DEVELOPMENT OF IMPLANTABLE SCAFFOLD FOR EAR TISSUE CARTILAGE WITH POTENTIAL APPLICATION IN BIONIC EARS

A thesis presented to the Department of Materials Science and Engineering

African University of Science and Technology, Abuja

In partial fulfillment of the requirements for the award

Master's Degree in

Materials Science and Engineering

By

Opeyemi Ogunkuade

Supervised by

Professor Wole Soboyejo

Dr. Shola Odusanya

African University of Science and Technology

www.aust.edu.ng

P.M.B 681, Garki, Abuja F.C.T

Nigeria

July, 2021.

Certification

This is to certify that the thesis titled “Development Of Implantable Scaffold Used For The Generation Of Ear Tissue Cartilage With Potential Application In Bionic Ears ” submitted to the school of postgraduate studies, African University of Science and Technology (AUST), Abuja, Nigeria for the award of the Master's degree is a record of original research carried out by **Opeyemi Ogunkuade** in the Department of Materials Science and Engineering.

CERTIFICATION

Development Of Implantable Scaffold for Ear Tissue Cartilage with Potential Application in Bionic Ears

By

Opeyemi Ogunkuade

A Thesis Approved by the Department of Materials Science and Engineering

RECOMMENDED: _____

Supervisor, Professor Wole Soboyejo

Supervisor, Dr. Shola Odunsanya

_____

Prof. Peter Azikiwe Onwualu

Head, Department of Materials Science and Engineering

APPROVED: _____

Chief Academic Officer

_____

Date

ABSTRACT

The technology of Organ regeneration and Bionics is fast being adopted to the 21st century medicine. While the regenerative organs will help overcome the challenge of donor and acceptor of organs, bionics present with an opportunity to have advanced function of this organ beyond biological capability

This novelle solution of organ printing and cybernetics is used to determine a prospective solution of hearing loss through bionic ears. An approach of 3-D printing is applied which is also a reliable method for cartilage growing of ear tissue. In this study, we confirmed the feasibility of fabricating the cell-printed structure made up of a framework 3-D printed PLA scaffold, growing of human chondrocytes cells on the scaffold, which was observed by various cell growth and viability techniques

KEYWORDS: hearing loss, organ printing, 3-D printing, cybernetics, PLA scaffold, bionics, biomaterials, tissue engineering.

Acknowledgements

All thanks to God for the grace and strength to finish a phase like this in my life,

My profound gratitude goes to **Professor Azikiwe Peter Onwualu,** for his encouragement, support and academic mentorship, through out the period of my MSc Thesis. Most especially for his sincere concerns towards our academic goals and future careers as the H.O.D of the department you are indeed a mentor sir.

To **Professor Winston Wole Soboyejo** your passion for Africa and solving problems keeps me moving, also remembering every lecture of yours relating to how we can solve that very problem in. You are indeed a role model and worthy of emulation.

Special Thanks to **Dr Sola Odunsanya**, for your support and availability at the Laboratory to ensure my work making progress, mostly thank you sir for every effort you made to put everything in place and the right working conditions.

Special gratitude to **Dr. Ali Salifu** from WPI, for conducting some of my research at the WPI laboratory, also thank you for taking out time out from your busy schedules to explain concepts and principles to my work.

Much gratitude to **Theresa Ezenwanfor** for her kind support and understanding, mostly for making herself available to communicate and help solve challenges I faced during my work. Also thanks to my project colleagues Chinaza, Alex, Gati, it was really amazing to work together, mostly being able to overcome every challenge we faced.

My profound gratitude goes to all faculty members of material science and engineering departments who lectured me during my course work at AUST, **Dr Anye Vitalis**, **Dr Abdulhakeem Bello**, without doubt you have impacted me with knowledge, and that knowledge will take me round the world. Thanks to **Ejemen Salami** the H.O. D secretary for always been available and helping.

Special thanks to my Dad and Mum, **Engr. and Mrs Gabriel and Justinah Ogunkuade** and all my siblings, thank you for the love, support, provision. I say thank you for making me always have the best of education and giving me the best of the best and mostly for believing in me.

To my friends whom I have met from all over Africa and different parts of Nigeria at AUST, **Daizy, Ronald, Dona, Nancy, Seun, Blessing, Pablo, Musa, Cyril, Jamael, Thomas, Gati, Freddie, Francis, Eleanor, Nonso, Nimota, Darius, Johnson, Kawathar, Gono, Azuka, Chinaza**. I say we made it our studying was not in vain, and let us do something greater on a global level.

Table of Contents

CHAPTER ONE: INTRODUCTION

1.1 INTRODUCTION

Bionics, the ages-old practice of replacing body parts with mechanical devices, has quietly grown more sophisticated over the past few decades, with successful integrations of artificial hands, knees and organs[1][2]. Even when some of these organs are obtained from donors, and replaced through surgery in individuals who need them, well one of the challenges is the availability of donors versus acceptors. The field of bionic organs has the potential to provide humans with organs that have capacity beyond the normal human biological capacity[3]. Therefore, looking for a better approach towards making organs and body parts available can be achieved through tissue engineering and regenerative medicine.

The concept of tissues engineering primarily involves the growing of cell into an appropriate scaffold in the presence of a favorable growth environment. These scaffolds are mimicked to the extracellular matrix and also the material used has to be cautiously put in place. Several attempts to produce this scaffolds to match the ECM has been put together, but amongst all methods the 3-D printing technology surpasses them all, because of certain advantages.[3], [4].The technology of 3- D printing is no new form of technology as it is been used across several industries, it is also considered to be evolving quickly and making graceful impact in the health care sector.

In the health care sector, the application of 3D printers is already been applied in the production of, biomedical devices, prosthetics implant, targeted drug delivery[5], bionic organs among other applications as researches discover daily. While this printing technology stirs a form enthusiasm in the medical field, a lot has to be put in place in order to achieve this, putting into consideration that a human life at stake.

The technology of organ printing and tissue generation involves various field coming together ranging from the material engineers, a biologist, biomedical engineer and medical doctors, on an overall base we would say a combination of material engineers and medical personnel. While the very process of organ printing starts from obtaining a CAD drawing or even an Image from an MRI scan that is readable for printing, more so this image can be obtained from the patient very own anatomical data [6] from which it can be printed.

While there are so many methods of fabricating scaffolds that replicate the ECM for an organ or tissue growth, the technology of 3-D printing remains a groundbreaking print strategy, that allows the fabrication of certain living constructs to a certain degree of complexity and accuracy[2].it is clearly seen that the engineered structure is considerable different from the native counterparts, and this is largely attributed to the inability of other methods to provide adequate spatial positioning and precision-made. More so, there are varying technologies of 3-D printing which exist depending on factors (I.e. speed, resolution)a user gives preference too[7].

Obtaining of cells from the human body, then proceeding to grow this cells into organs or tissues is a major part of regeneration. Whereas in conventional procedure of transplant cases of organ rejection occur, but in growing the cells from scratch there are better chances of compatibility. Traditional tissue engineering procedure involves isolating this cells from sources(e.g tissue, blood e.t.c), grow them in specific environment in the presence of certain growth factors and directly seeded onto scaffolds that promote cell proliferation and differentiation into functioning tissues. While this might be in its infancy[5] stage, another application is that of the 3-D bioprinting, but in this work we focus on the traditional; 3- D printing and seeding into scaffolds

In a given work of regenerative medicine, tissue engineering or organ printing, the use of Biomaterials is adoptable. These biomaterials could be synthetic or natural, as such this biomaterials interact with the human cells or tissues or organs and sometimes even carrying out their functions, while some of them degrade over a period of time upon reacting with certain enzymes in the body[8][9]. Furthermore in the aspect of tissue engineering and relative to the work described here, this biomaterials can printed via the 3-DPrinters and cells are cultured on them, this enables the bio-materials to harness the innate abilities of the cells to sense the local environment, through a cell to cell, and a cell to extracellular matrix (ECM) contacts[10].For many tissue engineering applications, it is desirable to have the scaffold material degrade, allowing the material to be broken down as the scaffold material is replaced with cells or tissue and newly deposited ECM. Importantly, these degradation by-products must be biologically inert, so that they can be rapidly cleared after they have been broken down[10]. Generally for the generation scaffolds, additives processes are used , in which a successive layer of material is laid on a surface and cured over a period of time, producing this 3- D structures in different

geometries or shapes[11].More so the use of additives process are been used , but modern technologies considers 3-D printing as a better approach.

Using this technologies of 3-D printing and regenerative medicine we can address the medical issue of partial or total deafness, by recreating a model of the human ear, by growing the chondrocytes on an appropriate seeded scaffold and also imitating the cochlear of the human ear, using materials that detect sound waves and radio frequency for deficient patients who suffer hearing impairments.

1.1 Statement of problem

According to WHO, the world's population suffering from disabling hearing loss is about 5% which is about 430 million individuals. It is also estimated that by 2050, over 700 million individuals or 1 in 10 people will have disabling hearing loss.[12] .A key threshold in solving this problem will be through prevention, but in a circumstance of existing cases, a suggested approach is the use of medication, hearing aids, cochlear implant and Bionic ears. We seek to provide this solution through the bionic ears, which we consider as more advanced ears and may also be able to exhibit greater functionality than the human ear. Although, Surveys from numerous health organizations have found that hearing aids are under-used, with cost and stigma being top reasons people do not wear them, we intend to provide long term hearing aids that will be affordable, through the approach of regenerative tissue engineering.[13].

1.2 **AIM**

Development of Implantable Scaffold that can be used for the generation of ear tissue cartilage with potential application in Bionic Ears

1.3 OBJECTIVES

- 3-D printing of Ear Scaffolds
- Mechanical Characterization of printed ear scaffold
- Cell tissue culturing into Scaffold

1.4 Contribution to knowledge and the African Society.

Regenerative tissue engineering is an uprising advancement in the field of medicine and surgery among several newly discovered technologies. In general, Africa form of traditional medicine does not create room for the regeneration of new organs or even cyborg organs. A dive in

regenerative medicine will help improve the health sector of the African economy ranging from various medical problems like loss of human ear, loss of hearing and so many other problems that can be addressed through regenerative medicine. Furthermore, as reported the United nation of a ratio of 1: 5 suffering from hearing loss gives us a total of 200 million individual suffering including Africa. So therefore, a solution to this helps eliminate the problem of hearing. Even the motto from the world hearing day celebrated every 3rd of March, says here the future and prepare for it.

References

[1] Krane Jim, "Bionic Eye Follows Bionic Ear," *government technology*, May 28, 2002. https://www.govtech.com/public-safety/Bionic-Eye-Follows-Bionic-Ear.html (accessed Mar. 10, 2021).

[2] A. Shapira and T. Dvir, "3D Tissue and Organ Printing — Hope and Reality," vol. 2003751, 2021, doi: 10.1002/advs.202003751.

[3] M. S. Mannoor *et al.*, "3D printed bionic ears," *Nano Lett.*, vol. 13, no. 6, pp. 2634–2639, 2013, doi: 10.1021/nl4007744.

[4] A. Do, B. Khorsand, S. M. Geary, and A. K. Salem, "3D Printing of Scaffolds for Tissue Regeneration Applications," pp. 1742–1762, 2015, doi: 10.1002/adhm.201500168.

[5] L. C. Ventola, "Medical Application for 3-D Printing: Current and Projected Use," *p&T*, vol. 39, no. 10, 2014.

[6] H. N. Chia and B. M. Wu, "Recent advances in 3D printing of biomaterials," pp. 1–14, 2015, doi: 10.1186/s13036-015-0001-4.

[7] B. Applications *et al.*, "Biomaterials Based on Marine Resources for 3D," 2019.

[8] S. Bose, D. Ke, H. Sahasrabudhe, and A. Bandyopadhyay, "Progress in Materials Science Additive manufacturing of biomaterials," *Prog. Mater. Sci.*, vol. 93, pp. 45–111, 2018, doi: 10.1016/j.pmatsci.2017.08.003.

[9] B. R. Ringeisen, S. J. Barry, and W. K. Peter, *Cell Organ and Printing*. 2010.

[10] P. Bajaj, R. M. Schweller, A. Khademhosseini, J. L. West, and R. Bashir, “3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine,” no. May, 2014, doi: 10.1146/annurev-bioeng-071813-105155.

[11] D. J. Thomas and T. C. Claypole, “18 3-D Printing,” pp. 293–306, 2016, doi: 10.1016/B978-0-323-37468-2.00018-X.

[12] World Health Organization Court Room, “Deafness and hearing loss,” *World Health Organization*, Mar. 02, 2021. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss (accessed Mar. 23, 2021).

[13] Joy Victory, “Hearing loss: Symptoms, causes and treatments,” *Healthy Haring*, 2021. https://www.healthyhearing.com/help/hearing-loss (accessed Mar. 23, 2021).

[14] J. Davis, “Cells as Products Cell Culture in Tissue,” 2007.

[15] M. Weisberger, “11 Body Parts Grown in the Lab | Live Science,” *Live Science*, 2017. https://www.livescience.com/59675-body-parts-grown-in-lab.html (accessed Jul. 14, 2021).

[16] G. Vogel, “Organs Made to Order | Science | Smithsonian Magazine,” *SMITHSONIAN MAGAZINE*, 2010. https://www.smithsonianmag.com/science-nature/organs-made-to-order-863675/ (accessed Jul. 14, 2021).

[17] K. Kiaee, Y. A. Jodat, and M. S. Mannoor, “Bionic Organs,” pp. 167–192, 2020.

[18] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, and D. S. Kumar, “Polymeric Scaffolds in Tissue Engineering Application : A Review,” vol. 2011, no. ii, 2011, doi: 10.1155/2011/290602.

[19] K. K. Sadasivuni, “A Comparative Review of Natural and Synthetic Biopolymer Composite Scaffolds,” 2021.

[20] P. Chen *et al.*, “The 3D-Printed PLGA Scaffolds Loaded with Bone Marrow-Derived Mesenchymal Stem Cells Augment the Healing of Rotator Cuff Repair in the Rabbits,” vol. 29, pp. 1–13, 2020, doi: 10.1177/0963689720973647.

[21] A. G. Namboodiri, “BIOMATERIAL FABRICATION TECHNIQUES.”

[22] J. Liu and C. Yan, “3D Printing of Scaffolds Scaffolds for for Tissue Tissue Engineering Engineering,” doi: 10.5772/intechopen.78145.

[23] N. Altaee, G. A. El-Hiti, A. Fahdil, K. Sudesh, and E. Yousif, “Biodegradation of different formulations of polyhydroxybutyrate films in soil,” *Springerplus*, vol. 5, no. 1, 2016, doi: 10.1186/s40064-016-2480-2.

[24] J. S. Lee, J. M. Hong, J. W. Jung, J. H. Shim, J. H. Oh, and D. W. Cho, “3D printing of composite tissue with complex shape applied to ear regeneration,” *Biofabrication*, vol. 6, no. 2, 2014, doi: 10.1088/1758-5082/6/2/024103.

[25] F. Melchels, *Organ Printing*. Elsevier Ltd., 2011.

[26] S. Goshal, “carbon Nanotubed for 3- D printing.pdf.” 2017.

[27] H. A. E.-S. Kaoud, “Introductory Chapter: Concepts of Tissue Regeneration,” *Tissue Regen.*, Jun. 2018, doi: 10.5772/INTECHOPEN.76996.

[28] H. Jian, M. Wang, S. Wang, A. Wang, and S. Bai, “3D bioprinting for cell culture and tissue fabrication,” *Bio-Design Manuf.*, no. March, 2018, doi: 10.1007/s42242-018-0006-1.

[29] M. Sun *et al.*, “3D cell culture — can it be as popular as 2D cell culture?,” doi: 10.1002/anbr.202000066.

[30] T. Xu, D. Reyna-soriano, J. I. Rodrı, M. Bhuyan, and T. Boland, *Organ printing*. 2019.

[31] K. Storck *et al.*, “Total reconstruction of the auricle: Our experiences on indications and recent techniques,” *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/373286.

[32] U. S. P. No, “alamarBlue ® Assay,” no. 5.

[33] S. Al-Nasiry, N. Geusens, M. Hanssens, C. Luyten, and R. Pijnenborg, “The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells,” *Hum. Reprod.*, vol. 22, no. 5, pp. 1304–1309, May 2007, doi: 10.1093/HUMREP/DEM011.

