3D PRINTING SCAFFOLDS FOR BREAST TISSUE ENGINEERING APPLICATIONS

A Thesis Submitted to the Department of Materials Science and Engineering
African University of Science and Technology, Abuja

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

By

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April, 2021

APPROVAL PAGE

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CERTIFICATION

This is to certify that the thesis titled "***3D Printing Scaffolds for Breast Tissue Engineering Applications***" submitted to the Department of Materials Science and Engineering in the 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 ***Alex, Azuka Amaechi***

ABSTRACT

Recently, the field of Tissue Engineering has explored the potential for the regeneration of many organs and tissues in the human body. The aim of this study applied to breast tissue engineering is to overcome the major criticalities practiced with conventional therapies (mastectomy, breast conserving therapy, and lipofilling). This Thesis is focused on the fabrication of biodegradable 3D implantable Scaffolds cultured with the breast cells for regeneration of damaged tissues. Poly Lactic Acid (PLA) is the polymer used for this study. In this work, the degradation of the 3D PLA Scaffolds immersed in Simulated Body Fluid (SBF). The mechanical properties of the scaffolds are also measured and after culturing with mammary breast cells. From the study, it was observed that the PLA scaffolds degrade over time, as the number of weeks of exposure increase. The mechanical properties before and after culturing with the breast cells showed a significant increase in their tensile strength and Young's modulus. However, the tensile strength and elastic modulus after culturing was 3.42 and 6.10 MPa for day 0 and 7 and 234.67 and 337.33 MPa for day 0 and 7, respectively.

KEYWORDS: Biomaterials, tissue engineering, breast cancer, scaffold, simulated body fluid, poly lactic acid, mechanical properties, Young's Modulus.

ACKNOWLEDGEMENTS

I am grateful to God Almighty for his grace and strength, this research was a huge success because of his divine mercy, love and grace upon my life. To my amiable supervisor Prof Wole Soboyejo, for his immerse impact throughout my research work. For his directives, encouragement, guidance and corrections, this project work would not have been close to completion. I am very grateful to you. To Dr Ali Salifu for his assistance and guidance to follow during the process of this research work and to Madam Theresa Ezenwafor for always making sure I am up to date, thank you so much. I want to express my sincere gratitude to the Head of Department Prof. P. A. Onwualu, not only for his fatherly advice and support throughout the duration of this project, but also for his endless contributions to my success in academics.

To the entire academic and non-academic staff of the African University of Science and Technology, I am grateful for your contribution towards my academic background and for your support throughout my duration of struggle.

Finally, I will not forget my lovely parents, Late Chief. Alex Amaechi and Mrs. Agnes Amaechi, for their prayers and unending love, caring and support, I am forever indebted to them. To my siblings, Collins Amaechi, Chibuike Amaechi, Victor Amaechi, Fidelis Amaechi, Chukuwdi Amaechi, Godspower King and Kenechukwu King. Thank you for all the love and care. To my friends and colleagues, Ayuk Colbert, Gono, Elly, Darius, who in one way or the other directly or indirectly contributed to the success of this project work, I say a big a big thank you.

DEDICATION

I dedicate this work to my Daddy G.O His Holiness The Most Hon. Dr. Rev. KING, who endowed me with the grace and strength to accomplish this task.

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CHAPTER ONE

1.0 Introduction

Tissue engineering involves combining scaffolds, cells, and biologically active molecules into functional tissues such as bones, breast, cartilages, skin etc. The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs [1]. TE has a wide scope of studies with potentials to offer early detection of pathological conditions, reduce the severity of therapy and result in an improved clinical outcome for the patient. This can lead to discovery of newer approaches for promotion of health and longevity with sustainable improvement in the quality of human life, with a reduction in the societal and economic cost associated with healthcare and life expectancy [2]. It is an important field of regenerative medicine for tissue repair (after damaged caused by a disease or an accident, for example). They only need the correct medium that will provide the desired stimulation to guided differentiation, a microenvironment with adequate temperature, pH, and a three-dimensional structure to provide the right microenvironment for cell proliferation, growth, and differentiation (scaffold) [3]. Furthermore, the designed physicochemical properties, morphology and degradation kinetics of Tissue Engineered Constructs (TEC) must be carefully measured. However, studies over the last 20 years by leading scientists and research laboratories in this field have served to clarify our understanding of the key factors associated with the full extent of TE's therapeutic vision. Particular challenges of the current TE involving large volume prefabricated scaffolds include the inability to mimic the cellular organization of natural tissues, upscale fabrication methods to the economically viable scale necessary for clinical application.

1.1 Background to the Study

The basic concept underlying tissue engineering (TE) is the use of a combination of cells, biomaterials (scaffolds) and physico-chemical factors to improve or replace a biological organ that are damaged in the human body. Cells are the building blocks of tissue, and tissues are the basic unit of function in the body. Generally, groups of cells make and secrete their own support structures, called extra-cellular matrix. This scaffold does not just support the cells; but also acts as a relay station for various signaling molecules. . Each signal can start a series of responses that will determine what happens to the cell by understudying

how these individual cells respond to signals, interact with their environment, and form into tissues and organisms. Researchers have been able to influence these processes to mend damaged tissues or even create new ones. Thus, cells receive messages from many sources that become available from the local environment. The process often starts with building a scaffold from a wide set of possible sources, from natural to synthetic. Once scaffolds are created and the environment is right, cells with or without a "combination" of growth factors introduced, a tissue will generate. In some cases, the cells, scaffolds, and growth factors are all mixed together at once, allowing the tissue to "self-assemble."

Another method to regenerate new tissue is by making use of existing scaffold. The cells of a donor organ are isolated and are used to grow new ones. This process has been used to bioengineer heart, liver, lung, breast, ear, bone and kidney tissue. This approach has posed a great impact on improving regenerative medicine as an alternative medicine by using scaffolding from human tissue of patient's own cells to make specific organs that would not be rejected by the immune system thereby mimicking the host tissue [3]. Poly (α-hydroxyacids) are bioresorbable synthetic polymers broadly known, studied and successfully employed as tissue engineering scaffolds for cell transplantation and tissue regeneration. The homopolymers poly glycolic acid (PGA) and poly lactic acid (PLA) and their copolymers (PLGA), are all poly (α-hydroxyacids) [5]. These polymers degrade by hydrolysis and the degradation rate of these polymers depends on configuration structure, molecular weight ratio, exposed surface area, crystallinity, stresses, site of implantation, and in the case of copolymers, the ratio of the hydroxy acid monomers. They demonstrate an excellent kind of mechanical properties and can be fabricated using various processing techniques as molding, extrusion, solvent casting and spin casting [4]. Biocompatibility is a key factor in the long and short term success of all implants; for biodegradable devices it is important that both the implant and its degradation products are biocompatible and non-toxic. This is why PLA, PGA and copolymers have been widely studied and several publications reported in-vitro and in-vivo studies of biocompatibility.

1.2 Breast Tissue Engineering

The breasts are medically known as the mammary glands made up of lobules, milk-producing glandular structures, and a system of ducts that transport milk to the nipple. Over the decade, the 21st century, impetus has been gradually growing towards TE-based regeneration of breast tissue post-mastectomy or Lumpectomy. Breast cancer is the most frequent cancer among women with an estimation of 1.67 million of new cases diagnosed worldwide in 2012 resulting in 522,000 deaths. [5] Due to their large number of clinical occurrences, breast tissue regeneration following lumpectomy (that is, partial removal of breast

tissue) or mastectomy (total removal of the breast) has become an alternative for women to regain back their confident. Most women tend to be deformed in size and shape of the breast hence, making tissue engineering a key to regenerate breast tissue using biodegradable and biocompatible scaffolds to help repair damaged tissues as a result of the surgery. Research has demonstrated that many women who have had a mastectomy or lumpectomy tend to suffer from a syndrome "marked by anxiety, insomnia, depressive attitudes, occasional ideas of suicide, and feelings of shame and value" [6]. The concept of breast tissue regeneration due to mastectomy has been established to ease the sense of damage and suffering that women experience after surgery. Thus, it has become a valuable alternative to any woman undergoing surgery for breast cancer or any other breast ailment.

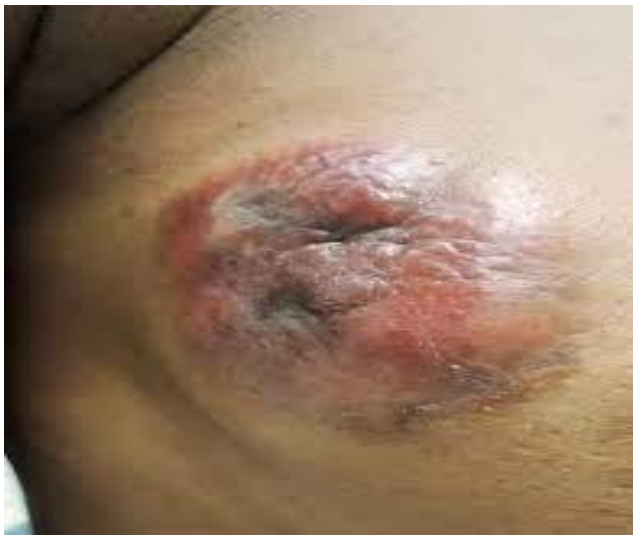


Figure 1.1; the breast of a patient diagnosed with breast cancer

1.3 Statement of Problem

Breast cancer and congenital defects or damage are serious problems that women have had to manage over time. There is also a need to regrow breast tissue, following operations to remove breast cancer tissue at different stages of development. This can be addressed by implanting resorbable 3D printed scaffolds with geometries that can be printed to occupy the space left behind after breast tissue resection.The scaffolds can be used to regenerate breast tissue with comparable mechanical properties to normal breast tissue.

