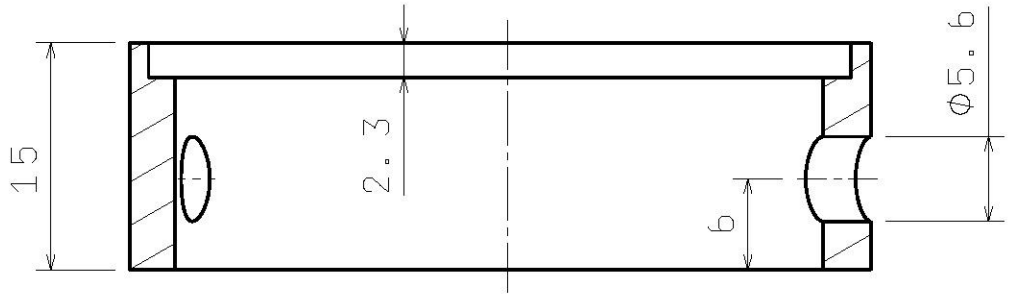
Section view G-G
Scale: 2:1

UNQUOTED RADIUS R 0.5

Ra 3.2

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	**TOP RING**		I –
CHECKED BY: XXX DATE: XXX			H –
			G –
			F –
SIZE A4	TBU in Zlín, Faculty of Technology		E –
			D –
SCALE 2:1	WEIGHT (kg) 0,15	DRAWING NUMBER 6	SHEET 1/6
			C –
			B –
			A –