[34] J. O'Brien, I. Wilson, T. Orton, and F. Pognan, "Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity," *Eur. J. Biochem.*, vol. 267, no. 17, pp. 5421–5426, 2000, doi: 10.1046/J.1432-1327.2000.01606.X/FULL.

[35] M. Abramowitz and M. W. Davidson, "Fluorescence - Overview of Fluorescence Excitation and Emission Fundamentals | Olympus LS," *Olympus*. https://www.olympus-lifescience.com/en/microscope-resource/primer/lightandcolor/fluoroexcitation/ (accessed Jul. 30, 2021).

Chapter two: Literature review

2.1 Regeneration of Ear Tissue using Polylactic acid scaffold, for Potential Bionic Ear Application

2.2 Tissue regeneration

The term reaeration and tissue engineering, is a whole context or course on its own, but here we look at it from a more narrowed perspective and not the study of the whole field. So therefore, we can say that tissue engineering is a field that evolved from biomaterial development, leading to the combination of scaffolds, cells and biologically active molecules into functional tissues[9]. Also, when talking about tissue engineering, we cannot ignore the term regenerative medicine but this is not within this scope of study[14].

Tissue engineering approaches are designed such that new or restored tissues and organs are produced; however, this is achieved when a scaffold(biomaterial) and a patient's cell is combined and placed within certain growth environment alongside some necessary factors. In the past several successes have been recorded. From a bladder creation and transplant by Dr. Atala Anthony, amongst several others which have been generated but yet to be clinical tried on a human. Below are some images of 3-D printed organs, with the bladder(B) as the only successful transplant on humans

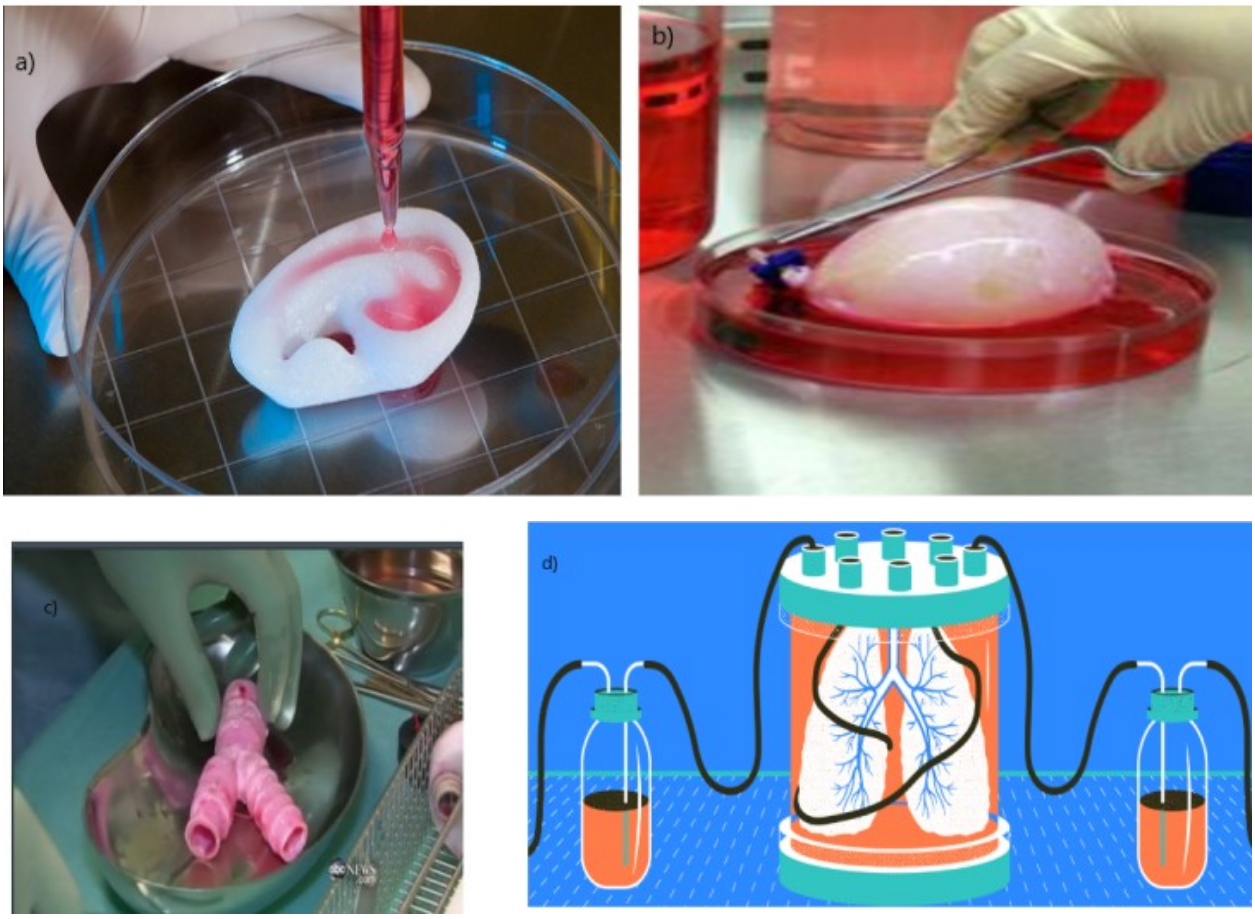

Figure 2-1a-d Illustration of 3-D printed organs and tissues.

The science of tissue engineering and regenerative medicine cannot be considered exactly new, even though it was developed in the 19th century. More or less it has been with nature but on a self-generating scale, an example is the limb of a starfish or a salamanders which can be re-grown after days, also a deer can re-sprout as much as 30 kilograms of antlers in 3 months, a zebrafish can regrow its heart within some days and a flat worm can re-grow its head[15]. While this seems totally impossible for humans the role of tissue engineering has come to bridge a gap, whereas if salamanders can do it then why not humans[16]

While scientist and researchers can print cells and biomaterials that form the human tissues, we still have a long way to go in printing functional organs. This is clearly seen in organs from 1-D organs or plain organs like skin, 2- dimensional organs, hollow body parts like veins then finally individual organs like kidney and hearts.

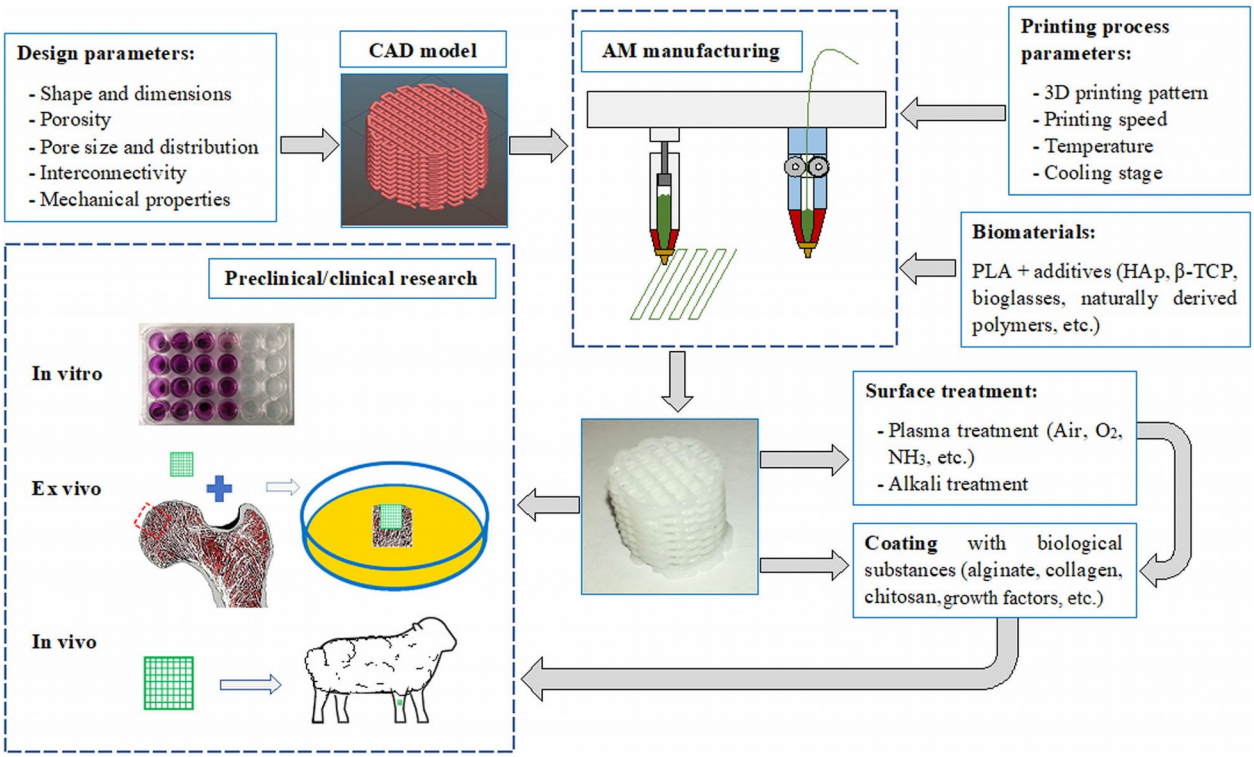

Figure 2-2 An Illustration chart showing stages for growing of cells on a scaffold

2.3 Tissue engineering a potential solution to Bionic Ears

With the area of tissue engineering evolving, there is also another field which seems to be growing alongside tissue engineering, this is the area of cybernetics which has led to novelle invention like the bionic ears, bionic hands and so many other body parts that can be mixed with artificial intelligence or technology. While it's still undergoing research, it has been mapped as the future of artificial intelligence alongside side a novelle in medicine. This newly developing body parts tend to exhibit functions greater than the human body as they have been modified to meet specific demand, according to a research by[3] he worked on the bionic ears which have the capacity to hear beyond the normal human hearing decibel of >85 decibels. Also the production of bionic organs /body parts , help assist humans with highly complex or hazardous task[17], overall the area of bionic organs or body parts have to serve human with varying functionalities that will be beneficial.

Figure 2-3 an Illustration of a bionic Eye

2.4 Challenges of Bionic organs

Some of the challenges of bionics is adding electronic devices to a natural giving organ, so therefore this organ has to be re-constructed exactly like the original organ, in order to avoid rejection or some from of modification beyond the human control.

2.5 Biomaterials for Scaffold Building and ECM replication

In tissue engineering, scaffolds are described as three-dimensional porous solid biomaterial which serves as a support to aid the growth of cells, proliferation of cells and tissues, promote cell interaction, cell adhesion, depositions of the ECM, through a means of in vivo or invitro culture. Whereas an implantable scaffold, is one that can be planted into a living human body such that the material is biocompatible, and should not be rejected by the human body[18]. Some of the essentials of scaffolds used in TE are illustrated in Figure 1 below

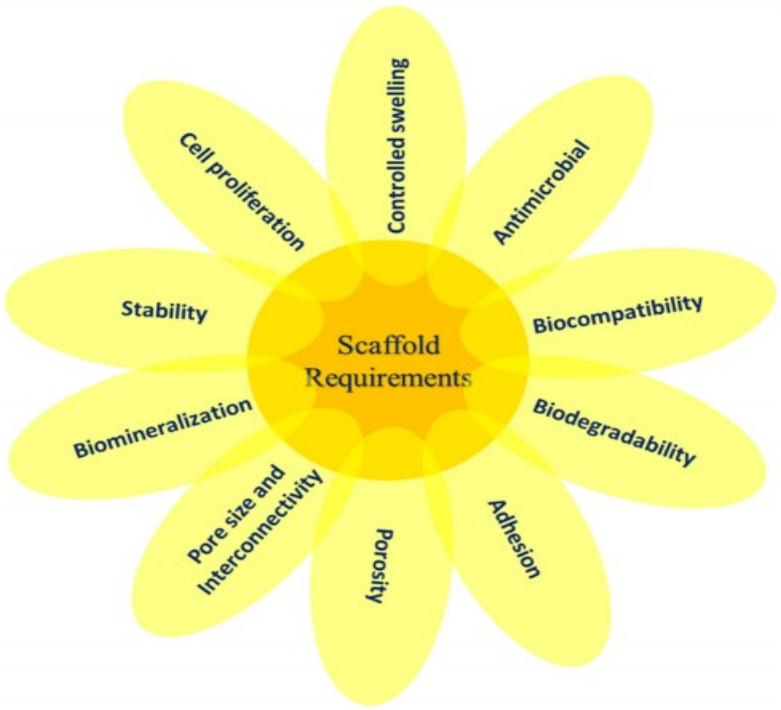

Figure 2-4 Essential characteristic properties for scaffold design in TE[19]

This implanted scaffold can as also serve as a mere representation of human extracellular matrix (ECM), to help growth of a lost body part, through a process of tissue engineering and regenerative medicine. Some of the properties of a good scaffold as suggested by [4], [20] are;

- Biocompatibility, that is it should not alter the biological composition of the body part that has be replaced and should also fit properly
- Excellent scaffold should not inhibit the proliferation of cells, tissues, organelles etc but rather enhance the infiltration in the needed site and overall improve the histologic score.
- the material should induce minimal inflammatory responses, thereby reducing the likelihood of rejection by the host's immune system
- It would also be beneficial if scaffold materials could behave as substrates that promote cellular attachment, proliferation and differentiation.
- As cells proliferate and differentiate, the scaffold must be able to withstand the forces being applied by the cells otherwise its collapse would result in poor diffusion of oxygen, nutrients and waste, leading to inefficient tissue formation.
- Should be able to produce biomimicry of the Extracellular Matrix

- Finally, the mechanical stability of the scaffold must be structurally sound so as to withstand daily activity and normal body movements
- Understanding the inflammatory responses of the polymer
- Cytotoxicity

Summarily, biodegradability, biocompatibility,pore size, porosity, mechanical properties, osteointegration ,osteogenesis, and osteoconductivity, are key design considerations for scaffolds design

2.5.1 Types of scaffold material in different application

Selecting a scaffold material varies depending on the functionality , which ranges from been a replacement of the extracellular matrix, to been an implant, also selection depends on certain properties like its mechanical properties, biological properties, cost, from this we have wide a range of materials to select from also considering its malleability in forming a desired morphology of scaffolds[4]. But most importantly, this material must be a biomaterial. These different classes of materials that can be used are explained as follows;

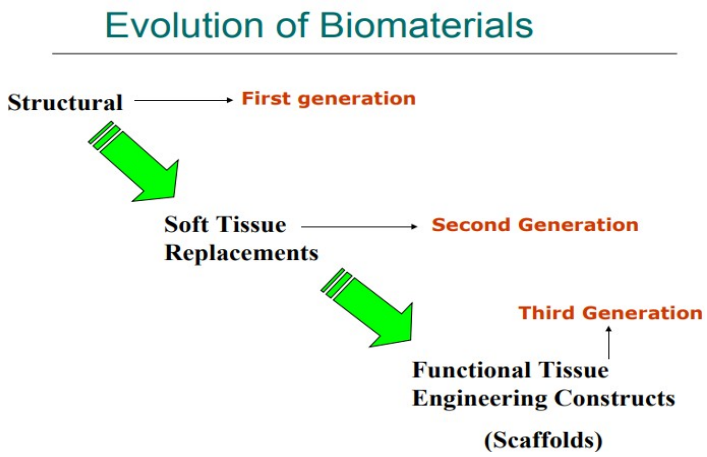

Figure 2-5 showing an Evolution of biomaterials over time*[21]*

2.5.2 Metals

Looking at the various metals across the periodic table, with their valence electrons and their different properties, specific metals are used in tissue engineering as both implantable scaffolds, external scaffold and prosthetics. Metals which generally have varying mechanical properties from an high young modulus, to their toughness as seen under a stress strain curve, then their elasticity, all these properties have narrowed the list to selecting certain metals that fit the need

of different application in tissue engineering, they include Titanium alloys, Vandanium, Cobalt, stainless steel, chromium alloys, nitinol, and others among which are used for majorly biomedical implants. In using these metals as scaffolds, they need to be passed through different metal work such as annealing, forge casting e.t.c depending on the use. In the case of 3-D printing of this metal scaffold, several works have to be done, as technology of metal printing is still been studied. On a general note, metallic scaffolds have glaring potentials such as the strength provided by the metals to support the cell growth, but limitation such as corrosion in metals will lead to the release of toxic metallic ions, which can degrade in the body. The use of metal is hindered also by its long-term degradation time, which results to the formation of functional tissues instead of a total replacement of tissue in the region. Ultimately, the limitation of metals is the fact that it is not exactly bio-degradable, for instance in the making of an ECM like scaffold which is meant to degrade into the body, and come out as waste is not possible. Notwithstanding, new “bio-degradable metals” have emerged[22]. A metal is considered to be a biodegradable metal if it has a suitable degradation rate in -vivo, secondly if they have the ability to metabolize via the body pathway easily, examples of this metals are usually magnesium-based, Iron-based, Zinc-based or calcium based and which consist of the pure metals themselves, alloys or metal matrix composite.