1.4 Aim and Objective of the Study

- To develop resorbable 3D Printed PLA Scaffolds for the regeneration of breast tissue from cultured with non-tumorigenic mammary breast cells.
- To study the initial stages of degradation of PLA scaffolds in Simulated Body Fluid
- To study the cell/surface interactions during the initial stages of exposure of 3D Printed PLA scaffolds to mammary breast cells
- To study the effects of simulated body fluid and cell/surface interactions on the mechanical properties of 3D Printed PLA scaffolds

1.5 Scope of Study

This study explores the possibility of regeneration of the mammary breast tissue from biodegradable breast scaffolds that are produced from PLA that is cultured with normal mammary breast cells.

1.6 Organization of Thesis

The five chapters in this thesis are presented in the following order:

- Chapter 1 – Introduction
- Chapter 2 – Literature Review
- Chapter 3 – Materials and Methods
- Chapter 4 – Results and Discussion
- Chapter 5 - Conclusions and Future Work

CHAPTER TWO

2.0 Literature Review

2.1 Current Approaches aimed at Regeneration of Mammary Breast Tissue

Over the last 10 years, the field of Tissue Engineering has improved significantly following the potentials for regeneration of many organs and tissues in the human body. Polymer scaffolds, tissue cells and

stimulation factors has been enormously adopted as an attractive therapeutic treatment for tissue defects. In recent years, biodegradable polymer have been to fabricate tissue scaffolds such PLA, PCL, PLGA etc due to their excellent properties such as absorbabilities, non-toxicity and biocompatibilities. PLA has also been approved by the US Food and Drug Administration as a biodegradable and biocompatible used for the human body. Biocompatible materials such as metals, ceramics and polymers have been extensively used for surgical implantation. However, metals and ceramics are not biodegradable and their processability is very limited. Polymer materials have received increasing attention and been widely used for tissue engineering because of easy control over biodegradability and processability [7, 8, 9]. Bioabsorbable polymers are preferred candidates for developing therapeutic devices such as temporary prostheses, three-dimensional porous structures as scaffolds for tissue engineering and as controlled/sustained release drug delivery vehicles. [5] Synthetic biodegradable poly-lactones such as poly-lactic acid (PLA), poly-glycolic acid (PGA), and poly-caprolactone (PCL) as well as their copolymers are now commonly used in biomedical devices because of their excellent biocompatibility.

Poly (L-lactic acid) (PLLA) is widely used in the biomedical field due to its biodegradability, biocompatibility, thermal plasticity and suitable mechanical properties [9, 10]. More recently, biodegradable materials have found enormous interest as supports because of the fact that the support disappears from the transplantation site with the passage of time, leaving behind a perfect patch of the natural tissue [11]. Three dimensional porous scaffolds of PLA have been created for culturing different cell types, using in cell based gene therapy for tissue regeneration and other treatments of cardiovascular, neurological, and orthopedic conditions [12, 13, 14].The mammary gland, which differentiates mammals from all other animals, functions to produce and secrete milk in order to nourish offspring. Indeed, studies of mammary gland development have presented a unique insight into the mechanisms regulating cell growth, cell and tissue polarity, differentiation, branching morphogenesis and the transformation of a functional organ. Moreover, many dysregulated pathways and processes observed in breast cancer progression mimic those observed during normal mammary gland development and tissue remodeling; as with most glandular tissues, the adult mammary gland is comprises of multiple cell types, including epithelial, adipose, fibroblasts, immune, lymphatic and vascular cells, which work together to shape and maintain a functional organ. [13]

Currently, there are 3 main surgical approaches for regeneration of mammary tissue following regeneration with autologous tissue (regeneration with scaffolds implants, free/pedicled flaps and lipofilling).

2.2 Prosthetic implant-based regeneration

Two different approaches may be adopted for breast tissue repair/reconstruction and regeneration. Both approaches may benefit from an appropriate selection of polymeric and composite materials, which are widely employed in the tissue engineering and prosthetic fields [14], as well as from the more advanced fabrication methods (i.e., additive manufacturing techniques). In this context, over the past years researchers' attention has been focused on the development of multifunctional devices in the form of gels/ hydrogels, semi-interpenetrating polymer networks [15-16] and 3D advanced scaffolds [17-23]. The breast implant must properly reproduce the exact shape and size of the defect as well as the mechanical features of the native tissue through a suitable material/geometry design. In this case, 3D scaffolds must be designed to possess suitable architectural features, tailored mechanical and mass transport properties according to the specific application. To this aim, aliphatic polyesters such as poly lactic acid (PLA) together with other synthetic biodegradable polymers commonly used for tissue engineering applications [23] can be properly considered.

2.3 Anatomy & Physiology of the Breast

The breast is an organ whose structure reflects its special function: the production of milk for lactation (breast feeding). The normal human breast consists of ductal epithelium and surrounding stroma. The stroma consists of two compartments (intralobular stroma and extralobular stroma), accounts for more than 80% of the breast volume, and provides nutrition and structural support for the normal epithelium. The epithelial component of the tissue consists of lobules, where milk is made, which connect to ducts that lead out to the nipple. These lobules and ducts are spread throughout the background fibrous tissue and adipose tissue (fat) that make up the majority of the breast. The blood supply from the breast comes primarily from the internal mammary artery, which runs underneath the main breast tissue. The blood supply provides nutrients, such as oxygen, to the breast tissue. The lymphatic vessels of the breast flow in the opposite direction of the blood supply and drain into lymph nodes. The dimensions and weight of the breast can vary substantially between individuals. A small to moderate breast weighs about 500 g or less [24], and large breasts weigh about 750 to 1000 g [25]**.** Some women have more glandular tissue in their breasts and some have less, and likewise, some have more fatty tissue or connective tissue than others, and the ratio of fat to connective tissue content determines the firmness of the breast. The size and shape also varies over time in the same woman because of the changes during menstrual cycle, pregnancy, after weaning, and during menopause [27].

2.4 Cellular Breast Regeneration

It involves cell seeding on a scaffold followed by culturing in vitro prior to implantation in vivo. The ideal scaffolds provide a framework and initial support for the cells to attach, proliferate and differentiate, and form an extracellular matrix (ECM) [27, 28]. It should be noted that scaffold surface topography and chemistry (wettability, softness and stiffness, roughness); microstructure (porosity, pore size, pore shape, interconnectivity, specific surface area) and mechanical properties [29] have been shown to significantly influence cell behaviors such as adhesion, growth and differentiation, and to affect the bioactivity of scaffolds used for in vivo regeneration applications of various tissues, such as breast, cartilage, skin and peripheral nerves. PLA has been utilized as ecological material as well as surgical implant material and drug delivery systems, and also as porous scaffolds for the growth of neo-tissue [30].

2.5 Mechanical Properties of Normal Breast Tissue

2.5.1 Basic Concepts

The biomechanical properties of tissue (ex. stiffness/elastic modulus) vary markedly between organs and tissues, and are inherently related to tissue function. Breast tissue has a unique rheology and optimum biomechanical properties, changing over the course of development in response to function (as during mammary gland lactation) or in pathological situations (such as tumors). Although, breast tumors are stiffer than normal breast. [31, 32] An important characteristic of breast tissue is their nonlinearity at high deformation [33]. For example, the tensile response of breast tissue exhibits nonlinear stiffening while undergoing high deformations. The mechanical characteristics of soft tissues consist, in general, of a complex combination of elastic and viscous components [34]. This combination controls the deformation of tissue [35].

2.6 Engineering Challenges

The complexity of mammary tissue and the variety of cells involved makes tissue regeneration an ambitious goal. Technical problems regarding the definition of supports (scaffolds), cells used and stability and culture medium has been a challenge to issues regarding regeneration in tissue engineering application. Hence, the need for a significant requirement for tolerant 3D models that could contribute to understanding both normal tissue function and changes that occur in disease, particularly cancer thereby helping to

overcome many of the shortcomings associated with experimentation and two-dimensional (2D) tissue culture. Clinical application is limited due to high machine cost, design and fabrication time involved.

2.6.1 Squared surface model

Essential parameters which scaffold should meet for a proper cell proliferation is sufficient and regular porosity, and imitation of the original architecture of tissue or organ that needs to be regenerated. [36] According to these conditions 2 types of scaffold structures for bone tissue regeneration were designed and printed. The reasons of different inner structures of both scaffolds are as follows:

Scaffold ST1 – Presumption that the scaffold will be seeded by cells from the top. thus individual filaments need to overlap each other vertically in each second layer to prevent the cells "fall" down through the scaffold structure (see the figure below - scaffold).