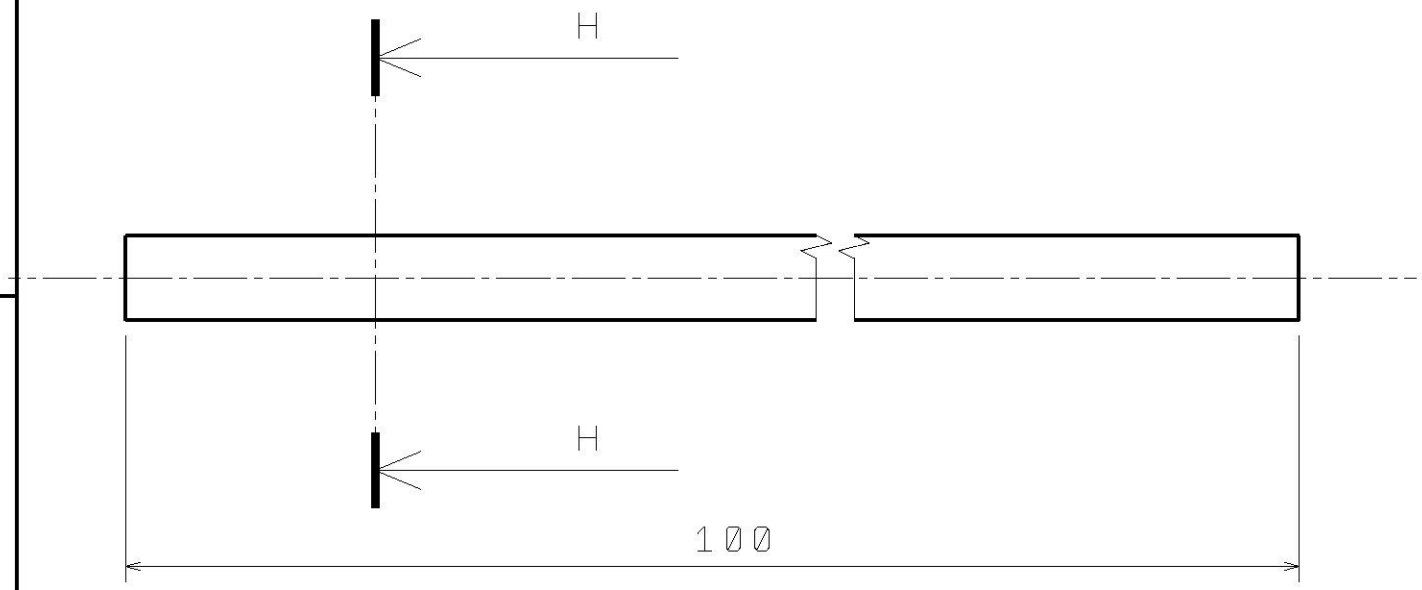


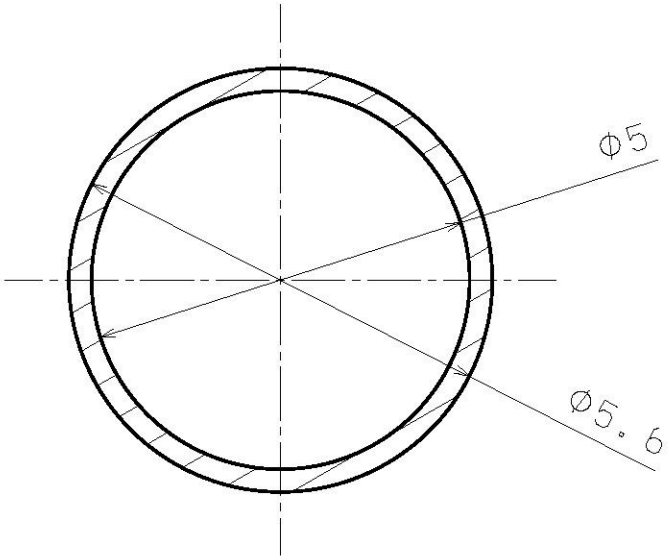
Section view J-J
Scale: 2:1

UNQUOTED RADIUS R 0.5

Ra 3.2

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	**BOTTOM RING**		I –
CHECKED BY: XXX DATE: XXX			H –
			G –
			F –
SIZE A4	TBU in Zlín, Faculty of Technology		E –
			D –
SCALE 2:1	WEIGHT (kg) 0,15	DRAWING NUMBER 7	SHEET 1/7
			C –
			B –
			A –

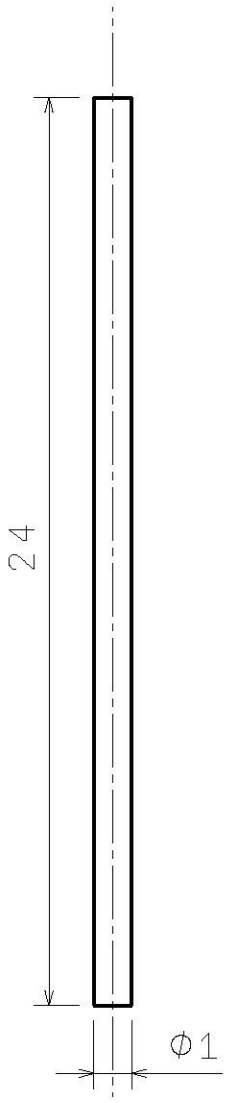




Section view H-H
Scale: 10:1

DESIGNED BY: Eva Hnátková DATE: 13.5.2012	**TEMPERING TUBE**		
CHECKED BY: XXX DATE: XXX			
SIZE A4	TBU in Zlín, Faculty of Technology		
SCALE 2:1	WEIGHT (kg) 0,15	DRAWING NUMBER **8**	SHEET 1/8

I	–
H	–
G	–
F	–
E	–
D	–
C	–
B	–
A	–



DESIGNED BY: Eva Hnátková DATE: 13. 5. 2012	**WIRE**		
CHECKED BY: XXX DATE: XXX			
SIZE: A4	TBU in Zlín, Faculty of Technology		
SCALE: 50:1	WEIGHT (kg): 0,15	DRAWING NUMBER: **9**	SHEET: 1/9

I	–
H	–
G	–
F	–
E	–
D	–
C	–
B	–
A	–