2.5.3 Ceramics

The use of ceramics for scaffolds in tissue engineering has adversely been explored. Ceramics which have a composition of metallic and non -metallic materials adds to the strength, also having an advantage of been biocompatible. The use of ceramics is commonly seen in teeth replacement, bone tissue regeneration due to their apatite-mineralization ability. An example of a ceramic material used for scaffold building is a hydroxyapatite, which is commonly found in the enamel of a human teeth and bone. Hydroxy apatite is a commonly used material in 3-D printing of scaffolds. According to HA allows the proliferation of cells growth for bone generation as seen in a study conducted using a mouse MC3T3-E1. Another study shows the combination of HA and Tricalcium phosphate (TCP) for scaffolds via 3- D printing, upon seeding of a human osteoblast to the scaffolds obtained from a human iliac crest cancellous bone, a high biocompatibility and cytotoxicity was observed, which are major properties of a good scaffold. In recent times ceramics like calcium silicate ($CaSiO_3$) is used, when compared to TCP, it was found that Casi03 could give better bone regeneration, when planted in vivo into femur defects in

rats' other ceramics that can be used include calcium phospahate and TCP. Scaffolds made from ceramics have very high prospects mostly if researched further on in areas of adequate compressive strength, ability to promote cell proliferation and differentiation that will be applicable in various uses of tissue engineering.[4]

2.5.4 Composites

Composites are another category of materials, with great prospects, they are important in tissue engineering because of their composition, i.e. mixing or combing different materials with varying properties to suit a certain specification. Generally, we can say that ceramics scaffold aids the increase in mechanical strength and more intricately designed scaffold.

2.5.5 POLYMERS

Polymers are with no doubt a major material for 3-d printing in tissue engineering. They are divided into natural polymers and synthetic polymers. Natural polymers do have bio-active properties and better interactions with cells owning to the fact that they are naturally produced and derived from living organism or the native ECM components such as collagen, Hyaluronic acid, Matrigel, fibrin, although they have been created from non-mammalian sources like alginate and seaweed. A major advantage of this bio-active properties is the interaction with cells, which gives room for enhanced performance in a biological system. Whereas synthetic polymers are often regarded as been cheaper than this natural polymer scaffolds, as mass production can be possible within allowed degree of uniformity, also allowing for long term storage. Coupled with the fact that these polymers are been produced with certain degree and factors, their properties can also be modified to suite certain conditions such as the tensile strength, porosity, elastic modulus and degradation rate. Some of these common polymers which are also co-polymers are PLA, PGA, PLGA. More so these synthetic polymers show physicochemical and mechanical properties compared to that of biological tissues.

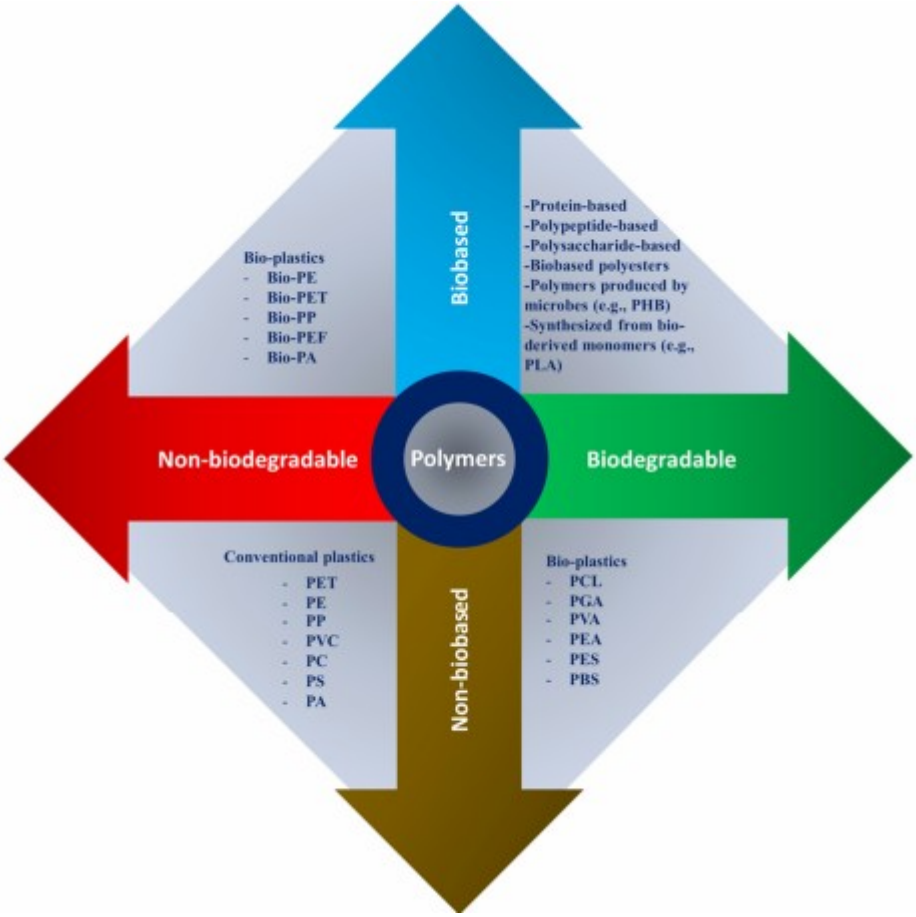

Figure 2-6 Showing A Combination Different Polymers for Scaffold According To Various Sub- Properties

2.5.6 Natural polymer scaffolds

With several varying properties of polymers as non-conductors, covalent bonds, carbon-hydrogen chains and so many other properties, we can classify some naturally existing materials as polymers. Also, we can classify them based on their monomeric units and molecular structure, and this classification is roughly classified into three they are;

1. **Polypeptide and protein-based biopolymers**, examples are fibrin, gelatin keratin, silk, actin, fibrinogen, elastin etc
2. **Polysaccharide based biopolymers** examples include chitin, chitosan, alginate, hyaluronic acid, cellulose, agarose, dextran, and glycosaminoglycans.
3. **Polynucleotide based biopolymers** examples are DNA, linear plasmid DNA, and RNA. These consist of long chains, including nucleotides, amino acids.

Table 2-1 Natural polymers used for scaffold fabrication with advantages and disadvantages

S/N	Polymer	Sources	Advantages	Disadvantages
1	Collagen	Native ECM	• Biodegradable • Excellent biocompatibility • Low toxicity • Low immunogenicity • Advantageous for cell adhesion, differentiation and ECM secretion • Weak antigenicity	• Scaffold deformation and contraction due to load bearing tissues • Inadequate stability in aqueous environment • Low mechanical strength • Difficult disinfection
2.	Silk Fibroin	Protein based polymer from Lepidoptera larvae(E,g Spider, Silk worm)	• Biocompatibility • Osteoconductivity • Good support for cell adhesion and proliferation with giving in to cell toxicity • Light weight with unique strength and elasticity • Vascularization of cells and easy migration • Averagely degradable • Thermostable (approx.. 250^{o}c) • Used mostly as cell carrier for cell seeding on scaffold	• Not best for long term usage as presence of silk can lead to deposition of unwanted residue which may prompt immune response
3	Fibrinogen and Fibrin	ECM	• Considerable cell adhesive and binding properties • No immunogenicity • Room for cellular interactions • Biocompatibility • Excellent affinity for biological surfaces	• Very fast degradation rate • Relatively low mechanical strength
4	Gelatin	Insoluble collagen(Alka line or acidic hydrolysis type 1 Collagen)	• Better infiltration, • Biodegradability • Low antigenicity • Ability to maintain stable temperature across varying PH.	• Increased quantity of gelatin reduces bioactivity processes in scaffold • Low stability
5	Keratin		• Speeds up cell	• Poor

			adhesion and proliferation • Cytocompatibility • Gradual degradation	mechanical properties
1	Starch	Cellulose in plants	• Biocompatible • Hydrophilicity • Serves as good substrate for cell adhesion • God degradation period • Non- cytotoxic • Thermoplastic behavior	• High water absorption rate • Low mechanical strength • Release of by products when modified chemically • Not suitable for long term use
2	Chitin and Chitosan	Crustacean shells Chitosan- deacetylation of chitin	• Speeds up the repair of tissues • Promotes cell adhesion • Prevents scar formation • Bioactivity • Anti-inflammatory • Oestoconductivity • Hemostatic potential • Non toxic • Little/no allergies	• High viscosity • Rapid in vivo degradation • Poor mechanical strength
3	Agarose		• Excellent biocompatibility • Suitable for cell medium encapsulation • Non immunogenic	• Low cell adhesion • Nondegradability due to the absence of an appropriate enzyme in the body.
4	Alginate	Algae Sea weed	• Mimics the function of the ECM of body tissues • Biocompatibility • Biodegradability • Cytocompatibility • Thickener/ gel forming agent	• Difficult to sterilize • Low cell adhesion • Poor mechanical strengths
5	Cellulose	Plants	• Appropriate mechanical strength • Hydrophilicity • Biocompatibility • Stable for matrix for tissue engineering application	• Behaves non degradable when present in the human body
6	Hyaluronic acid	Extracellular tissues	• Noninmmuniogenic • HA scaffolds	• Brittle; mechanical properties need

			are frequently used in the case of both hard and soft tissue regeneration • Biocompatibility • nonantigenic	fine-tunning via chemical modification.
7	Glycosaminoglycans		• Anticoagulant activity • Biocompatibility • Anti-inflammatory • Controlled inflammatory processes •	• Very fast degradation • Possibility of risk contamination with infectious agents

Synthetic polymers are often cheaper than biologic scaffolds; it can be produced in large uniform quantities and have a long shelf time. Many commercially available synthetic polymers show physicochemical and mechanical properties comparable to those of biological tissues. Synthetic polymers represent the largest group of biodegradable polymers, and they can be produced under controlled conditions.

Table 2-2 Synthetic polymers used for scaffold fabrication with advantages and disadvantages

Synthetic Polymers					
S/N	Polymer	Sources	Advantages	Disadvantages	Degradation Rate
1	Poly(E-caprolactone) PCL		• Non-toxic • Cytocompatibility • Degrades by hydrolysis or bulk erosion • Good mechanical properties • Slower degradation rate compared to PLA and PLGA	• Low bioactivity • Hydrophobicity which hinders wound healing	2-4 years by hydrolysis
2	Polylactic acid(PLA)	Lactic Acid(from fermentation of Grain, maize, wheat)	• Cytocompatibility • Biocompatibility • Thermal stability • Excellent	• Brittleness • Hydrophobicity • Poor thermal stability • Lack of ideal surface	3-6 months by hydrolysis

			mechanical strength • Good degradation rate • Non-toxic degradation products	chemistry which aids cell adhesion	
3	Polylactic-co-glycolic acid(PLGA)		• Remarkable cell adhesion and proliferation • High mechanical strength • More faster degradation rate compared to PGA or PLA • Varying rates of degradation	• Likely not to yield biocompatibility • Low Osteoconductivity	12weeks
4	Polyglycolic acid(PGA)		• Possess Biocompatibility features • High melting point • Bulk degradation • Hydrophilicity properties	• Very sensitive to hydrolysis • Difficulty in creating pores without the use of Toxic solvent	1-2 months by hydrolysis
5	Polyhydroxybutyrate(PHB)		• Biocompatible • Biostable • No toxicity • Slow degradation rate • Advantages over PLA and PGA • Obtained naturally from b-hydroxy acid	• Cumbersome to handle due to viscous liquid nature at 21°C	3weeks[23]
6	Polypropylene carbonate(PPC)		• A biodegradable amorphous • No inflammatory response • Impact resistance • Biocompatibility	• cumbersome as well due to viscous flow at room temperature • Large brittleness at low temp. • Poor thermal and processing properties • Cell attachment is hindered	Enzymatic hydrolysis

				due to highly hydrophobic nature	
7	Polyurethane(PU)	Urethane moiety	• Biocompatibility and hemocompatibility • Biodegradable • Non-allergenic • Excellent mechanical strength	• Limited stability invivo	-
8	Poly(Ethylene glycol)(PEG)		• Biocompatibility • Elasticity • Bio adhesive • Non-ionic • Hydrophilic • Non-immunogenic • Can be easily modified to different moieties to pass different requirements of a skin. • Non-immunogenic	• Non-reactive, creates an insoluble network • Bio-inert nature which cause no cell-interaction.	-
9	Polyvinyl alcohol(PVA)		• Biocompatible • Noncarcinogenic • Similar tensile strength to human articular cartilage • Good lubrication • High water content, highly hydrated water soluble polymer	• No cell adhesiveness • Less ingrowth of bone cells	-
10	Polypropylene fumarate(PPF)		• Biocompatibility • Non-ionic • Non-toxic • When degradation occurs remnants are easily passed out of the body through	• Viscous liquid at room temperature making the polymer handling somewhat cumbersome	-

			metabolic processes • Bio-adhesive • Mucoadhesive • Elasticity		

2.5.7 Properties of Polymers for Tissue generation

In selecting a polymer for any given functions in tissue engineering, the following properties have to be considered which are the material Intrinsic nature, Processing condition and Final processing[19]. The intrinsic properties are material property dependent on its chemical structure and composition (e.g temperature, mechanical strength, gas barrier properties, transparency, solubility, density, crystallinity). While the processing characteristics include melt flow, index, and melting strength. Also key constraints to considers are the biological properties such as Immuniogenity, biocompatibility,biodegradability e.t.c

With the listed table above, we can therefore pick a suitable polymer based on availability, bio-fabrication processes, and interaction among other factors. This work places us in the scope of selection of implantable scaffold for cartilage generation, so therefore we have considered the scaffold PLA which tends to suit our usage. One major factor considered here is the fabrication method, which will require the use of 3-D printing an uprising technology, which allows the printing of a part according to what has been drawn and sent. Secondly, the degradation rate of the PLA which is about 4-6 months gives sufficient timing for the growth of this cartilaginous tissue body part. Lastly the mode of degradation of PLA is not toxic as this allows hydrolysis in the presence of simulating body fluids (SBF).Also, due to the crucial features that PLA offers, it has gained approval by the FDA (Federal Drug Administration).