Scaffold ST2 – Porosity is approx.. 50–60% higher than in case of ST1 in order to determine whether the cells attach individual filament even if there are vertical gaps between layers (see the figure below)

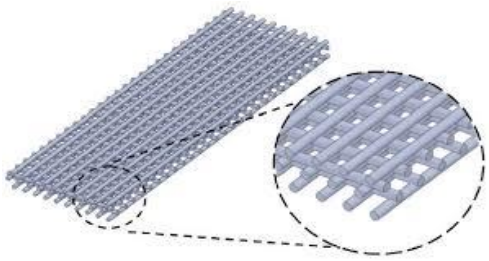


Figure 2.1; Scaffold design; the porosity of ST1 scaffold was expected around 30% and intended diameter of the filament is 0.35 mm and pore size 0.35 mm

In fact, we have recently confirmed that the variation of scaffold geometry from an orthogonal configuration (squared pores) to a diagonal configuration (triangular pores) (see Fig. 1) affects both macrophages morphology and cytokine expression (data not shown). Furthermore, orthogonal scaffolds promoted the presence of rounded multinucleated giant cells, whereas diagonal ones lead to elongated macrophages.

2.6.2 Scaffold design and porosity

To repair damaged tissues and organs, tissue engineering currently utilizes artificial supporting structures called "scaffolds", which serve as carriers of cell cultures and control their growth. Scaffolds are fabricated

as porous structures of pre-defined shapes. Their structure properties include external geometry, porosity, porous interconnectivity, individual pore size, and surface area [37]. Scaffolds are prepared using biodegradable materials, allowing the material gradually disintegrates (degrades) after the formation of a new tissue or organ. Scaffolds are seeded with suitable cells (depending on the type of tissue) in vitro and then implemented in vivo into the place of damage. Here, through the porous structure of the scaffold a cell proliferation occurs, which enables the formation of a new tissue [38]. Design and inner architecture of the 3D structure strongly depends on its final application. An important parameter affecting cell response is the scaffold geometry including pores size, shape, and struts size and orientation among others. Scaffolds architecture not only affects their mechanical performance but also affects their permeability, nutrients diffusion and cell response [39]. Sufficient and regular porosity is required for uniform cell proliferation both in the space of scaffolds and in time the speed of cell proliferation and degradation of the material should ideally be uniform. Current studies report that ideal scaffold porosity should be around or more than 90% (especially for bone tissue engineering) and pores should provide good interconnectivity to ensure good proliferation of cells [40].

2.6.3 Scaffold Manufacturing

Materials currently used for scaffold manufacturing are split into several types; entirely synthetic materials, natural materials, ceramics, and their combinations. Natural fibres used in scaffolding include collagen, the protein that creates the majority of extracellular matrix; alginate, a plant polymer derived from algae; chitosan, derived from chitin found in insects and fibrin gel [41]. Synthetic materials allow for a better control of chemical, physical and mechanical properties, as well as degradation rate. In addition, fabrication methods can process synthetic materials into scaffolds of desired porosity, morphologies, and anisotropies with well improved cell attachment and migration. The synthetic materials that scaffolds are usually made of are polymeric. The most popular polymers are linear aliphatic polyesters. This group includes polyglycolic acid (PGA), polylactic acid (PLA), and their co-polymers polylactic co-glycolic acid (PLGA). [42] One of the most promising techniques for an “ideal” scaffold structure fabrication is Rapid prototyping due to its excellent control over the geometry of the created sample while industrial 3D printers have reached extremely high resolution in the past few years. Unfortunately, porosity reduces mechanical properties such as compressive strength, and increases the complexity for reproducible scaffold manufacturing. Mechanical properties constitute another important feature of the scaffold. This importance has multiple reasons; growing cells may exert force, and certain cell types such as fibroblasts generate

substantial force, a mechanically weak scaffold might be broken down under the load of these forces and change the shape of the final tissue structure [43].

2.6.4 Scaffold Requirements and Fabrication Methods

Scaffold design plays an important role in manipulating cells behavior and guiding tissue formation in tissue engineering. The optimal scaffold should mimic the target tissue in its native condition. Tissue engineering uses porous 3D scaffolds to provide the appropriate environment for the regeneration of tissues and organs. These scaffolds usually act as a support for biomolecules decoration and cell loading and eventually for tissue formation, as is shown in figure below [42]. Although there is discrepancy to this effect which is why there is a need to take into consideration certain conditions and requirement because different site of body makes it difficult for exact mimic composition, organization and multiple functions of native tissues. Thus following are significant when designing or determining the feasibility of a scaffold for a specific application in tissue engineering:

a) Biocompatibility
b) Proper degradation
c) Suitable mechanical strength
d) Scaffold architecture
e) Easy modification
f) Easy processing

2.6.5 Scaffold Fabrication Methods

Several methods have been developed to engineer biomaterials into desirable complex architectures for specific usage in tissue engineering which includes solvent casting and particulate leaching, Phase separation, 3D printing, freeze drying, electrospinning, self-organization, microfabrication. But for the purpose of this study, our focus is on the fabrication of scaffolds using 3D printing.

2.7 3D Printing

Rapid prototyping (RP), also known as additive manufacturing (AM), has been well received and adopted in the field of biomedical application of which the techniques to fabricate customized 3D structures with

complex geometries and excellent reproducibility has revolutionized implantology and regenerative medicine. In particular, nozzlebased systems allow the fabrication of high-resolution polylactic acid (PLA) structures that are of interest in regenerative medicine. The 3D in vitro provides platforms for studying cell response to different scaffolds conditions. The approach consists of a system integrated with pumping technology and a CAD/CAM system for the fabrication of 3D structures with well-defined predetermined geometries. Scaffolds obtained gives platforms for studying the effect of various parameters such as scaffolds architecture, pore size, geometry, topography, wettability, and mechanical properties among others, on cells behavior including inflammatory response.[44, 45]

2.8 Scaffold Biomaterials

Implantable 3D scaffolds are used for restoration and reconstruction of different anatomical defects of complex organs and functional tissues. . Scaffolds are three-dimensional (3D) porous, fibrous or permeable biomaterials intended to permit transport of body liquids and gases, promote cell interaction, viability and extracellular matrix (ECM) deposition with minimum inflammation and toxicity while bio-degrading at a certain controlled rate. Based on their chemical composition, biomaterials used for 3D scaffolds are classified into metals, ceramics and glass-ceramics, natural and synthetic polymers, and composites [46, 47]. Also, biomaterial scaffolds are used for delivering therapeutic agents like proteins, growth factors, drugs, etc. and the anchorage of these substances to the scaffold is of high importance for loading. As biomaterial-cell interactions are key to cell viability, proliferation and differentiation, characteristics of biomaterials such as surface chemistry, charge, roughness, reactivity, hydrophilicity, and rigidity need to be considered. [48, 49]

2.9 Formation of Tissue Constructs

The major aspect of tissue engineering is the design and fabrication of constructs for the replacement of non-functional or damaged tissue. In an additional feature, the creation relates to a tissue construct having a composite structure. The tissue construct includes:

(a) A biodegradable substrate, in which the substrate is modified to allow deposition or growth of a plurality of cells.

(b) A vascularized layer comprising a plurality of blood vessels therein.

In advance aspect, tissue construct for the growth and structuring of new tissue or for the repair of damaged tissue is developed. The tissue construct may be configured into various shapes as long as it is moderately flat and adequately flexible to influence the shape of the tissue construct to conform to the anatomical site

of interest and to be sutured. The tissue construct described can be used for implantation or regeneration in mammals (such as a human, dog, cat, rabbit, mouse, rat, etc.). The layer thickness of contrast should be in the micrometer range about 10 to about 500 μιη (microns) not thicker than about 1,000 μιη. The substrate consists of a biodegradable material, such as a biodegradable polymer. Biodegradable material is readily prone to biological processing in vivo.[42] Biodegradable material may result in the formation of primary degradation products such as compounds of low molecular weight, which then decay further through the action of a living organism.

2.9.1 Polymers in Tissue Engineering

Different materials have been used to produce scaffolds for numerous applications. These include natural and synthetic polymers. Ceramic materials are also used, particularly in mixture with polymers especially in bone tissue applications, as a result forming composite materials with enhanced mechanical and biological properties. Moreover, natural or synthetic polymers can be used to form the matrix used in breast tissue engineering applications, although synthetic polymers are preferred for reproducibility and controlled release kinetics. Synthetic polymers that can be used include biodegradable polymers such as poly (lactide) (PLA), poly (glycolic acid) (PGA), Poly lactide-co-glycolide (PLGA), poly (caprolactone), polycarbonates and so on. [50]

2.9.2 Natural Polymers

Polymer materials such as collagen, fibrin, glycosaminoglycans (GAGs), chitosan, alginates and starch, can be extracted from plants, animals or human tissues; they demonstrate good biocompatibility, low toxicity and a low chronic inflammatory response. They can be combined into a composite with other natural materials or synthetic materials and can be degraded by naturally occurring enzymes. Disadvantages include poor mechanical properties and they often require chemical modification to increase strength such as cross-linking by dehydrative methods or chemical methods (glutaraldeyde). [51]

2.9.3 Synthetic Polymers

Synthetic polymers represent the largest group of biodegradable polymers. Recent developments in the synthetic biodegradable polymers have significant interest for macromolecular science in both environmental and biomedical perspectives. One of the most important polymeric candidates is the biodegradable poly (lactic acid) (PLA) that is described as aliphatic polyester.