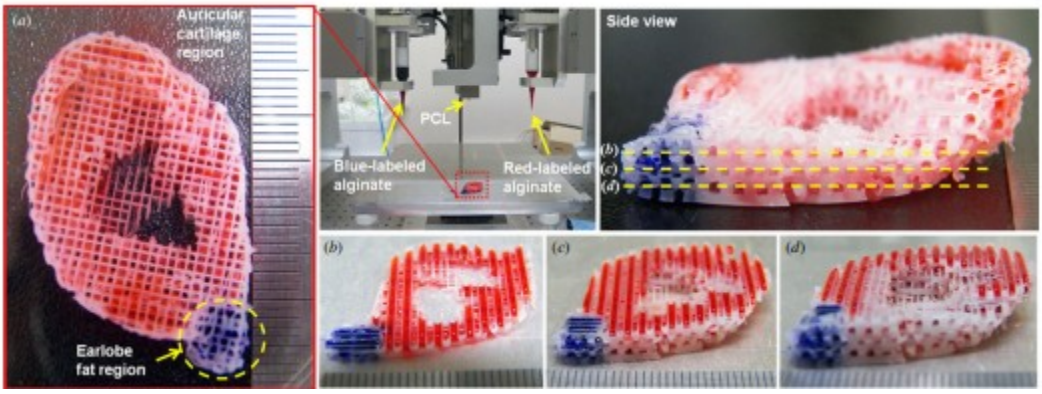

Figure 2-73-D printed Ear from PEG AND PCL*[24]*

2.5.8 DEGRADTION RATE OF POLYMERS

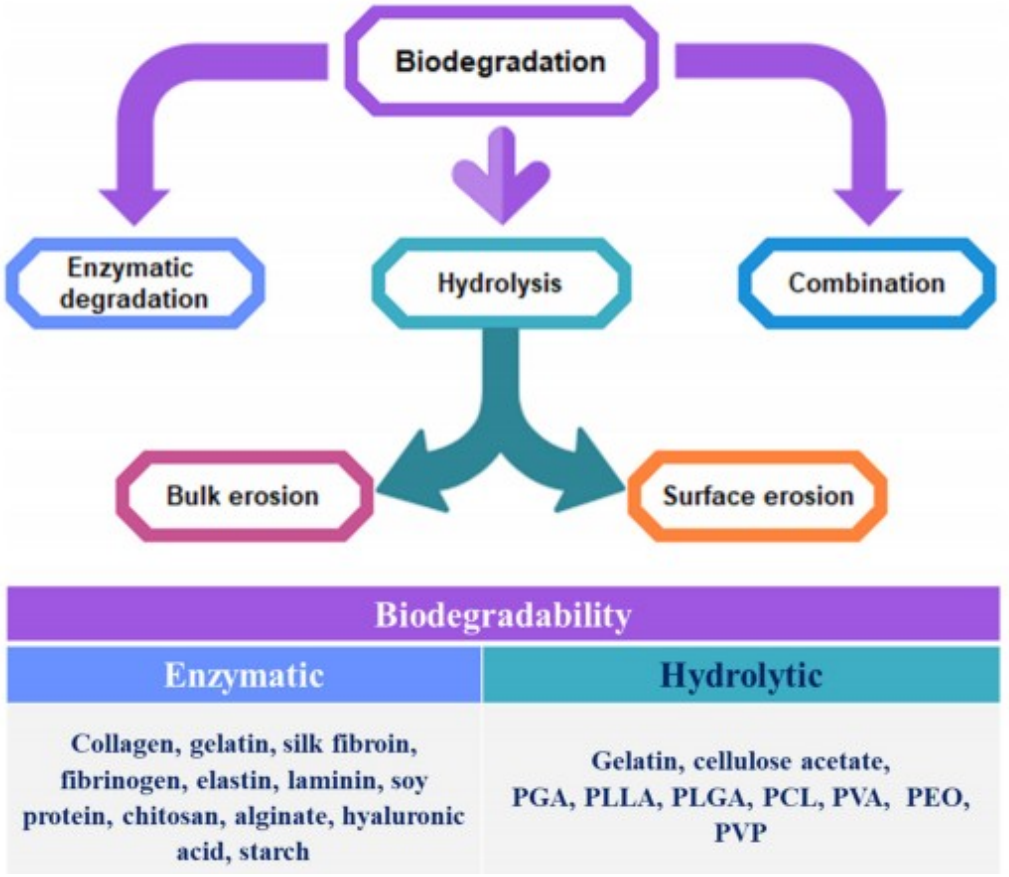

Figure 2-8 Biodegradation mechanisms of polymers *[19]*

2.6 Scaffold fabrication

The process of scaffold fabrication is without doubt an important stage in tissue engineering, as this scaffold tends to mimic the ECM of the human body for either in vivo or in vitro culturing. Also, these scaffolds are porous and serves as a matrix for initial cell attachment and afterwards for tissue growth. Over a period, time several techniques have been adapted in making of this scaffolds and they include self-assembly, electrospinning, solvent casting/ particulate leaching, phase separation, gas foaming and melt molding[25][26][4].

A. **Solvent Casting/ Particulate Leaching**: This involves mixing the polymer solution with salt(NaCL), upon hardening it is then placed in a solvent that can dissolve the salt. Porosity produced is about 87% while spacing is over 100µm up to 500 µm

B. **Melt Molding** ; this method of fabrication involves the use of a mold with polymer melt and gelatin micro-spheres, the polymer attains the shape of the mold and when it solidifies the mold is removed and the gelatin is leached. obtaining the shape of the mold when it solidifies obtaining.

C. **Gas Foaming**: this process is a combination of Particulate leaching and Melt Molding. In this case Ammonium bicarbonate is added to the polymer solution in chloroform. The resultant mixture is then highly viscous and can be shaped with a mold. The solvent then undergoes evaporation and the composites is immersed in hot water or vacuum dried. Porosity obtained is as high as 90% with pore sizes between 200-500 µm.

D. **Freeze** D**rying**: this method is achieved by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to the gas phase. Porosity is up to 95% while pore size is between 13-35 µm

E. **Fiber Bonding:** Fiber materials are immersed in polymer solution. Porosity is up to 81%, pore size is up to 500µm.

F. **For Nano technologies:** Material fabrication is also geared toward nanotechnology, some of this fabrication process include electrospinning and self-assembly. Whilst self-assembly comprises of spontaneous organization of individual component into an ordered and stable structure with pre-programmed non covalent bonds, while electrospinning involves the use of electric charge to form a mat of fine fibers.

G. **Rapid Prototyping technique:** this is a family of fabrication process developed to make the scaffold available within a short period of time, and this is achieved through CAD drawing processes available. The Rapid prototyping method is advantageous as earlier detection helps in reduction of design errors, there is improved ability to visualize the geometry due to physical existence, ability to create assemble parts. Rapid Prototyping is further divided into;

   i. Three-dimensional printing(3DP)
   ii. Stereolithography (SLA)
   iii. Selective Laser Sintering (SLS)
   iv. Fused Deposition Modelling (FDM)
   v. Organ Printing

vi. Membrane Lamination

Three-Dimensional printing: this method was developed in MIT by bredt in 1998, here a layer of powder is spread on the platform, the ink jet printer head deposits drops of binder on cross section, the binder dissolves and join adjacent powder particles then the table is lowered by layer thickness. The dimensional printing is tied to several advantages such as easy processing, achievable pore size (45-500 μm), high porosity, with various options of materials.

Stereolithography: this comprises of a photocurable monomer by a laser beam, its part are constructed in layers of thickness. The UV laser solidifies parts in cross-section. Photopolymerization occurs by the exposure of liquid resin to laser.

Selective Laser Sintering: here there is a moving laser beam, which sinters heat-fusible powders in areas corresponding to the CAD geometry which models one layer at a time to build the solid part. This printer helps us achieve pore sizes of 45-200 μm. High surface area to volume ratio, a complete pore interconnectivity, wide range of materials used here. While some of its disadvantages remain, high processing temperature.

Fused Deposition Modelling: this printer makes use of a nozzle to extrude a fiber of polymeric material (x-axis and y-axis control), the material is built layer by layer. The model is lowered(Z-axis) and the procedure is repeated. Some of it advantageous features include 250-1000 μm pore sizes, macro shape control, solvent and very good compressive strength. A disadvantage here is limited material range, inconsistent pore opening in x-y and z directions

Organ Printing/3-D Bioprinters: this printer is similar to the ink jet, its prints gels that are thermo responsive, cells are sprayed onto the solidifying thin layer of polymer solution. Some of its disadvantage is that cells can be damaged and choices of material is limited.

Membrane lamination technique: here a laser is used to cut out the exact shape needed, another layer is then cut and placed on top of it and pressure is applied to adhere both layers, finally the solvent is evaporated

2.7 Organ and body part regeneration through cell culture

The regrowing of a body part involves a lot of processes, but one major procedure is that which has to do with the cells, firstly obtaining the cells from a source, preserving it and the growing

into a seeded r a pre-seeded biomaterial scaffold. While other species have capabilities to regenerate parts or organs, by studying this and having a great understanding of the molecular basis of regeneration mechanisms we can apply to the human specie[27]. The regeneration of ear via tissue engineering is considered promising, the human ear which mainly consist of elastic cartilage and fat tissue and has a very complex shape is quite difficult to regenerate[24]. In as much, the cartilage of the ear auricle has a non-vascular structure which is supported by the ECM and collagen fibers. More so, the proliferation of chondrocytes and cell density has a slower rate than other cells, making the cartilage to have a slow self-renewal. Damages and diseases like microtia, anotia and even accidents are replaced through the use of prosthesis or carved rib cartilage, while this treatment has varying disadvantage, the use of tissue engineering has come to bridge this gap.

2.8 Cell to tissues and Organs

Every organ or tissue generation starts from the collection of cells, in most cases, these cells are usually obtained from a donor cell in the case of an organ transplant, whereas they can also be obtained from other individuals for the purpose of study and research. Majorly we have three different cell sources, the (i) Primary cells;(ii) Secondary cell (iii) Cell lines. After the cells are obtained from any others sources a culturing technique will now be decided. The cells generally behave like a normal living being, just that they are being studied as a single unit, also just like the human body the cells have human characteristics MR NIGER D

Movement: every cell in a cell culture medium moves as it grows, depending on the medium, while some attach themselves to cell walls others float above the culture medium, which is also similar to humans.

Nutrition: cells are fed with nutrients like amino acid, carbohydrates, vitamins minerals serum and so unlike the human body has to feed with all seven different nutrients

Irritability: different factors make cells have irritation ranging from factors like their PH buffer, osmotic condition, also the nature of scaffolds materials as explained earlier.

Growth: the growth of cells during cell culture often leads to a term called differentiation, as when cells grow in a cell medium, they increase over a period of weeks depending on the cell line and room is created for the cells to further subdivide

Excretion: Just like human's cells excrete, in this case they produce CO which is not healthy and this flushed away

Reproduction: cells multiply/reproduce provided they are in a good and favorable environment

Death: Cells die when these nutrients for growth are not provided for them, as well when intoxicated in the release of metabolic waste.

2.8.1 Types of Cell Culture

2.8.1.1 2-D Culture

2- d culture was the first from of culturing embraced amongst scientist for addressing several problems like drug screening and testing, studying of different cellular types. In this technique, the monolayer allows cell growth over a polyester or glass flat surface, which presents a medium that feeds the growing cell population. Several biological breakthroughs have occurred through the 2-D culturing techniques, even with the presence of certain limitations.[28] due to simplicity, this technique does not provide accurately and simulate the rich environment and complex processes observed in vivo, this includes properties or instances like cell signaling, chemistry and geometry cells. Conclusively data gathered from 2-D culture are invariantly misleading and not predictive for in vivo application.

2.8.1.2 3-D Culture

3-D culturing comes in different cell culturing techniques, but one major advantage of this over the 2_D culturing technique is that there is room for growth. Also 3- D cell culture facilitates cell differentiation and tissue formation cause of organization unlike the 2-D technique.[29] This 3-D technique shows clearly morphological and physiological changes when viewed in a image processing technique. Furthermore, researchers have said that the geometry and composition of this cellular support can help in influencing genes expression and also enhance cell communication. Finally, using 3D cell culture **make it easier to control and monitor the growing cells micro-environment parameters** (temperature, chemical gradients, oxygen rate, pH, etc.) to a certain extent while remaining as close to reality as possible thanks to micro-engineering (microfluidic).

Every cell culture needs certain favorable conditions to be grown in, they are, a substrate (source of nutrition), ideal temperature range (controlled), gworth medium, and, ideal pH among others necessities' within the environment[30].

2.9 Tissue engineering and approach to Bionics (Bionic Ears

Relatively the term bionics is regarded as a combination of biology and electronic. In recent times, scientist and researches are coming to terms that organs or tissue which are bionics tend leave us in position of deciding whether this new organ ends as a tissue and begins with electronics.

More recently, varying action have been conducted by various researches on developing new bionic organs, a given instance is a bionic tissue developed by a group of Harvard researchers. The technology of bionics has rather come to stay, though challenging so many progresses is being made. The bionic ear designed by [3] was 3-D printed by fully interweaving a functional biological tissue and an electronic components and a cell seeded hydrogel. To demonstrate this proof of concept this samples were printed alongside a cochlea shaped electrode, upon analysis it was found to transmit signals across the extended frequency spectrum. Having this information, we consider the bionic ears as devices that help restore or enhance the human hearing capacity, which falls in the field of cybernetic and this is an area of interest to many scientist and researches. Ongoing research on the bionic ears is even more enthusiastic because of its special ability to hear beyond the normal biological hearing range[3]

However, attaining seamless three dimensionally (3D) entwined electronic components with biological tissues and organs is significantly more challenging. Tissue engineering is guided by the principle that a variety of cell types can be coaxed into synthesizing new tissue if they are seeded onto an appropriate three-dimensional hydrogel scaffold within an accordant growth environment. Following in vivo or in vitro culture, tissue structures form which possess the morphology of the original scaffold. A major challenge in traditional tissue engineering approaches is the generation of cell-seeded implants with structures that mimic native tissue both in anatomic geometries and intratissue cellular distributions. Techniques such as seeding cells into non adhesive molds or self-folding scaffolds have been used to fabricate three-dimensional tissue constructs with complex 3D geometries. Yet, existing techniques are still incapable of easily creating organ or tissue parts with the required spatial heterogeneities and accurate

anatomical geometries to meet the shortage of donor organs for transplantation. For instance, total external ear reconstruction with autogenous cartilage with the goal of recreating an ear that is similar in appearance to the contralateral auricle remains one of the most difficult problems in the field of plastic and reconstructive surgery[31].

[1] Krane Jim, “Bionic Eye Follows Bionic Ear,” *government technology*, May 28, 2002. https://www.govtech.com/public-safety/Bionic-Eye-Follows-Bionic-Ear.html (accessed Mar. 10, 2021).

[2] A. Shapira and T. Dvir, “3D Tissue and Organ Printing — Hope and Reality,” vol. 2003751, 2021, doi: 10.1002/advs.202003751.

[3] M. S. Mannoor *et al.*, “3D printed bionic ears,” *Nano Lett.*, vol. 13, no. 6, pp. 2634–2639, 2013, doi: 10.1021/nl4007744.

[4] A. Do, B. Khorsand, S. M. Geary, and A. K. Salem, “3D Printing of Scaffolds for Tissue Regeneration Applications,” pp. 1742–1762, 2015, doi: 10.1002/adhm.201500168.

[5] L. C. Ventola, “Medical Application for 3-D Printing: Current and Projected Use,” *p&T*, vol. 39, no. 10, 2014.

[6] H. N. Chia and B. M. Wu, “Recent advances in 3D printing of biomaterials,” pp. 1–14, 2015, doi: 10.1186/s13036-015-0001-4.

[7] B. Applications *et al.*, “Biomaterials Based on Marine Resources for 3D,” 2019.

[8] S. Bose, D. Ke, H. Sahasrabudhe, and A. Bandyopadhyay, “Progress in Materials Science Additive manufacturing of biomaterials,” *Prog. Mater. Sci.*, vol. 93, pp. 45–111, 2018, doi: 10.1016/j.pmatsci.2017.08.003.

[9] B. R. Ringeisen, S. J. Barry, and W. K. Peter, *Cell Organ and Printing*. 2010.

[10] P. Bajaj, R. M. Schweller, A. Khademhosseini, J. L. West, and R. Bashir, “3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine,” no. May, 2014, doi: 10.1146/annurev-bioeng-071813-105155.

[11] D. J. Thomas and T. C. Claypole, “18 3-D Printing,” pp. 293–306, 2016, doi:

10.1016/B978-0-323-37468-2.00018-X.

[12] World Health Organization Court Room, “Deafness and hearing loss,” *World Health Organization*, Mar. 02, 2021. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss (accessed Mar. 23, 2021).

[13] Joy Victory, “Hearing loss: Symptoms, causes and treatments,” *Healthy Haring*, 2021. https://www.healthyhearing.com/help/hearing-loss (accessed Mar. 23, 2021).

[14] J. Davis, “Cells as Products Cell Culture in Tissue,” 2007.

[15] M. Weisberger, “11 Body Parts Grown in the Lab | Live Science,” *Live Science*, 2017. https://www.livescience.com/59675-body-parts-grown-in-lab.html (accessed Jul. 14, 2021).

[16] G. Vogel, “Organs Made to Order | Science | Smithsonian Magazine,” *SMITHSONIAN MAGAZINE*, 2010. https://www.smithsonianmag.com/science-nature/organs-made-to-order-863675/ (accessed Jul. 14, 2021).

[17] K. Kiaee, Y. A. Jodat, and M. S. Mannoor, “Bionic Organs,” pp. 167–192, 2020.

[18] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, and D. S. Kumar, “Polymeric Scaffolds in Tissue Engineering Application : A Review,” vol. 2011, no. ii, 2011, doi: 10.1155/2011/290602.