2.9.4 Biodegradable Polymers

Biodegradable synthetic polymers offer a number of advantages over other materials for emergent scaffolds in tissue engineering. The major advantages include the ability to adapt mechanical properties and degradation kinetics to suit various applications. Synthetic polymers represent the largest group of biodegradable polymers. They exhibit predictable and reproducible mechanical and physical properties such as tensile strength, elastic modulus and degradation rate. Synthetic polymers are also attractive because they can be fabricated into various shapes with desired pore morphologic features conducive to tissue in-growth. Moreover, polymers can be designed with chemical functional groups that can induce tissue in-growth. Biodegradable synthetic polymers such as poly (glycolic acid), poly (lactic acid) and their copolymers, poly (p-dioxanone), and copolymers of trimethylene carbonate and glycolide have been used in a number of clinical applications.[51, 52] An unusually wide range of polymeric implant devices are used in soft tissue sites. Just a few examples include mammary prostheses, drug delivery systems, sutures and reconstructive (plastic surgery) materials. Degradation rate of scaffolds can be adapted to the specific applications by selecting specific polymers, copolymers or blends. Most of these polymers undergo to a simple hydrolytic degradation. The biocompatibility of soft tissue implants has generally been associated with their toxicology (i.e. the leaching or extraction of cytoreactive components into the body space). [53]

Diagram of some polymers;

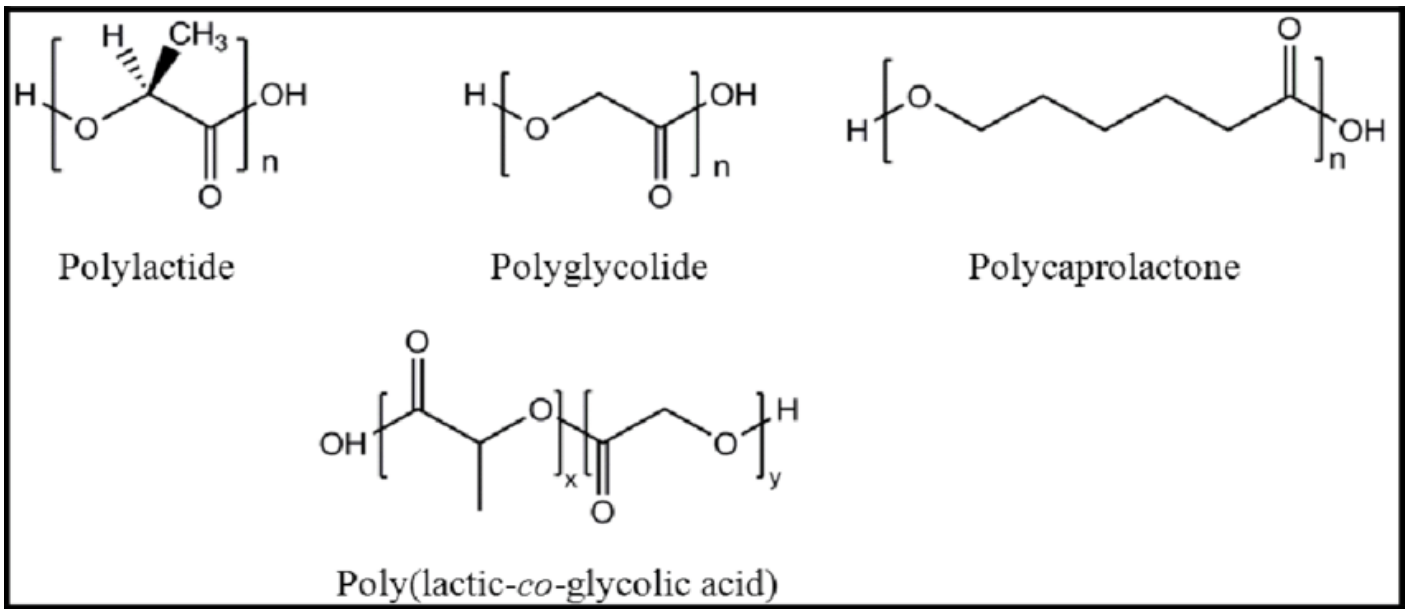


Figure 3.1; schematic showing the chemical structure of some main biodegragradable polymer used in biomedicine

2.9.5 Physiochemical Properties of PLA

Poly lactic acid is a bioplastic produced by totally renewable sources that belongs to the family of poly (alpha-hydroxyl esters). Although it was first synthesized in 1932 by Carothers (DuPont), its patenting occurred in 1954, as a higher molecular weight was achieved. Its monomer is the lactic acid molecule, and its esterification leads to the existence of stereoisomers, such as poly (L-lactide) (PLLA), poly (D-lactide) (PDLA), and poly (DL-lactide) (PDLLA). The eco-friendly characteristics of PLA, in terms of renewability, recyclability, non-toxicity and compostability, make it very promising in the perspective of green chemistry applications, whereas its cytocompatibility and the biocompatibility of its degradation products make it attractive as material for biomedical and drug delivery applications. Depending on these characteristics, PLA may be amorphous or semi-crystalline and its crystallinity may depend on intrinsic chemical-physical properties, such as stereochemistry, or preparation conditions, such as thermal history [53, 54]. PLA tends to be crystalline when the amount of PLLA is higher than 90%; otherwise, it tends to be amorphous. The content of PLLA affects even melting temperature (Tm) and glass transition temperature (Tg), as well as mechanical properties. Elastic modulus proved to increase from less than 1–3.5 to 2.7–4.1 GPa as a function of L-lactide content, whereas tensile strength was found to vary from 20– 50 to 60–80 MPa. Notably, elongation at break of PLA seems to be scarcely affected by stereoisomer content and equal to 2–10%. Physical characteristics such as density, heat capacity, and mechanical and rheological properties of PLA are dependent on its transition temperatures. [55]

2.9.6 Hydrolysis of PLA

The degradation properties of a scaffold are of essential importance for biomaterial selection and design especially for the long-term success of a tissue engineered construct. Polymers have been shown to degrade mainly by simple hydrolysis of the ester bond into acidic monomers, which can be removed from the body by normal metabolic pathways. Other factors that affect degradation include hydrophobicity and molecular weight [53]. The chain cleavage reaction during the hydrolytic degradation of PLA proceeds preferentially in amorphous regions, which leads to an increase in the polymer crystallinity and it, has also been noted that the crystallinity of PLA tends to increase as it degrades. In aqueous solutions, the hydrolytic degradation of PLA proceeds via random cleavage of the ester bond, which is controlled by four basic parameters: the rate constant, the amount of absorbed water, the diffusion coefficient of chain fragments within the polymer, and the solubility of degradation products. In general, the hydrolytic degradation of PLA-based solid polymer matrices can proceed through under two different mechanisms: (i) surface or heterogeneous reactions and (ii) bulk or homogeneous erosion [54]. Hydrolytic rate was dependent on the molecular weight of the oligomer along with pH and temperature of the media.

(B)

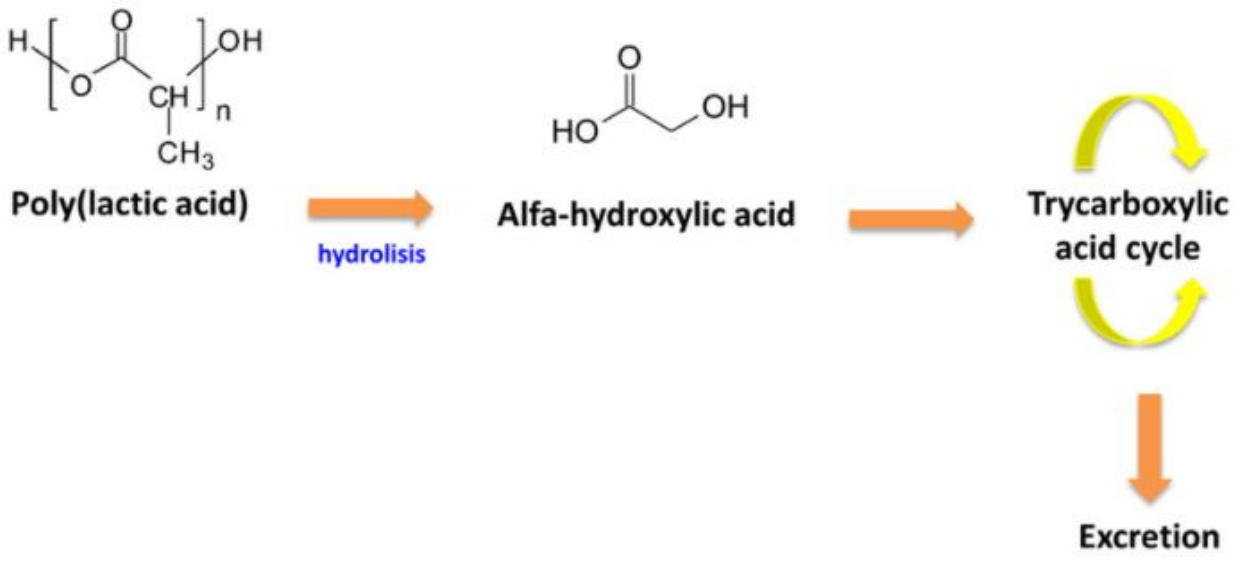


Figure 4.1; the hydrolysis of PLA

2.9.7 Rate of Degradation

The main intent of engineered tissue is to replace and regenerate damaged tissue or organ. In order to comply with this requirement, the scaffold material of the transplanted tissue should be subjected to remodeling and absorption. They should be able to degrade in equal or similar pace with the regeneration of extracellular matrix and differentiation of cells. This phenomenon depends on several factors, including hydrophilicity of the scaffold, surface area, porosity, degree of crystallinity, presence or absence of certain enzymes, etc. The most critical part here is harmonization in these factors, so that the degradation of biomaterial and stress release to the surrounding tissue is well synchronized, to ensure healing of the damaged tissue. [54-58]

2.9.8 Effects of pH

It is simple to understand that the material degradation strongly depends on temperature. By examining the half-life of MW of PLA in the form of porous scaffolds under different temperatures [54], we confirmed that the biodegradation of porous scaffolds obeys the Arrhenius equation with activation energy. The pH effect is also not unexpected. PLA degrades prevailingly via chemical hydrolysis, and a low pH or very high pH causes a significant effect to catalyses hydrolysis of an ester bond. So, temperature and pH should be strictly controlled for a convincing *in vitro* degradation test.