[19] K. K. Sadasivuni, “A Comparative Review of Natural and Synthetic Biopolymer Composite Scaffolds,” 2021.

[20] P. Chen *et al.*, “The 3D-Printed PLGA Scaffolds Loaded with Bone Marrow-Derived Mesenchymal Stem Cells Augment the Healing of Rotator Cuff Repair in the Rabbits,” vol. 29, pp. 1–13, 2020, doi: 10.1177/0963689720973647.

[21] A. G. Namboodiri, “BIOMATERIAL FABRICATION TECHNIQUES.”

[22] J. Liu and C. Yan, “3D Printing of Scaffolds Scaffolds for for Tissue Tissue Engineering Engineering,” doi: 10.5772/intechopen.78145.

[23] N. Altaee, G. A. El-Hiti, A. Fahdil, K. Sudesh, and E. Yousif, “Biodegradation of different formulations of polyhydroxybutyrate films in soil,” *Springerplus*, vol. 5, no. 1,

2016, doi: 10.1186/s40064-016-2480-2.

[24] J. S. Lee, J. M. Hong, J. W. Jung, J. H. Shim, J. H. Oh, and D. W. Cho, “3D printing of composite tissue with complex shape applied to ear regeneration,” *Biofabrication*, vol. 6, no. 2, 2014, doi: 10.1088/1758-5082/6/2/024103.

[25] F. Melchels, *Organ Printing*. Elsevier Ltd., 2011.

[26] S. Goshal, “carbon Nanotubed for 3- D printing.pdf.” 2017.

[27] H. A. E.-S. Kaoud, “Introductory Chapter: Concepts of Tissue Regeneration,” *Tissue Regen.*, Jun. 2018, doi: 10.5772/INTECHOPEN.76996.

[28] H. Jian, M. Wang, S. Wang, A. Wang, and S. Bai, “3D bioprinting for cell culture and tissue fabrication,” *Bio-Design Manuf.*, no. March, 2018, doi: 10.1007/s42242-018-0006-1.

[29] M. Sun *et al.*, “3D cell culture — can it be as popular as 2D cell culture?,” doi: 10.1002/anbr.202000066.

[30] T. Xu, D. Reyna-soriano, J. I. Rodrı, M. Bhuyan, and T. Boland, *Organ printing*. 2019.

[31] K. Storck *et al.*, “Total reconstruction of the auricle: Our experiences on indications and recent techniques,” *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/373286.

[32] U. S. P. No, “alamarBlue ® Assay,” no. 5.

[33] S. Al-Nasiry, N. Geusens, M. Hanssens, C. Luyten, and R. Pijnenborg, “The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells,” *Hum. Reprod.*, vol. 22, no. 5, pp. 1304–1309, May 2007, doi: 10.1093/HUMREP/DEM011.

[34] J. O’Brien, I. Wilson, T. Orton, and F. Pognan, “Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity,” *Eur. J. Biochem.*, vol. 267, no. 17, pp. 5421–5426, 2000, doi: 10.1046/J.1432-1327.2000.01606.X/FULL.

[35] M. Abramowitz and M. W. Davidson, “Fluorescence - Overview of Fluorescence Excitation and Emission Fundamentals | Olympus LS,” *Olympus*. https://www.olympus-

lifescience.com/en/microscope-resource/primer/lightandcolor/fluoroexcitation/ (accessed Jul. 30, 2021).

Chapter Three: Materials and Methodology.

3.1 Brief Introduction

This chapter gives an overall view of the materials used, procedures adopted and the equipment used to undergo this study or research. While some of the procedures were adopted from literature, some where incorporated based on manuals used by equipment, some methods adapted where also based on personal encounter and finally from a guide assistant at Worchester Polytechnique Institute.

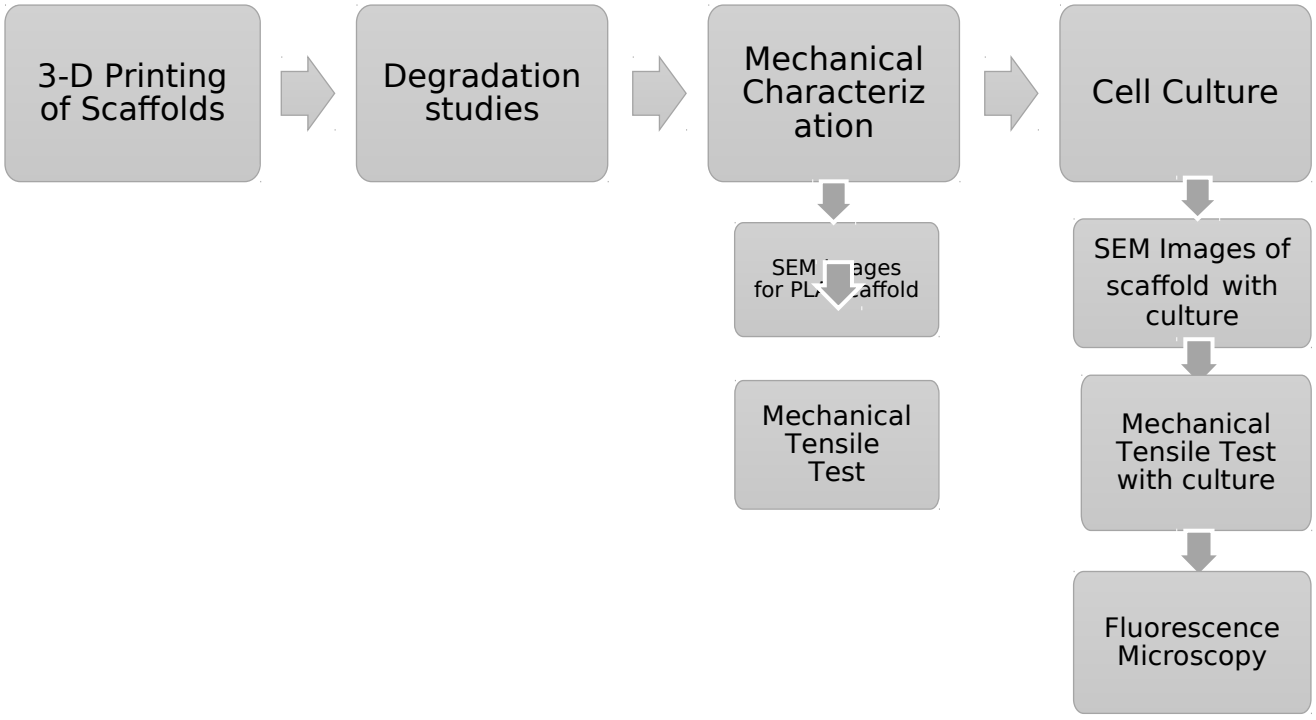

Figure 3-9 Illustrating the Overall Work done

3.2 CAD Drawing and 3-D Printing

Before printing the scaffold, a design is obtained using a 3- D CAD drawing (AutoCAD fusion 360) and converted to a stereographic format (STL). This drawing can also be obtained online, but the availability of meshed CAD drawing is scarcely available. For cell culturing, the mesh spacing required is usually 200 microns. A square scaffold of 5mm by 5mm was printed with 200 microns spacing in order to obtain the dog bone shape since there is no exact ASTM standard for 3-D printers, a micron spacing of 700micron was used in this work as opposed to the earlier stated 200microns required for cell culturing, this was because the 200micron mesh consumed larger space and could not be read in the 3-D printer. An application Cura Lulz bolt version 3.6.18 was downloaded (open source) for the purpose of translating the drawing to the 3-D printer Taz 6 Single Print Extruder V2.1. This software application gives details of print speed, time of print, selection of material as well as setting the sample on a flatbed for printing., also the material can be scaled down to different sizes compared to its original size ratio.

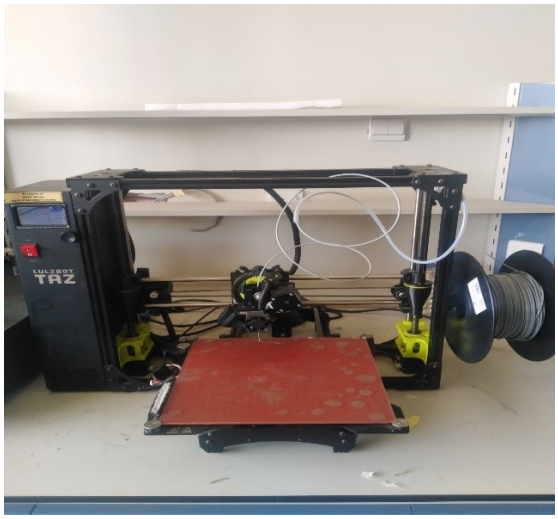

Figure 3-10 Front view of the 3-D Printer

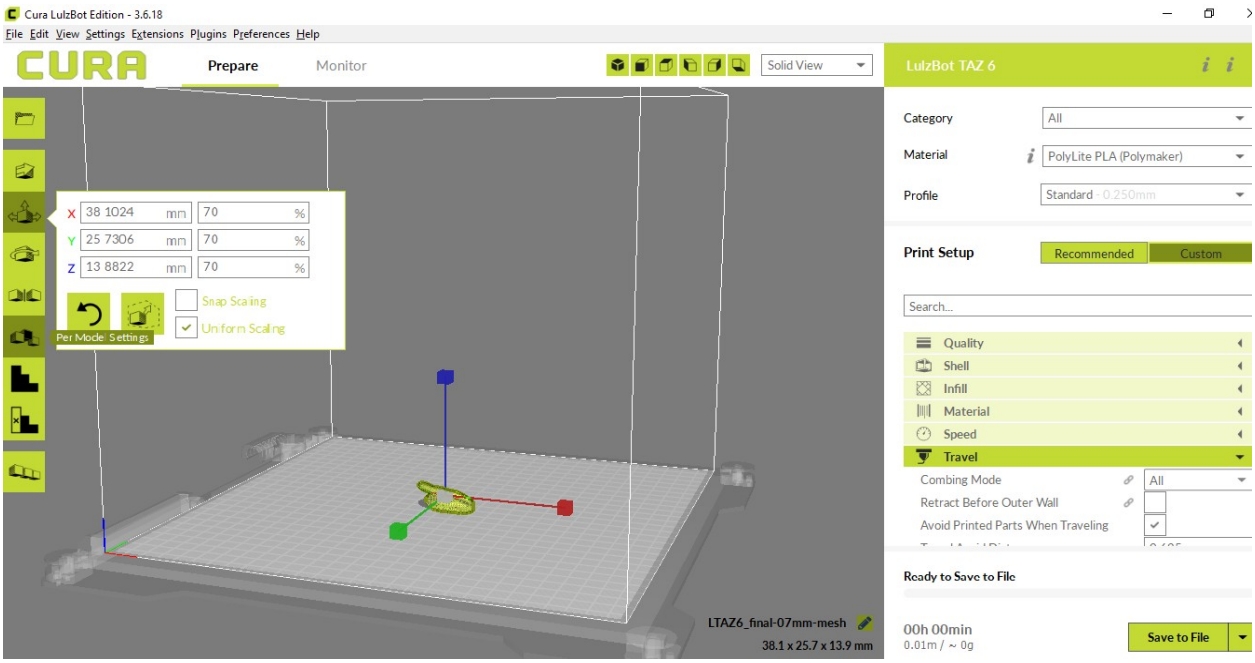

Figure 3-11 A viewing interface for the 3- D printer

As shown is the interface between the application that coverts the CAD drawings to a readable format that is accepted by the 3-D printer.

Table 3-3 Showing the configuration used for the 3-D printers.

S/N	Parts	Specification
1	Material	PolyLite PLA
2	Extruder	250°C
3	Bed	60°C
4	Print time per ear sample(700micron spacing)	45mins 06second
5	Print time for scaffold sample(200micronspacing)	10mins 14seconds

Table 3-4 Showing Materials and Sample Specification

S/N	Material/ Equipment/software application	Sample Specification
1	3-D Printer Taz Luz bot 6	Square scaffold

		5mmX5mmX0.2mm(200micron spacing)
2	Cura Loz But	Dog bone: Web:20mm Flange:160mm Breadth:200mm
3	Auto Cad fusion 360	Ear Mesh: Mesh spacing 200-700 microns
4	Venier Caliper	

Figure 3-12 showing the printed ear

3.3 Degradation Study of Polylactic Acid Scaffold

3.3.1 Materials

I. Water Bath @ 37degree Celsius
II. Sample holder
III. Water
IV. Weighing Balance
V. Vernier Caliper’s

VI. Drying Oven

Methodology

In order to determine the bio activeness and study the degradation of the PLA sample, the use of water bath is needed alongside simulated body fluids which is prepared or bought. The simulated body fluid (SBF) is a solution with an ion concentration close to that of human blood plasma, kept under mild conditions of pH and identical physiological temperature. Here a temperature of 37degree Celsius is maintained by the water bath. While the samples are placed inside sample holder containing SBF, and then placed in the water bath, after a week the sample is taken out and rinsed with distilled water, then oven dried, the weight is then measured. After this the sample is taken for SEM viewing to obtain the morphology, EDX analysis Is obtained, furthermore the tensile test of the sample is obtained this was done for a period of 8weeks. Degradation according to literature takes about 6 months in

PLA, but considering this study pitting was observed around the PLA samples from the SEM images, as shown below, also the EDX analysis showed reduction in elements in terms of percentages on the final 8weeks result.

Figure 3-13 Samples of PLA in Sample holders for observing degradation purpose

Although the changes in weight was not very significant and can vary based on water absorption by the PLA samples.

3.3.2 SEM/EDX Analysis

The samples are coated with Gold Nano particles using a sputtering machine, to enable the samples to be a conducting material when placed in the SEM. Upon placing this samples in the SEM, via the sample holders available, the morphology was viewed, in figures below we can see images of that of week 0 with practically smooth surface, while that of week 8 seems to have pitting around, which clearly shows some percentage of degradation.

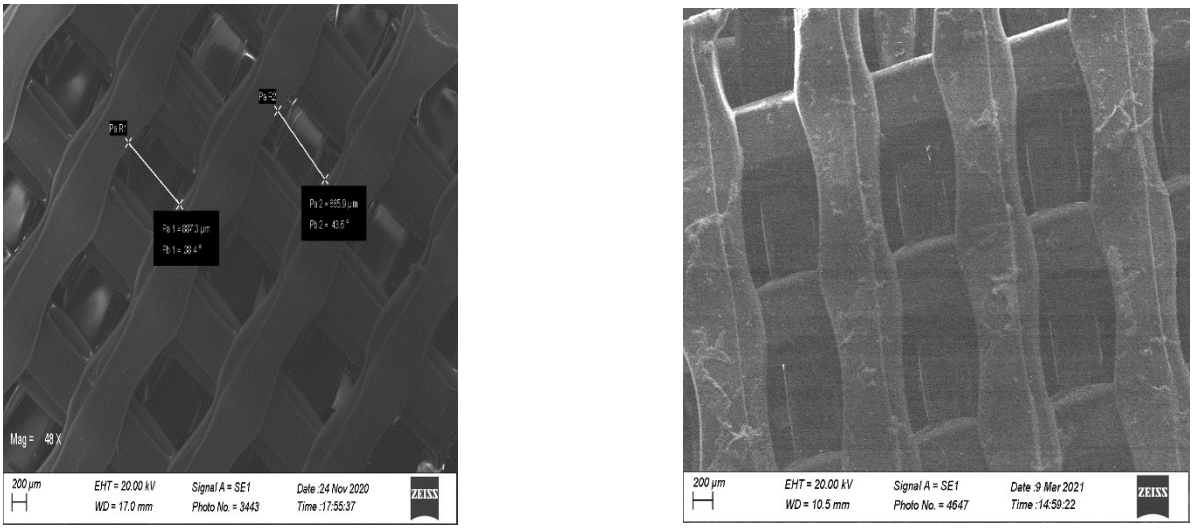

Figure 3-14a-b Morphology of PLA samples obtained from the SEM at Week 0 and Week 8

EDX Analysis

The EDX analyzer is installed alongside the SEM, it is a qualitative and quantitative analysis indicating the elements present in the sample, it gives a graphical representation and percentage of each element and also a percentage composition of each element.

3.4 Mechanical Characterization

3.4.1 Tensile Testing

In order to obtain the tensile properties of the PLA samples before culturing and after culturing, dog bones samples were obtained by printing out square shaped scaffolds and cut out to the dog bone shape. Some of the samples where cultured for a period of 7days.The mechanical characterization test was carried out using the universal testing machine Instron to determine the tensile strength and young's modulus on the PLA Scaffold before culturing and after culturing, the bar graphs below show the results. Before testing, the dimensions were taken using a vernier

caliper, then placed between the grips of the machine, a constant load was applied at 1mm/min, the samples where then strained until failure occurred. The instrument generated the maximum tensile strength, young modulus, other parameters like the stiffness was generated from the linear region of the load-elongation curves.

3.5 CELL CULTURING

The procedure for cell culture was adopted from literature and conducted at the cell laboratory, data was collected and analyzed by me, this data include the alamar blue assay, fluorescence microscopy and finally the tensile test of material attached with the cells this is explained below.

3.6 ALAMAR BLUE ASSAY

AlamarBlue assay is considered an excellent redox indicator among other assays. It is used for the purpose of studying cell proliferation and viability studies. One adavantage of this assay, is its ability not to interfere with reaction of the electron transport chain. Also when using AB the functionality of the cells is retained as well as its viability, this makes it preferred amongst other assays.[32]

METHODS

The AB is usually added directly into to the culture medium of the incubated test cells, at varying time intervals. From here absorbance is determined within a certain range.[33]

Resazurin is used as an oxidation-reduction (REDOX) indicator that undergoes colorimetric change in response to cellular metabolic reduction. The reduced form, resorufin, is pink and highly fluorescent, and the intensity of fluorescence produced is proportional to the number of living cells respiring.[34]

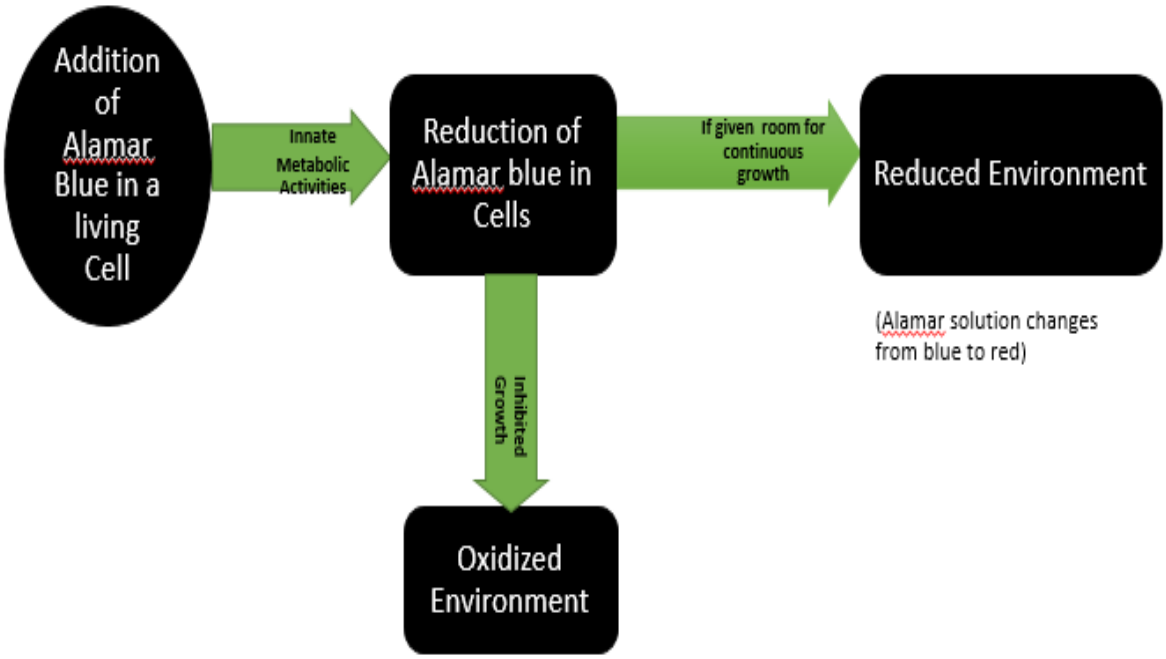

Figure 3-15 showing alamar blue reaction with cells, a change to red or blue

3.7 Fluorometer Microscopy

The fluorometer microscopy helps in the identification of cells and sub-microscopic components (i.e. nucleus and cytoplasm) giving a high degree of specificity amid non-fluorescing material. It is capable of revealing the presence of a single molecule. Though it has the inability to provide spatial resolution below the diffraction limit of specific specimen features, notwithstanding the detection of fluorescing molecules below such limits is readily achieved.

In order to achieve maximum fluorescence intensity, a fluorophore (often termed a **dye**) is usually excited at wavelengths near or at the peak of the excitation curve, and the widest possible range of emission wavelengths that include the emission peak are selected for detection.[35].

Figure 3-16 a Fluoromete

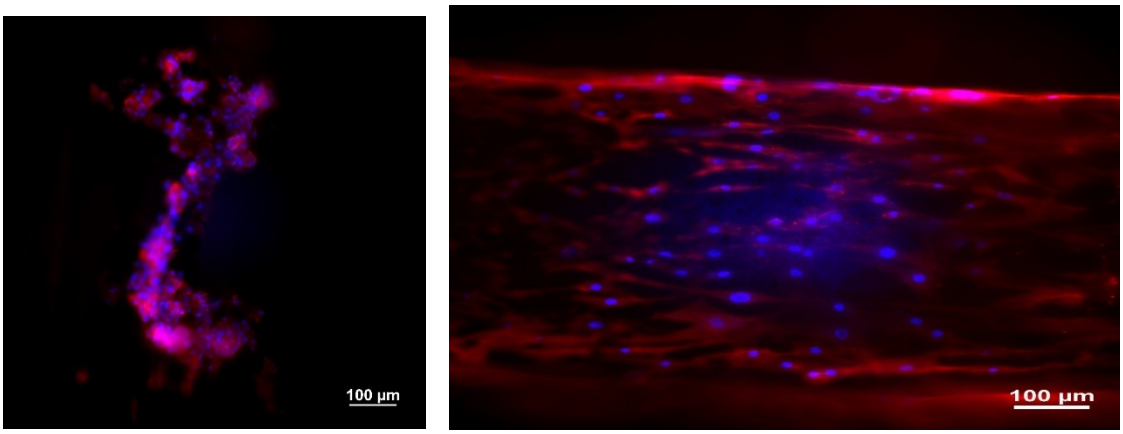

Figure 3-17 image after staning at day 0 and Day 7 respectively

REFERENCES

[1] Krane Jim, “Bionic Eye Follows Bionic Ear,” *government technology*, May 28, 2002. https://www.govtech.com/public-safety/Bionic-Eye-Follows-Bionic-Ear.html (accessed Mar. 10, 2021).

[2] A. Shapira and T. Dvir, “3D Tissue and Organ Printing — Hope and Reality,” vol. 2003751, 2021, doi: 10.1002/advs.202003751.

[3] M. S. Mannoor *et al.*, “3D printed bionic ears,” *Nano Lett.*, vol. 13, no. 6, pp. 2634–2639, 2013, doi: 10.1021/nl4007744.

[4] A. Do, B. Khorsand, S. M. Geary, and A. K. Salem, “3D Printing of Scaffolds for Tissue Regeneration Applications,” pp. 1742–1762, 2015, doi: 10.1002/adhm.201500168.

[5] L. C. Ventola, “Medical Application for 3-D Printing: Current and Projected Use,” *p&T*, vol. 39, no. 10, 2014.

[6] H. N. Chia and B. M. Wu, “Recent advances in 3D printing of biomaterials,” pp. 1–14, 2015, doi: 10.1186/s13036-015-0001-4.

[7] B. Applications *et al.*, “Biomaterials Based on Marine Resources for 3D,” 2019.

[8] S. Bose, D. Ke, H. Sahasrabudhe, and A. Bandyopadhyay, “Progress in Materials Science Additive manufacturing of biomaterials,” *Prog. Mater. Sci.*, vol. 93, pp. 45–111, 2018, doi: 10.1016/j.pmatsci.2017.08.003.

[9] B. R. Ringeisen, S. J. Barry, and W. K. Peter, *Cell Organ and Printing*. 2010.

[10] P. Bajaj, R. M. Schweller, A. Khademhosseini, J. L. West, and R. Bashir, “3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine,” no. May, 2014, doi: 10.1146/annurev-bioeng-071813-105155.

[11] D. J. Thomas and T. C. Claypole, “18 3-D Printing,” pp. 293–306, 2016, doi: 10.1016/B978-0-323-37468-2.00018-X.

[12] World Health Organization Court Room, “Deafness and hearing loss,” *World Health Organization*, Mar. 02, 2021. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss (accessed Mar. 23, 2021).

[13] Joy Victory, “Hearing loss: Symptoms, causes and treatments,” *Healthy Haring*, 2021. https://www.healthyhearing.com/help/hearing-loss (accessed Mar. 23, 2021).

[14] J. Davis, “Cells as Products Cell Culture in Tissue,” 2007.

[15] M. Weisberger, “11 Body Parts Grown in the Lab | Live Science,” *Live Science*, 2017. https://www.livescience.com/59675-body-parts-grown-in-lab.html (accessed Jul. 14, 2021).

[16] G. Vogel, “Organs Made to Order | Science | Smithsonian Magazine,” *SMITHSONIAN MAGAZINE*, 2010. https://www.smithsonianmag.com/science-nature/organs-made-to-order-863675/ (accessed Jul. 14, 2021).

[17] K. Kiaee, Y. A. Jodat, and M. S. Mannoor, “Bionic Organs,” pp. 167–192, 2020.

[18] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, and D. S. Kumar, “Polymeric Scaffolds in Tissue Engineering Application : A Review,” vol. 2011, no. ii, 2011, doi: 10.1155/2011/290602.

[19] K. K. Sadasivuni, “A Comparative Review of Natural and Synthetic Biopolymer Composite Scaffolds,” 2021.

[20] P. Chen *et al.*, “The 3D-Printed PLGA Scaffolds Loaded with Bone Marrow-Derived Mesenchymal Stem Cells Augment the Healing of Rotator Cuff Repair in the Rabbits,” vol. 29, pp. 1–13, 2020, doi: 10.1177/0963689720973647.

[21] A. G. Namboodiri, “BIOMATERIAL FABRICATION TECHNIQUES.”

[22] J. Liu and C. Yan, “3D Printing of Scaffolds Scaffolds for for Tissue Tissue Engineering Engineering,” doi: 10.5772/intechopen.78145.

[23] N. Altaee, G. A. El-Hiti, A. Fahdil, K. Sudesh, and E. Yousif, “Biodegradation of different formulations of polyhydroxybutyrate films in soil,” *Springerplus*, vol. 5, no. 1, 2016, doi: 10.1186/s40064-016-2480-2.

[24] J. S. Lee, J. M. Hong, J. W. Jung, J. H. Shim, J. H. Oh, and D. W. Cho, “3D printing of composite tissue with complex shape applied to ear regeneration,” *Biofabrication*, vol. 6, no. 2, 2014, doi: 10.1088/1758-5082/6/2/024103.

[25] F. Melchels, *Organ Printing*. Elsevier Ltd., 2011.

[26] S. Goshal, “carbon Nanotubed for 3- D printing.pdf.” 2017.

[27] H. A. E.-S. Kaoud, “Introductory Chapter: Concepts of Tissue Regeneration,” *Tissue Regen.*, Jun. 2018, doi: 10.5772/INTECHOPEN.76996.

[28] H. Jian, M. Wang, S. Wang, A. Wang, and S. Bai, “3D bioprinting for cell culture and tissue fabrication,” *Bio-Design Manuf.*, no. March, 2018, doi: 10.1007/s42242-018-0006-1.

[29] M. Sun *et al.*, “3D cell culture — can it be as popular as 2D cell culture?,” doi: 10.1002/anbr.202000066.

[30] T. Xu, D. Reyna-soriano, J. I. Rodrı, M. Bhuyan, and T. Boland, *Organ printing*. 2019.

[31] K. Storck *et al.*, “Total reconstruction of the auricle: Our experiences on indications and recent techniques,” *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/373286.

[32] U. S. P. No, “alamarBlue ® Assay,” no. 5.

[33] S. Al-Nasiry, N. Geusens, M. Hanssens, C. Luyten, and R. Pijnenborg, “The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells,” *Hum. Reprod.*, vol. 22, no. 5, pp. 1304–1309, May 2007, doi: 10.1093/HUMREP/DEM011.

[34] J. O'Brien, I. Wilson, T. Orton, and F. Pognan, "Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity," *Eur. J. Biochem.*, vol. 267, no. 17, pp. 5421–5426, 2000, doi: 10.1046/J.1432-1327.2000.01606.X/FULL.

[35] M. Abramowitz and M. W. Davidson, "Fluorescence - Overview of Fluorescence Excitation and Emission Fundamentals | Olympus LS," *Olympus*. https://www.olympus-lifescience.com/en/microscope-resource/primer/lightandcolor/fluoroexcitation/ (accessed Jul. 30, 2021).

CHAPTER FOUR

4.1 DISCUSSION OF RESULT/ANALYSIS

This chapter presents the results and discussion of the work done in this thesis. It includes results obtained right from the inception, some of which are 3-D printing of scaffolds, the degradation studies carried on the scaffold. Other material properties determined included the scaffolds and cells morphology, the tensile properties of scaffold. Assays like the Alamar Blue assays were carried out as well. More so other analysis like cell activation, protein concentration. While some of the analyses were done at the African university of science and technology others were carried at the Worcester Polytechnique institute.

4.2 Fabrication of Ear shaped Scaffold

While there are several other fabrication technologies. The 3-D printing technology is able to produce precise stereographical sample print, also considering the fact that the ear tissue cartilage is one of the greatest challenges in facial plastic surgery[31]. This 3-D print approach using a PLA sample, allows the growth of cells, then with time this PLA samples dissolve upon the addition of SBF. Other fabrication method which involves the use of molds do not give precise stereographs and in most cases curing of the samples, more so the use of 3-D prints allows the creation of pores alongside printing of the scaffolds.

4.3 TENSILE TEST ANALYSIS

4.3.1 Tensile Test Analysis Without Cells

Tensile test on scaffolds without cells, at each point the dog bone samples removed from the water bath, then oven dried. The analysis was obtained over a period of 8 weeks and data was collected bi-weekly,

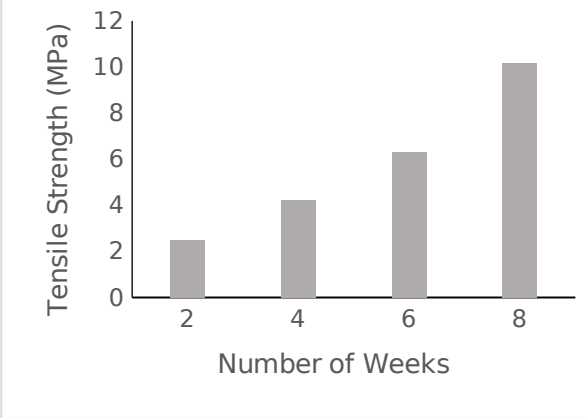

Figure 4- 1 The Tensile strength versus number of weeks for degradation study

4.3.2 Tensile Test On Scaffolds With Cells

The analysis of the tensile stress, young modulus were obtained for the samples with cells, the average tensile stress of the sample at day 0 for the 3 specimens was 4.9Mpa and 3.44Mpa at Day 7, for the young modulus an average result for the 3 specimen was 201Mpa at Day 0 and 285Mpa at day 7.