2.10 Cell Culture

Cell lines used in 3D experiments are transduced with genetic constructs driving expression of fluorescent proteins, in order to allow monitoring of the cells during cultivation. Since no common protocol to generate labeled cell lines was generated, but a variety of working protocols exist [56]

2.11 Key Factors about Scaffolds that Affect Cell/Surface Interactions

The following characteristics of scaffolds affect cell/surface integration during tissue engineering:

2.11.1 Cell Responses Due to the Surface Chemistry of Tissue Engineering Scaffolds

These phenomenon as listed below shows the various reaction which takes place on the surfaces of the scaffolds.

2.11.1.2 Surface Hydrophobicity

The surface hydrophobicity is well known as a key factor to govern cell response. The surface hydrophobicity can be assessed by measuring contact angle through water spread of a droplet on a surface. The lower the contact angle, the more hydrophilic the surface is. Previous studies showed the more hydrophilic surface of material films is the much more cell adhesion on the surface [59]. For example, osteoblast adhesion was reported decrease when the contact angle of surface increased from 0° to 106°. Fibroblasts were found to have maximum adhesion when contact angles were between 60° and 80° [60]. Furthermore, surface hydrophobicity is related to the rate of cell spreading and differentiation. On hydrophilic surfaces, cells generally showed good spreading, proliferation and differentiation.

2.11.1.3 Surface Charge

After surface hydrophobicity, surface charge has been recently described a lot in the cell attachment phenomenon. Firstly, the amount of surface charges can influence cell behavior. Secondly, many researchers reported the improved biocompatibility, cell affinity and cell differentiation on the implanted surfaces by using the positive ions and the negative ions [61]. Positively charged surfaces, for example, modified with quaternary amine, have been proved to largely enhance cell adhesion and cell spreading with or without serum on hydrophilic surfaces or even on hydrophobic surfaces in the presence of serum [62]. The best cell adhesion, growth and spreading rate were recorded on polar and positively charged surfaces (amine group grafted PE) while the negatively charged surface (carboxylic acid group-grafted PE) still had poor growth. Moreover, the surfaces grafted with neutral amide and hydroxyl groups showed a similar number of cell attachments; however; the morphology of cells attached on the surfaces was quite distinct. The cells were spread much more on the hydroxyl group grafted surface than the amide group-grated one. On the other hand, surface charge may modulate protein adsorption to direct integrin binding and specificity, thereby controlling cell adhesion. [63]

2.11.1.4 Protein Adsorption

Many proteins, including immunoglobulins, vitronectin, fibrinogen, and fibronectin (Fn), adsorb onto implant surfaces immediately upon contact with physiological fluids and modulate subsequent

inflammatory responses. For example, adsorbed adhesive proteins mediate the attachment and activation of neutrophils, macrophages, and other inflammatory cells. [61] Hydrophobic surfaces tend to absorb more proteins, while hydrophilic surfaces tend to resist protein adsorption. Absorption onto various polymer substrates and the maximal protein absorption were observed on surfaces with water contact angle ranging from 60^{o} to 80^{o} [62]

2.11.1.5 Surface Topography

Material surface roughness or Topography plays an important role in regulating cell adhesion, migration, proliferation, and differentiation on the substrates [63]. Material surface roughness has a direct influence in vitro as well as in vivo on cellular morphology, proliferation, and phenotype expression. Studies have been reported that cells grown on micro rough surfaces were stimulated towards differentiation; as shown by their gene expression in comparison with cells growing on smooth surfaces. Considering the fundamental method of cell responses to scaffold surface with specific topography is a key to successful regeneration of tissues with optimal structures and functions. The most prominent phenomenon might be the contact guidance of cells: namely, cell alignment on an anisotropic surface. Usually, this leads cells to elongation along groove or ridge structures. Cell viability and proliferation are also regulated by surface topographical features. As a basic cellular event, cell migration is also significantly sensitive to the topographic cues called topotaxis. Depending on the scale of irregularities of the material surface, surface roughness can be divided to macro roughness (100 μm – millimeters), micro roughness (100nm – 100 μm), and nano roughness (less than 100 nm), each with its specific influence [63, 64]. The response of cells to roughness is different depending on the cell type. For smaller cells, surfaces at nanometer scale (10–102nm) while for large cell, we have between (200 nm–8.0 μm) of which the human mammary epithelial cells (hTERT-HME1) cultured surface is about (4.0 nm). Hence, the selectivity of cells on surface roughness could be greatly of improvement on the development of implanted devices.

2.11.1.6 Surface Softness and Stiffness

Numerous studies have reported that cell attachment, proliferation and differentiation are all modulated by the substrate rigidity to a degree dependent upon the substrate stiffness in relation to the stiffness of the native tissue. To obtain good mimic of *in vitro* environment, it is essential to fabricate scaffolds with similar stiffness of targeted sites because scaffolds poses a significant effect on cell performance, for example, cell spreading, migration and differentiation. Stiffness also exhibits certain environmental factors (e.g.

temperature, pH and electric field). Therefore, mechanical cells response is altered due to the cell projection area and polarity ranging from stiffness changes [65].

2.11.1.7 3D Architecture

On a macroscopic level, the overall shape of the scaffold provides boundaries for tissue regrowth. On a microscope level, the material provides a framework and capillary networks for local cell growth and tissue organization, permitting cell attachment, distribution and proliferation within a controllable microenvironment [65]. Altering the micro-architecture, such as the material crystallinity or the microporosity, and/or the macro-architecture of the scaffold can be achieved by changing the pores size, porosity, pore interconnectivity and tortuosity, to match the characteristics of the native tissue whilst retaining integrity. Scaffold porosity in particular controls the key processes of nutrient supply to cells, metabolite dispersal, local pH stability, mechanical stability at this critical interface and cell signaling. The size of the pores can affect how close the cells are at the initial stages of cultivation (allowing for cell-cell communication in three dimensions), but also influences the amount of space the cells have for 3-D organization in the later stages of tissue growth. Porous structure allows cells to grow and migrate in 3D space within scaffold as they do *in vivo and in vitro*. Suitable pore size and good interconnectivity can allow efficient diffusion of nutrients and removal of metabolic wastes, thus promoting cell proliferation in 3D space. [66] The porous scaffolds can also promote the structure regeneration and function realization of tissues.

2.12 Micro-environment

A microenvironment is comprised of stem cells, localized signaling cells, soluble glycoprotein mediators, and the extracellular matrix (ECM) [56]. Within the mammary gland the localized signaling cells consist of epithelial cells, both luminal and basal, myoepithelial cells, fibroblasts and the cells of the stromal compartment including adipocytes. The mammary gland is an intricate network of interconnected ducts and alveolar structures. In the ducts, the luminal cells are surrounded by a continuous layer of myoepithelial cells, However, in the alveolar structures, the luminal cells are surrounded by discontinuous layer of myoepithelial cells [66,67] allowing the luminal cells of the alveolar structures to interact with and receive signals from the different microenvironment components. Such interactions facilitate the further differentiation of alveolar luminal cells into milk-producing cells. This extensive regenerative potential of the mammary gland is due to the presence of the primitive mammary stem cells, which can give rise to both

luminal and myoepithelial cells that make up the ductal and alveolar structures The maintenance and differentiation of the various mammary gland cell types is also dependent on the features and properties of the local tissue microenvironment, in particular those of the surrounding ECM. The importance of the ECM and stroma in mammary gland development and function were proposed several decades ago, reviewed by Varner and Nelson (2014) [68, 69]. The ECM transduces the interaction signals required for normal functioning and undifferentiated cell. Without the interactions, including chemical and physical, provided by the surrounding signaling cells the stem cells will not behave normally. The mammary microenvironment can be regarded as the essential functional building block required for the complete development of a functional mammary gland. Understanding the intricate interactions between all the components of the microenvironment is fundamental in the early detection of pathologies and for future tissue engineering technologies in regenerative medicine. Large amount of data suggest that cell-cell and cell-microenvironment interactions modify the proliferation, survival, polarity, differentiation, and invasive capacity of mammary epithelial cells. However, the molecular mechanisms underlying these effects are poorly understood. Another source of intercellular signals that influence the normal mammary microenvironment is the immune system. [70]

2.13 Cell Adhesion

The mammary gland has long served as a valuable model system for studying cell adhesion in epithelial morphogenesis and tumor biology [71]. Mature mammary ducts exhibit simple epithelial architecture, with a bilayer of inner luminal and outer myoepithelial cells, each expressing distinct adhesion proteins [72, 73]. Studies in the mammary gland have focused on a component of the adherens junction, E-Cadherin and the intracellular molecules (catenins) which associate with it and on a family of molecules which are involved in cell-matrix interactions, called the integrins. Early functional analyses of cell adhesion frequently relied on function perturbing antibodies which in turn has an impact on the morphogenesis of the normal mammary tissue.