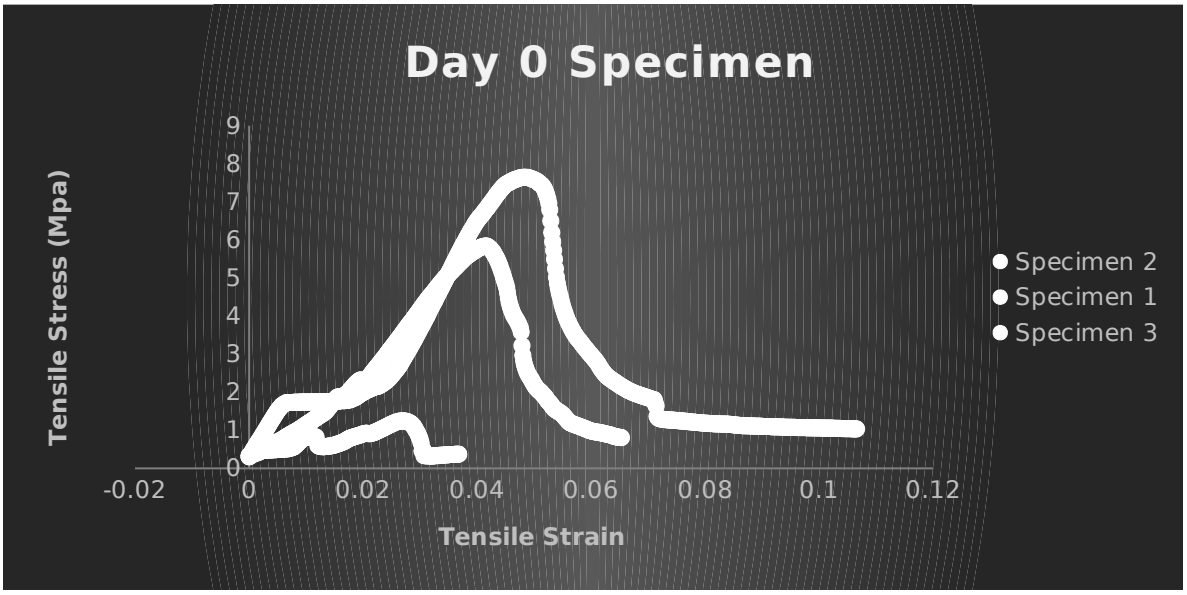

Figure 4- 2 Illustration of Tensile stress versus tensile strain with cells

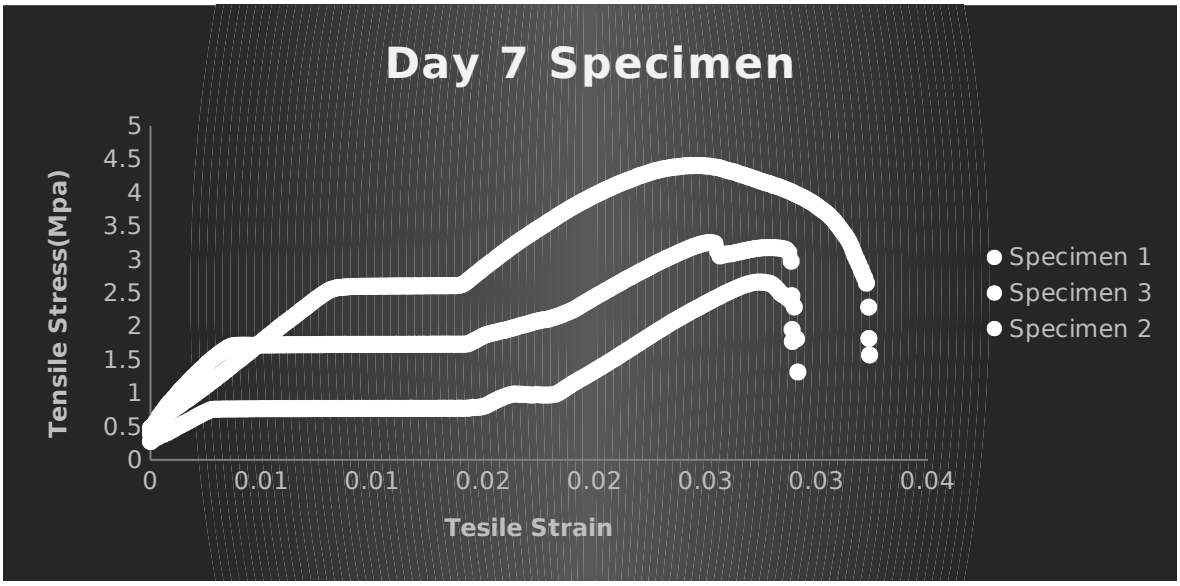

Figure 4- 3 Illustration of Tensile stress versus tensile strain without cells

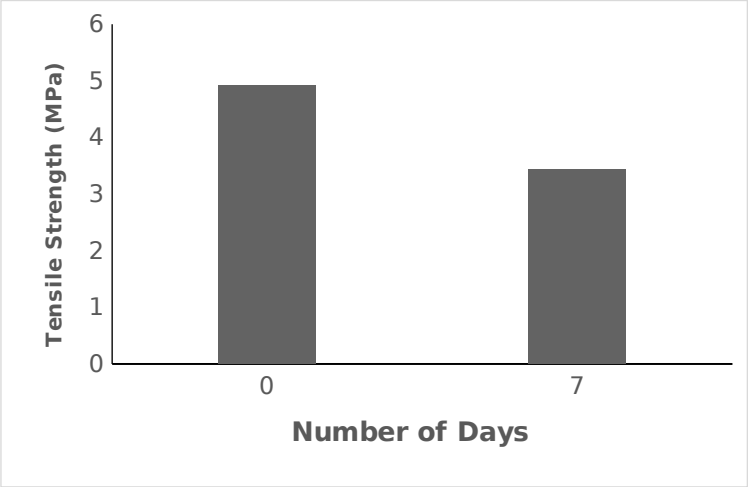

Figure 4- 4 Showing the Tensile test analysis versus number of weeks in culture

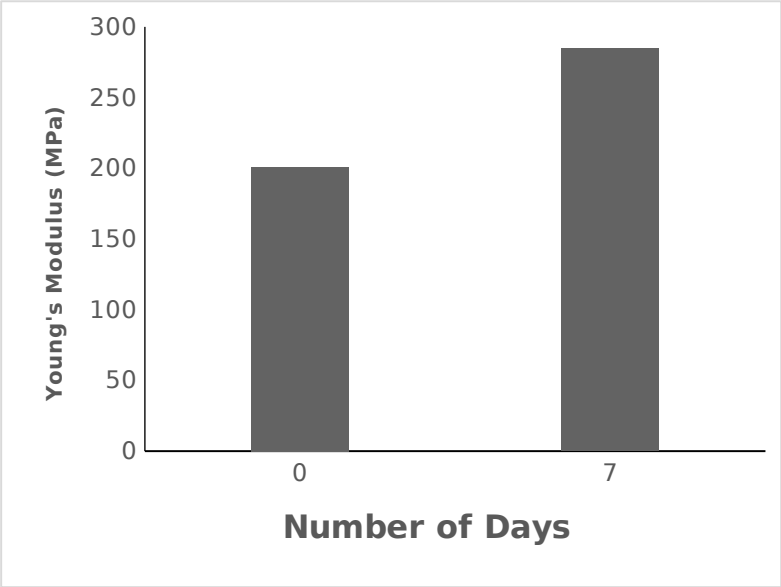

Figure 4- 5 Showing the Young Modulus analysis versus number of days in culture

4.3.3 ALAMAR BLUE ASSAY

The Alamar blue assay measures quantitatively proliferation In cells. The alamar blue assay is considered superior to other cell viability assays, such as the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide test, because it is not toxic to cells and does not lead to the dying of the cells during the procedure. Moreover, AB can also be reduced by cytochromes, whereas tetrazolium salts such as MTT are not. According to[33] This might explain the higher sensitivity and reproducibility of AB in detecting low cell concentrations. Here we have been able to observe the increase in cells over the period of 7 days.

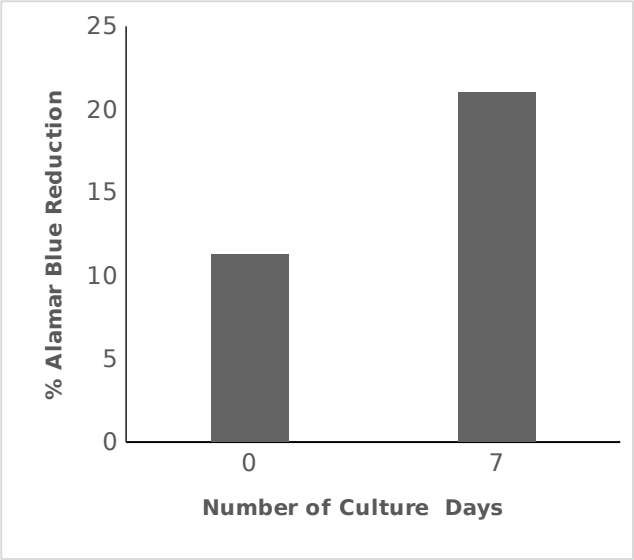

Figure 4- 6 Showing the %Alamar Blue versus number of days in culture

4.4 Fluorescence Microscopy

The fluorometer microscopy helps in the identification of cells and sub-microscopic components (i.e nucleus and cytoplasm) giving a high degree of specificity amid non-fluorescing material. Using the fluorophore dye, we have been able to determine the protein concentration figure and also indicating the nucleus and cytoplasm, furthermore it is seen that there is a increase in the concentration of cells at the day 7 data collection.

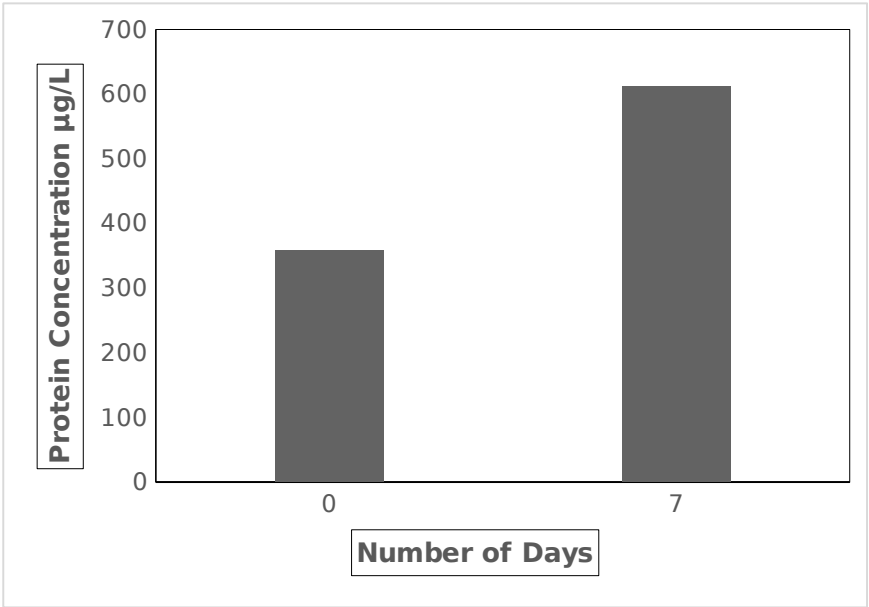

Figure 4- 7 Showing the % Protein concentration versus number of days in culture

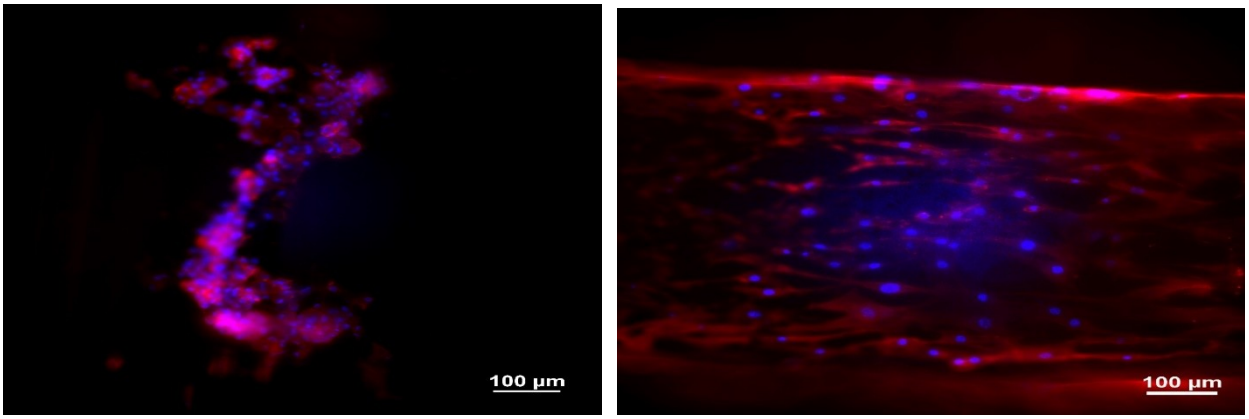

Figure 4- 8a-b Showing an image(Day 0 & Day 7)of the cells after staining with 4′,6-diamidino-2-phenylindole(DAPI) and Rhodamine

DAPI and Rhodamine are dyes used in staining the cells, the former stains and give a blue color which indicates the nucleus of the cell, while the later stains and gives a red color which shows the cytoplasm.

4.5 SEM ANALYSIS

The SEM analysis gave the morphology of the samples before and after culturing as illustrated in

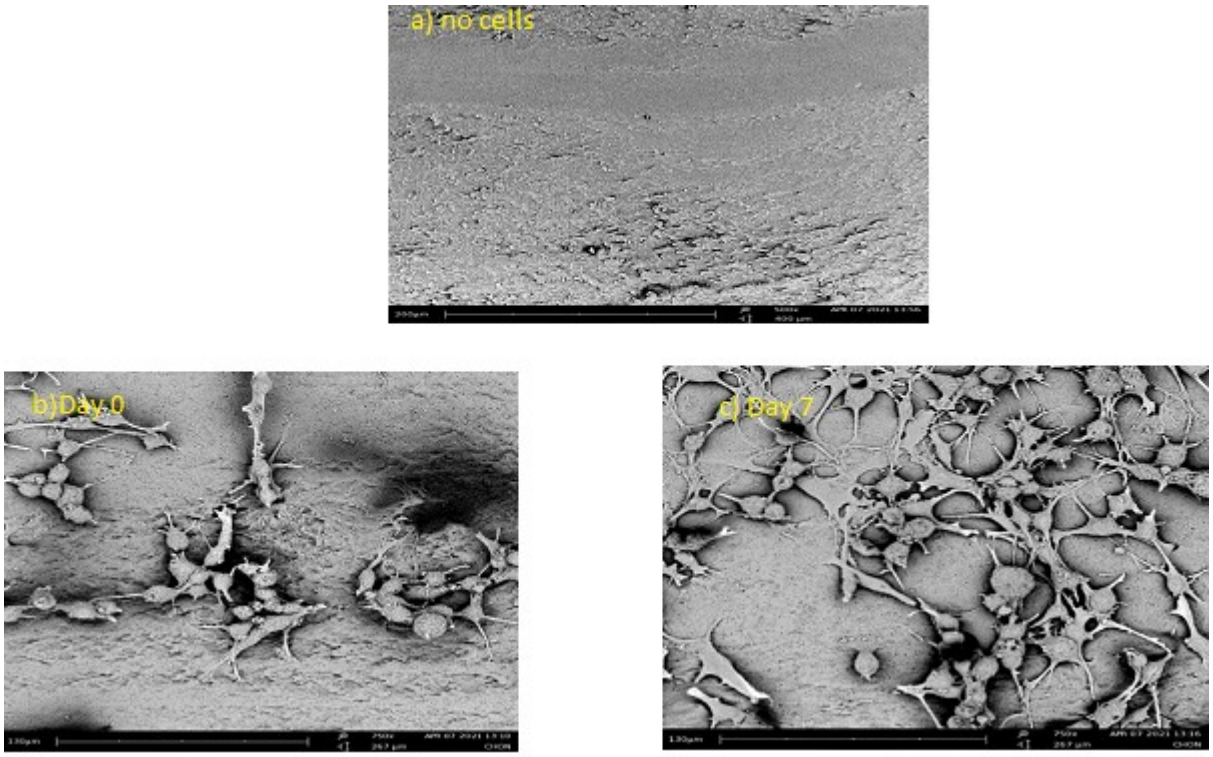

Figure 4- 9a-c) Morphology of samples from SEM viewing, without cell, and with cells at day 0 and day 7 Respectively

4.5.1 The Energy Dispersive X-Ray Analysis (EDX):

This is an x-ray technique used to identify the elemental composition of specimen, as illustrated below form the graph. While the chemical composition of PLA is established as been made up of carbon and hydrogen elements, other elements present are from the presence of simulating body fluid and the conductive particles used for coating of samples .