2.14 Homeostasis

In the mammary gland, homeostasis involves the renewal of somatic stem cells (basal and luminal). Volume homeostasis within a closed, fluid-filled space is a common physiological problem for multicellular organisms, and maintenance of volume–space homeostasis typically requires two kinds of feedback networks: which includes multiple organs and those that are tissue-autonomous. Disturbances of volume–space homeostasis contribute to pathologies such as hypertension, glaucoma, hydrocephaly, cystic fibrosis,

mastitis, and polycystic kidney disease. In the case of milk filling in the mammary glands, homeostatic regulation of volume is a major practical problem for the dairy industry. [74, 75] In the breasts, volume–space homeostasis is achieved by complex interactions among signals that travel through neuroendocrine pathways and signals strictly within the local environment of the glands [76-80]. Milk synthesis is regulated within the alveolar units of the breast so as to control the degree of alveolar distension in the short term, and adjust milk secretion to the demands of the offspring, in the long term.

2.15 Immunohistochemistry

Histological examinations of in vivo systems using implanted PCL/PLA scaffolds showed that cells intruded into the PCL/PLA scaffolds seem to increase the thermostability of the material. [81]

2.16 Vascularisation

The mammary gland is intercalated with extensive vascular and lymphatic networks present throughout the fat pad. Immune cells, such as macrophages and eosinophils, are also required for branching morphogenesis, and they are recruited to the branching tips of the epithelium to mediate invasion into the fat pad [82].Vascular implants and soft tissues present unique challenges, whereby combining strength, flexibility, and cellular compatibility have led to the use of materials which degrade and are replaced by native tissue overtime. Small and simpler organ printing has been successful, without much difficulty. However, it is not simple when comes to bigger and complex organ, due to difficulty in vascularization. Small tissues are avascular, and most of the time, aneural, alymphatic, and thin or hollow. They can receive nutrition from host vasculature. But when the transplanted tissue is thicker than 150–200 μm, oxygen cannot be diffused from host tissue to it. As such, to create a functional bigger and complex tissue or organ, an integrated vascular system is to be created, which is still not in place [83-84]. Engineering vasculature poses the greatest clinical need in tissue engineering, as without adequate vascularization, any large cell-containing implant will fail from insufficient nutrient exchange. [85]

2.17 Regenerative Therapies

The delivery of therapeutic cells that directly contribute to the structure and function of new tissues is a principle model of regenerative medicine to date [86, 87]. The cells used in these therapies are either autologous or allogeneic and are typically differentiated cells that still maintain proliferative capacity. Materials plays an important role in current regenerative medicine strategies because the material

can mimic the native extracellular matrix (ECM) of tissues and direct cell behavior, contribute to the structure and function of new tissue, and locally present growth factors. [88] For example, 3D polymer scaffolds are used to promote expansion of cells in mammary breast tissue repair. Regenerative medicine approaches, including stem cells therapies and tissue engineering, holds the potential to modernize the management of numerous diseases and trauma in the upcoming years. There are several key technologies and methodologies used in tissue regeneration therapy of which of the first key technology is for the preparation of scaffolds for cell proliferation and differentiation for in *vivo* tissue regeneration. The scaffold is a temporary platform of cell activities. The long-term retention of cell scaffold sometimes causes physical interference against the natural process of tissue regeneration.

CHAPTER THREE

3.0 Materials and Methods

3.1 Introduction

Rapid prototyping (RP) can also be referred to as additive manufacturing (AM), emerged in the field of biomaterials as a new tool for the fabrication of scaffolds with a reputable architectures. An RP technique provides the possibility of building customized scaffolds based on patient-specific tissue defects. These techniques combine computer-aided design together with automated printing technology. The capacity of this family of techniques to fabricate customized 3D structures with complex geometries and excellent reproducibility has proven to be the alternative for implantology and regenerative medicine. [89]

3.2 Materials

Lulzbot TAZ (made in North Dakota, USA) available at African University of Science and Technology, Abuja Nigeria was used for printing the PLA Filament with grey colour pigment (diameter 2.85 mm, density 1.25 g/cm3, printing temperature 195^{o}C – 230^{o}C, printing speed 40-90 mm/s, net weight 3kg (as specified by the manufacturers). [90] Universal Testing Machine (UTM) designed and built by Instron a division of Illinois Tool work (ITW) of USA with maximum capacity of 5000N/500N was used to carry out the mechanical property. Introducing Scanning Electron Microscope (SEM, ZEISS EVO LS10 USA) was used to investigate the morphology of the sample.

3.2.1 Scaffold Design

Fusion Autodesk 360 (San Rafael, California USA) software was created to design the scaffold in .stl files. The base layer of each design was first created as an .f3d file and then using a bottom up approach consecutive, alternating layers were printed to form the 3D scaffold.

3.2.2 Scaffold Fabrication

A desk top Lulzbot TAZ 3D Printer (Lulzbot, North Dakota, USA) a desktop 3D printer system was used to fabricate the 3D PLA scaffolds. The print head dispenses molten material through the nozzle which receives

PLA filament through a thermoplastic extruder to the heated bed which moves in the x–y plane. Computer-aided design-based software fusion Autodesk 360^{TM} was used to design the 3D scaffold structure. The 3D CAD models saved in a .stl format was converted into G-code using Cura software files that enabled the Lulzbot TAZ (made in North Dakota, USA) software to command and Control the printing process. All parameters were left in the default condition.

Table II. Printing Processing Parameters Used

Parameter	**Value**
Filament diameter	2.85mm
Printing speed	60mm/s
Printing temperature	205^{o}C
Bed temperature	55^{o}C

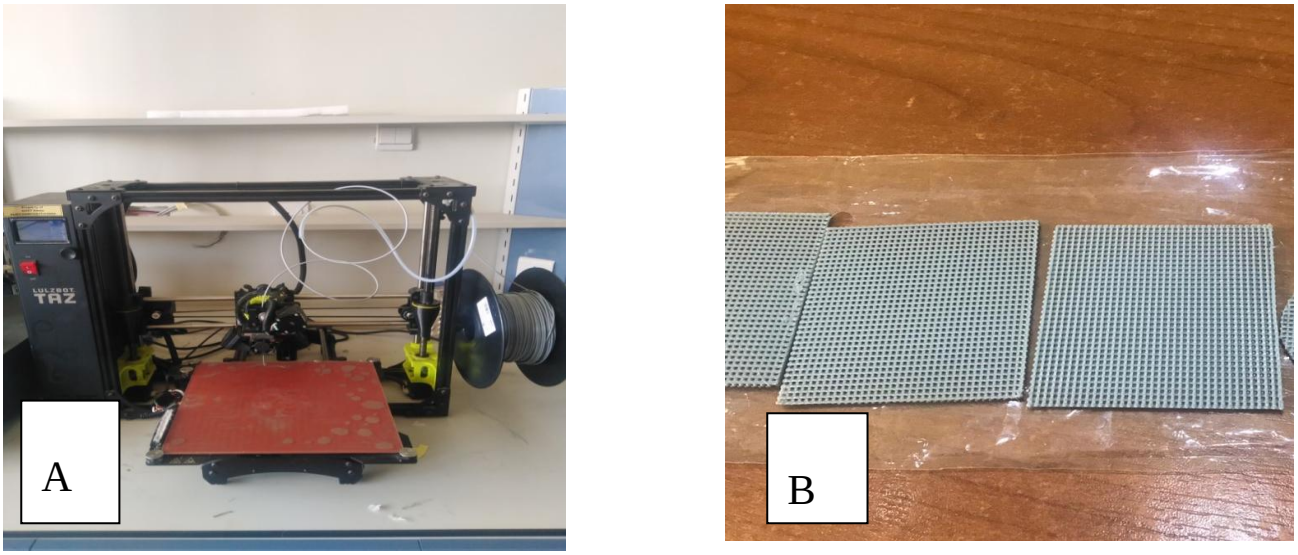


Figure 3.1: (A) Lulzbot TAZ 3D printer, (B) Printed Scaffolds

3.3 Characterization of Samples

Samples characterization was obtained on the 3D PLA structures in order to get better analysis of the surface properties and compositions.

3.3.1 Morphology Analysis

The scaffold was air-dried, and the surface was ready for observation with the Scanning Electron Microscope (SEM, ZEISS EVO LS10 USA) at AUST for the beginning and end of the degradation period at time (t) = 0, 2, 4, 6 and 8 weeks to visualize possible surface changes due to degradation. The scaffold sample was coated with gold/palladium targets using a sputter coater (Quorum SC 7620 sputter coater) set at 10mA for a total time of 60secs for the SEM analysis.

3.3.2 Water Adsorption

the scaffolds were pre-wetted by SBF solution and then immersed in 30 mL of SBF solution at 37 °C in water bath for different periods (2, 4 , 6, and 8 weeks) in a water bath. After immersion, the scaffolds were carefully wiped with filter paper to remove the surface water, and then the weights of the scaffolds were measured as W_{wet}. The dry weights of the scaffolds were measured as W_{dry} before the absorption test.