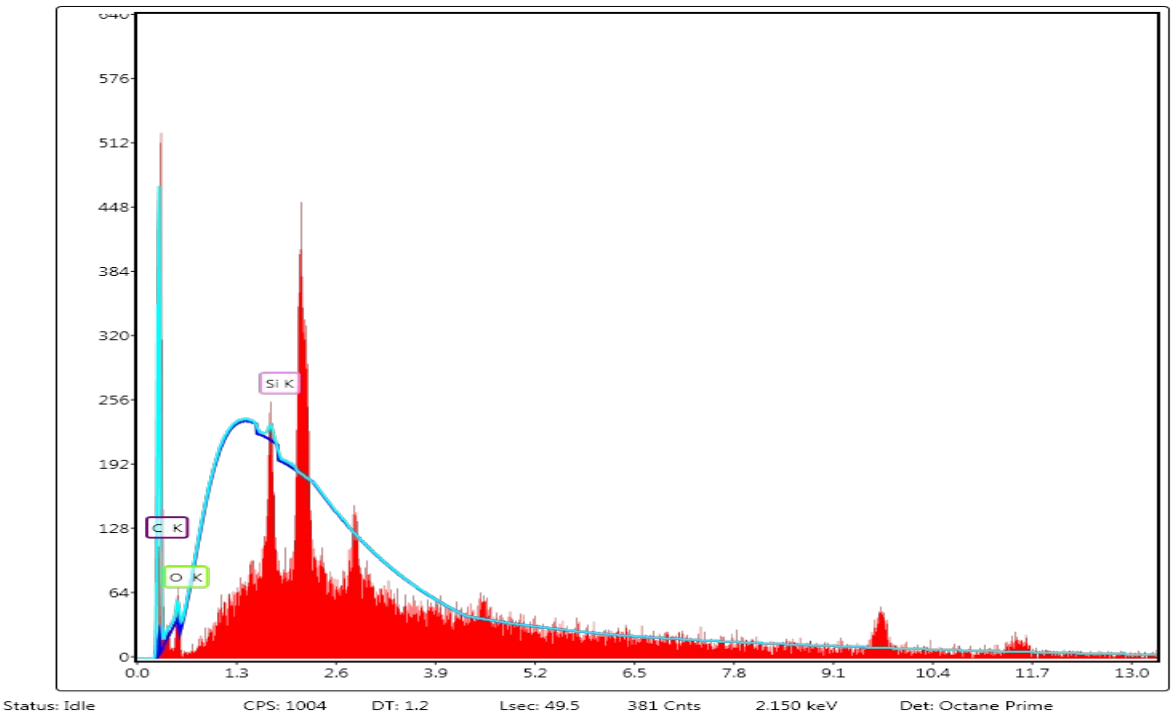

Figure 4- 10 EDX analysis of the PLA scaffolds

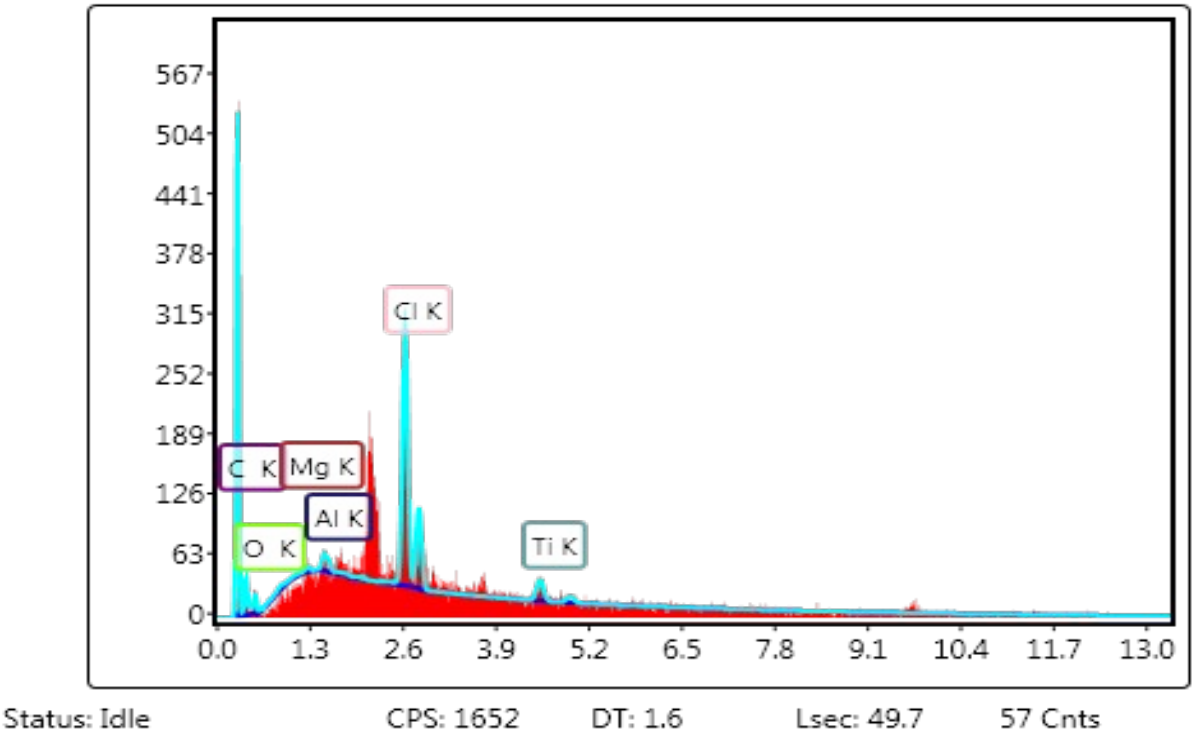

Figure 4- 11 EDX analysis of the PLA scaffolds after degradation studies

4.6 DEGRADATION STUDIES

Degradation studies is carried out using a water bath where by the samples are placed in samples holders, this samples where cut into dog bone shapes, with no specific ASTM standard as there is none presently for the studies of 3-D printing.

Studies were carried out for period of 0 -8 weeks, and the SEM Morphology was obtained as well as the tensile tests,

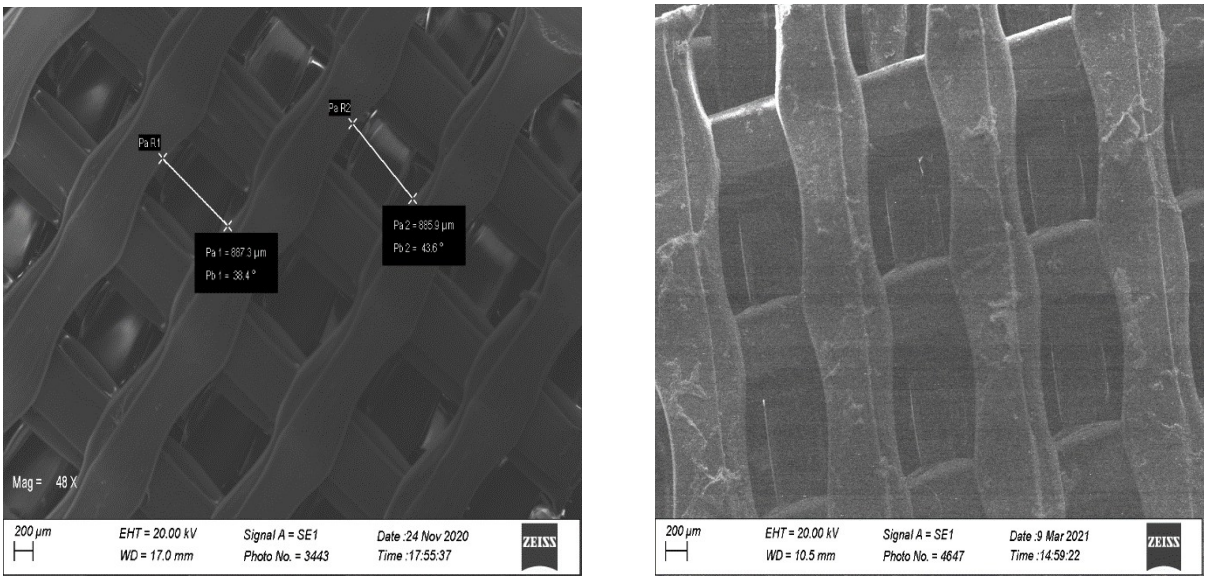

Figure 4- 12 SEM Morphology of PLA samples, comparison, between 0 and 8th week

References

[1] Krane Jim, “Bionic Eye Follows Bionic Ear,” *government technology*, May 28, 2002. https://www.govtech.com/public-safety/Bionic-Eye-Follows-Bionic-Ear.html (accessed Mar. 10, 2021).

[2] A. Shapira and T. Dvir, “3D Tissue and Organ Printing — Hope and Reality,” vol. 2003751, 2021, doi: 10.1002/advs.202003751.

[3] M. S. Mannoor *et al.*, “3D printed bionic ears,” *Nano Lett.*, vol. 13, no. 6, pp. 2634–2639, 2013, doi: 10.1021/nl4007744.

[4] A. Do, B. Khorsand, S. M. Geary, and A. K. Salem, “3D Printing of Scaffolds for Tissue

Regeneration Applications," pp. 1742–1762, 2015, doi: 10.1002/adhm.201500168.

[5] L. C. Ventola, "Medical Application for 3-D Printing: Current and Projected Use," *p&T*, vol. 39, no. 10, 2014.

[6] H. N. Chia and B. M. Wu, "Recent advances in 3D printing of biomaterials," pp. 1–14, 2015, doi: 10.1186/s13036-015-0001-4.

[7] B. Applications *et al.*, "Biomaterials Based on Marine Resources for 3D," 2019.

[8] S. Bose, D. Ke, H. Sahasrabudhe, and A. Bandyopadhyay, "Progress in Materials Science Additive manufacturing of biomaterials," *Prog. Mater. Sci.*, vol. 93, pp. 45–111, 2018, doi: 10.1016/j.pmatsci.2017.08.003.

[9] B. R. Ringeisen, S. J. Barry, and W. K. Peter, *Cell Organ and Printing*. 2010.

[10] P. Bajaj, R. M. Schweller, A. Khademhosseini, J. L. West, and R. Bashir, "3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine," no. May, 2014, doi: 10.1146/annurev-bioeng-071813-105155.

[11] D. J. Thomas and T. C. Claypole, "18 3-D Printing," pp. 293–306, 2016, doi: 10.1016/B978-0-323-37468-2.00018-X.

[12] World Health Organization Court Room, "Deafness and hearing loss," *World Health Organization*, Mar. 02, 2021. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss (accessed Mar. 23, 2021).

[13] Joy Victory, "Hearing loss: Symptoms, causes and treatments," *Healthy Haring*, 2021. https://www.healthyhearing.com/help/hearing-loss (accessed Mar. 23, 2021).

[14] J. Davis, "Cells as Products Cell Culture in Tissue," 2007.

[15] M. Weisberger, "11 Body Parts Grown in the Lab | Live Science," *Live Science*, 2017. https://www.livescience.com/59675-body-parts-grown-in-lab.html (accessed Jul. 14, 2021).

[16] G. Vogel, "Organs Made to Order | Science | Smithsonian Magazine," *SMITHSONIAN MAGAZINE*, 2010. https://www.smithsonianmag.com/science-nature/organs-made-to-order-863675/ (accessed Jul. 14, 2021).

[17] K. Kiaee, Y. A. Jodat, and M. S. Mannoor, “Bionic Organs,” pp. 167–192, 2020.

[18] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, and D. S. Kumar, “Polymeric Scaffolds in Tissue Engineering Application : A Review,” vol. 2011, no. ii, 2011, doi: 10.1155/2011/290602.

[19] K. K. Sadasivuni, “A Comparative Review of Natural and Synthetic Biopolymer Composite Scaffolds,” 2021.

[20] P. Chen *et al.*, “The 3D-Printed PLGA Scaffolds Loaded with Bone Marrow-Derived Mesenchymal Stem Cells Augment the Healing of Rotator Cuff Repair in the Rabbits,” vol. 29, pp. 1–13, 2020, doi: 10.1177/0963689720973647.

[21] A. G. Namboodiri, “BIOMATERIAL FABRICATION TECHNIQUES.”

[22] J. Liu and C. Yan, “3D Printing of Scaffolds Scaffolds for for Tissue Tissue Engineering Engineering,” doi: 10.5772/intechopen.78145.

[23] N. Altaee, G. A. El-Hiti, A. Fahdil, K. Sudesh, and E. Yousif, “Biodegradation of different formulations of polyhydroxybutyrate films in soil,” *Springerplus*, vol. 5, no. 1, 2016, doi: 10.1186/s40064-016-2480-2.

[24] J. S. Lee, J. M. Hong, J. W. Jung, J. H. Shim, J. H. Oh, and D. W. Cho, “3D printing of composite tissue with complex shape applied to ear regeneration,” *Biofabrication*, vol. 6, no. 2, 2014, doi: 10.1088/1758-5082/6/2/024103.

[25] F. Melchels, *Organ Printing*. Elsevier Ltd., 2011.

[26] S. Goshal, “carbon Nanotubed for 3- D printing.pdf.” 2017.

[27] H. A. E.-S. Kaoud, “Introductory Chapter: Concepts of Tissue Regeneration,” *Tissue Regen.*, Jun. 2018, doi: 10.5772/INTECHOPEN.76996.

[28] H. Jian, M. Wang, S. Wang, A. Wang, and S. Bai, “3D bioprinting for cell culture and tissue fabrication,” *Bio-Design Manuf.*, no. March, 2018, doi: 10.1007/s42242-018-0006-1.

[29] M. Sun *et al.*, “3D cell culture — can it be as popular as 2D cell culture?,” doi: 10.1002/anbr.202000066.

[30] T. Xu, D. Reyna-soriano, J. I. Rodrı, M. Bhuyan, and T. Boland, *Organ printing*. 2019.

[31] K. Storck *et al.*, “Total reconstruction of the auricle: Our experiences on indications and recent techniques,” *Biomed Res. Int.*, vol. 2014, 2014, doi: 10.1155/2014/373286.

[32] U. S. P. No, “alamarBlue ® Assay,” no. 5.

[33] S. Al-Nasiry, N. Geusens, M. Hanssens, C. Luyten, and R. Pijnenborg, “The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells,” *Hum. Reprod.*, vol. 22, no. 5, pp. 1304–1309, May 2007, doi: 10.1093/HUMREP/DEM011.

[34] J. O’Brien, I. Wilson, T. Orton, and F. Pognan, “Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity,” *Eur. J. Biochem.*, vol. 267, no. 17, pp. 5421–5426, 2000, doi: 10.1046/J.1432-1327.2000.01606.X/FULL.

[35] M. Abramowitz and M. W. Davidson, “Fluorescence - Overview of Fluorescence Excitation and Emission Fundamentals | Olympus LS,” *Olympus*. https://www.olympus-lifescience.com/en/microscope-resource/primer/lightandcolor/fluoroexcitation/ (accessed Jul. 30, 2021).

CHAPTER FIVE

5.1 Conclusion

3-D printing has the potential to play an important role in upcoming medical trends, through its varying use of organ printing, drug delivery, while also removing the practice of animal butchering for the purpose of clinical trial and drug testing. The technology of 3-D printing is highly anticipated as many problems can be resolved.

Looking at the cyborg ears, with the technology of 3-D printers merged with tissue engineering and cybernetics, humans who have lost the ability to hear due to partial or total deafness can regain this sense back, this is been achieved by ear reconstruction and replacement from the outer ear cartilage to the inner ear membrane, further more with this technology advance features in this organs can be created i.e. hearing in a higher dimension than the regular hearing capacity

In this study, we confirmed the feasibility of fabricating the cell-printed structure made up of a framework 3-D printed PLA scaffold, growing of human chondrocytes cells on the scaffold, which was observed by various cell growth and viability techniques. Although not been able to conclude if the approach of using extrusion 3-D printers as been the most effective, we can say that the technique is plausible. But the technology was effective in fabricating or generating an exact ear ECM made of PLA and giving us certain degree of porosity. We can conclude that complex shapes and multiple cell types can be created using this approach of 3-D printing by extrusion-based printers, then the cells grown on them and the scaffold is being dissolved by a simulating body fluid overtime.

5.2 Recommendation.

For further researches I will recommend that faster materials which can be used to degrade PLA samples can be worked on or even a material that degrades the PLA alongside promoting of cell growth.