The water adsorption capacity of the PLA scaffolds was characterized in terms of the swelling percentage (S_w), which was calculated using the following equation;

$$\text{Swelling Ratio } (S_w) = \frac{W\text{wet.} - W_D}{W_D} \; X \; 100$$

$$= \frac{0.0858 - 0.2351}{0.2351} \; X \; 100$$

$$= -63.5\%$$

Where W_{wet} is the weight of the wet sample and WD is the weight of the dried sample. At least three samples were tested for each sample to obtain an average value.

3.3.3 Mechanical Properties of the 3D-scaffolds

Universal testing machine (built by Instron Illinois tool Work USA) with a 5000N/500N load cell was used to evaluate the tensile Strength of the developed scaffolds. For each scaffold composition, three samples were tested although; their original dimensions were measured using a vernier caliper before the test. A

speed of 1 mm/min was used and a preloading of 0.5N was applied. Load-Extension data were computed from load displacement measurements.

3.3.4 Degradation Studies of PLA Scaffold

Degradation studies were performed by immersing the scaffolds in simulated body fluid (SBF), an acellular solution whose chemical composition is similar to that of blood plasma. Samples were immersed in SBF at 37 ºC in a water bath for 8 weeks. The degradation of the materials was evaluated by means of weight loss measurements and SEM analysis.

Figure 3.2: Isotemp 2341 water bath

3.3.5 Weight Loss

Materials' weight loss during degradation was calculated from the changes in the specimens' dry weight before and after the incubation time periods. After 2, 4, 6 and 8 weeks of immersion in SBF, the samples were removed from the fluid, rinsed with distilled water and dried in a furnace at 37°C for 12h or until complete weight stabilization. The percentage of weight loss was computed according to the following equation:

$$\%W = \frac{W_o - W_t}{W_0} X\ 100$$

$$= \frac{0.2351 - 0.1478}{0.2351} X\ 100$$

$$= 37\%$$

Where Wo is the initial dry weight and Wt is the dry weight of the specimen at different degradation times. Values are expressed as the average of three replicates.

3.3.6 Cell Culture Experiments

The assay employs using a DAPI dye and Rhodamine phalloidins which stains the nucleus and the cytoskeleton of the cells embedded in the scaffold respectively thereby produces large fluorescence enhancement of cell growth.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Introduction

This chapter presents the results obtained from the cell culture and degradation experiments. The potential implications are also discussed for the development of 3D-printed scaffolds for breast tissue regeneration.

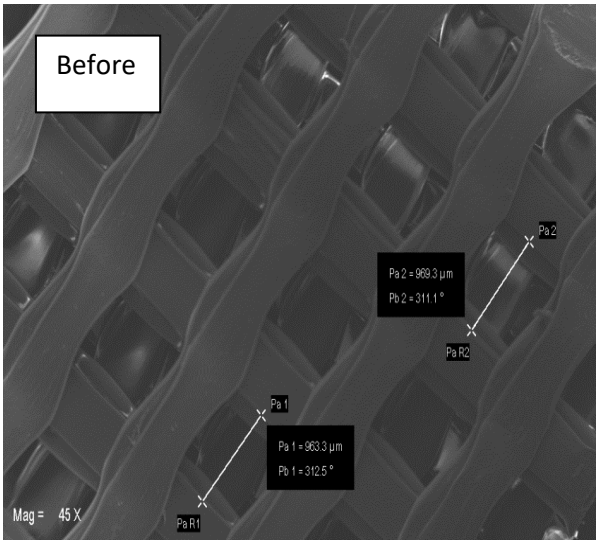
4.2 Characterization

4.2.1 Surface morphology

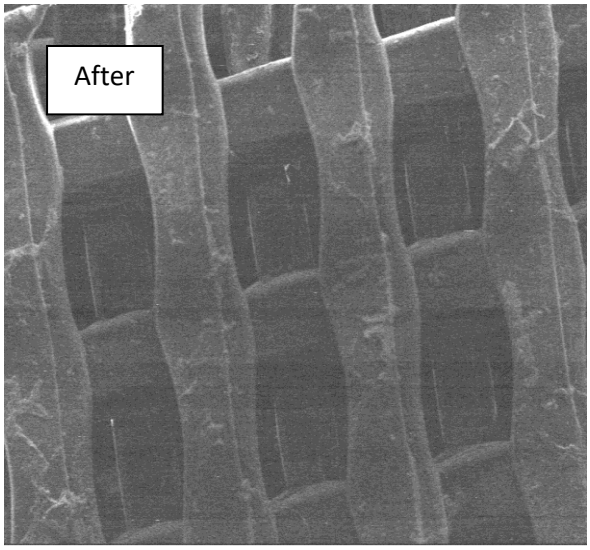
SEM images of 3D PLA scaffolds are presented in Figures 4.1a – 4.1d for scaffolds before and after the degradation studies. The initial surface morphologies of the scaffolds are presented in Figure 4.1. The initial scaffold surfaces are smooth (Fig. 4.1a). However, after exposure to SBF for 8 weeks, clear evidence of surface debris is present on the surfaces (Fig. 4.1b). Spherical cell morphologies are also observed on the surfaces of the scaffolds after days 0 and 7 of exposure of the scaffolds to the mammary breast cells. These are consistent with limited cell spreading during the initial stages of cell spreading and integration, following the insertion of the implant.

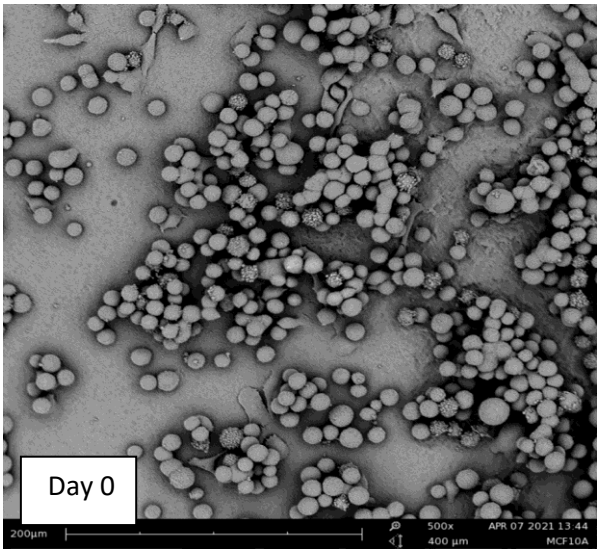
EDS results obtained from the scaffold are presented in Figure 4.1e. These reveal the presence of C, O, Mg, Cl and Ti on the scaffolds. The SEM image in Fig. 4.1f also reveals the surface morphology of the PLA scaffold without cells.

(a)

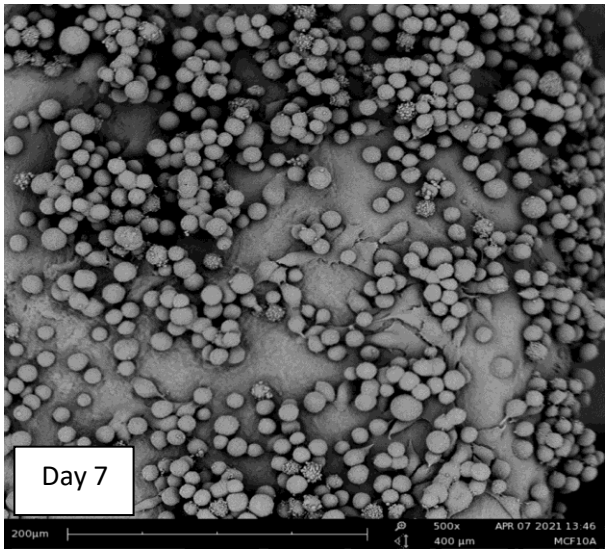

Before
Pa 2
Pa 2 = 969.3 µm
Pb 2 = 311.1 °
Pa R2
Pa 1
Pa 1 = 963.3 µm
Pb 1 = 312.5 °
Mag = 45 X
Pa R1

(b)

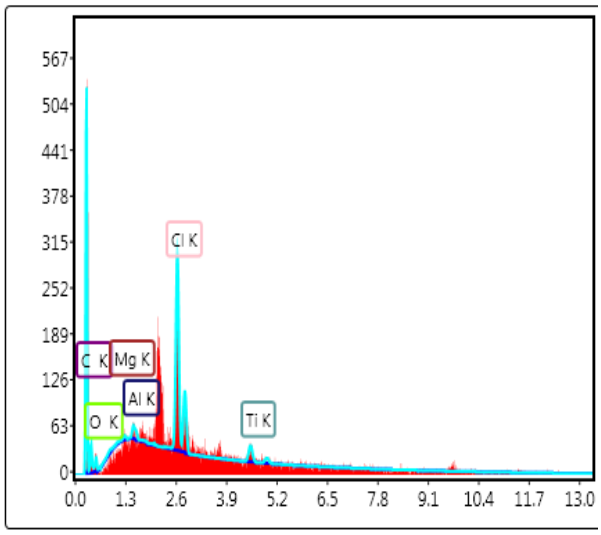

After


Day 0
200µm
500x
400 µm
APR 07 2021 13:44
MCF10A

(c)


Day 7
200µm
500x
400 µm
APR 07 2021 13:46
MCF10A

(d)



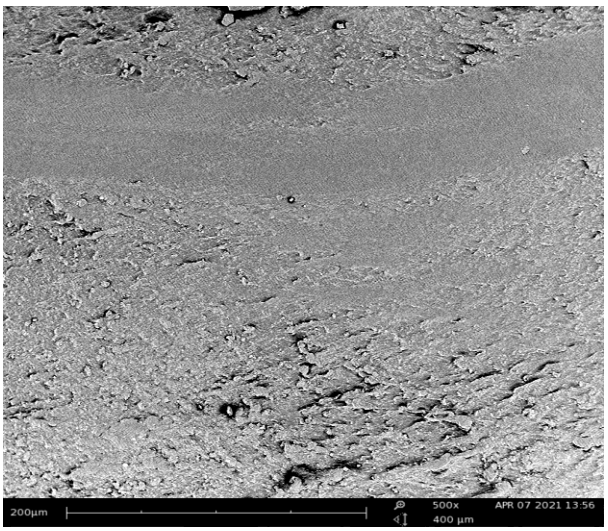


Figure 4.1; ; SEM images showing (a)week 0 immersing in SBF, (b) week 8 of immersing in SBF, (c) Day 0 of scaffold cultured with the cells, (d) Day 7 of scaffold cultured with cells, (e) EDX image of scaffold immersed in SBF, (f) no cells

(e) (f)

4.2.2 Effect of 3D Scaffold on Porosity

The shape of the pores on the PLA scaffolds became longer and narrower and for a longer duration, the clusters will be evenly distributed on the surface of the PLA scaffolds. Therefore degradation time significantly influences the pore sizes and porosities of the scaffolds.

4.2.3 Standard Curves

The plot of absorbance against protein concentration (µg/L) (Fig 4.1) gives a linear line graph with a slope of 0.001 L/µg. These results show the cell proliferation assay of the scaffolds embedded with the breast cell.

4.2.4 Absorbance versus Protein Concentration

The graph of Absorbance against Albumin Protein Concentration (standard curve) for samples is shown below.

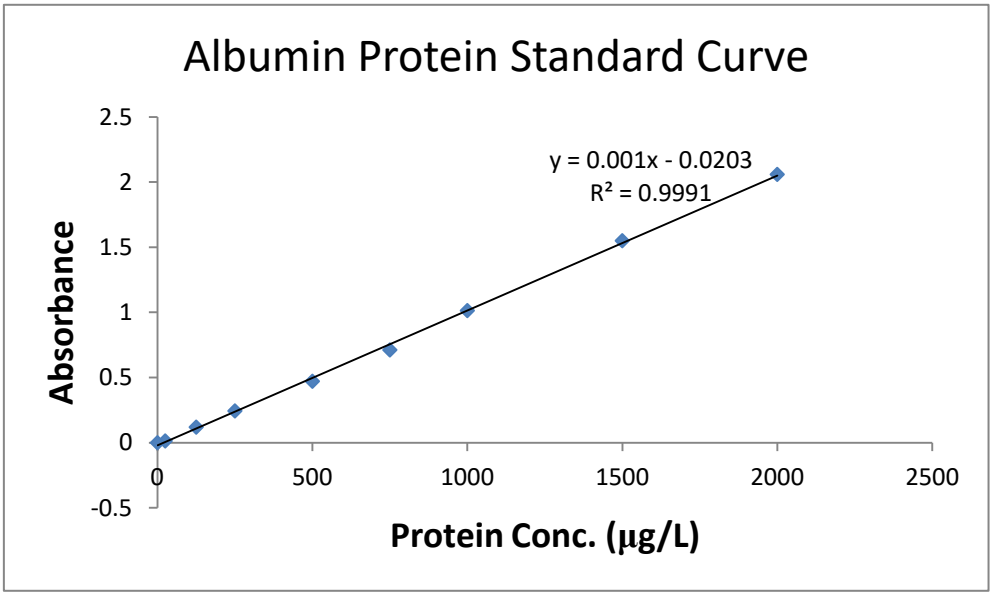


Figure 4.2: 4.2: Plot of absorbance versus protein concentration

(a) (b)

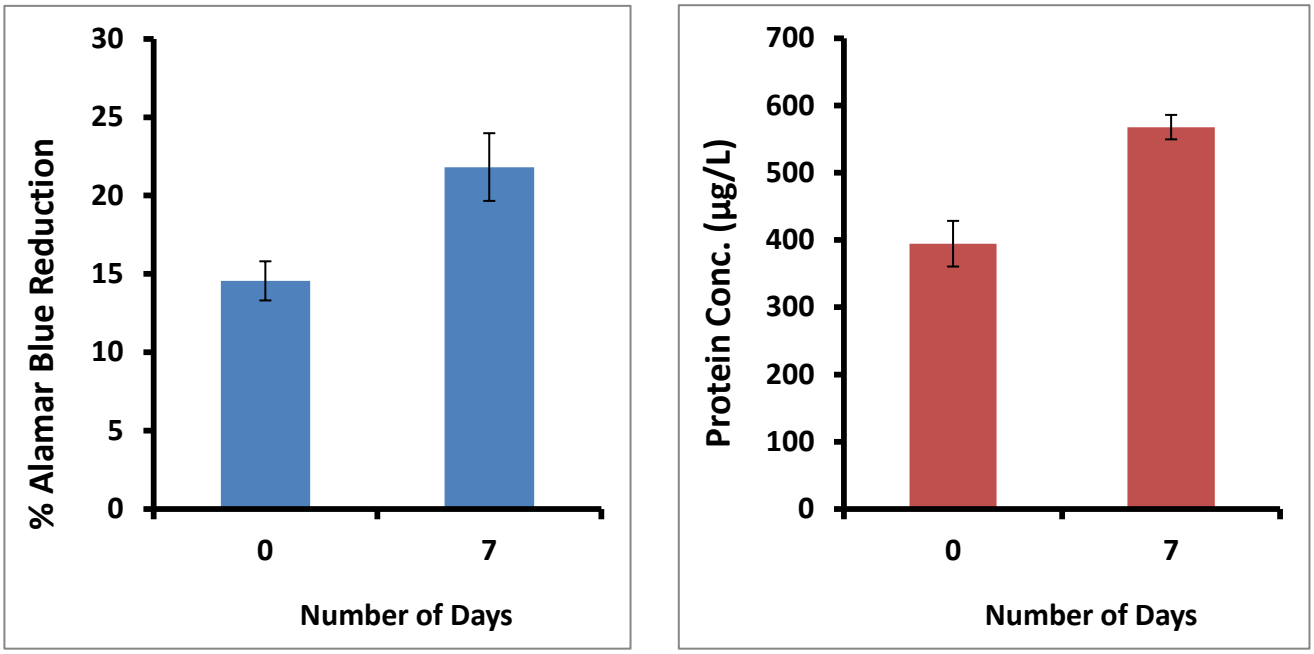


Figure 4.3; gives (a) % Alamar Blue Reduction against number of days of scaffolds embedded with cells, (b) Protein Concentration against number of days of scaffolds embedded with cells

4.2.5 Fluorescence Microscopy

The Fluorescence micrograph or staining gives a visual representation showing that the cells are actually increasing or growing in numbers. Fluorescence micrograph images are show below.

The DAPI dye shows the blue dots colour while Rhodamine phalloidins give red/pink dots colour. The red stain means more cells and the pink stain means fewer cells as seen in (Fig. 4.4).

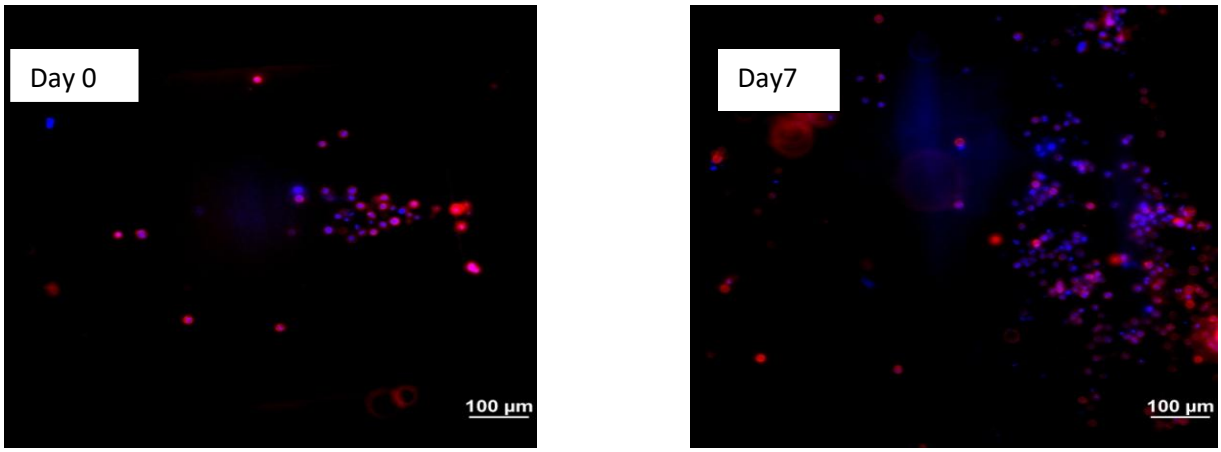


Figure 4.4: Fluorescence micrographs: (C) day 0 and (D) day 7

4.2.6 Weight Loss Versus Time

Material weight loss calculated during the degradation studies showed that the weight of the sample decreases with number of weeks (Fig. 4.5). The results below indicate a significance decrease in weight as the number of week's increases.

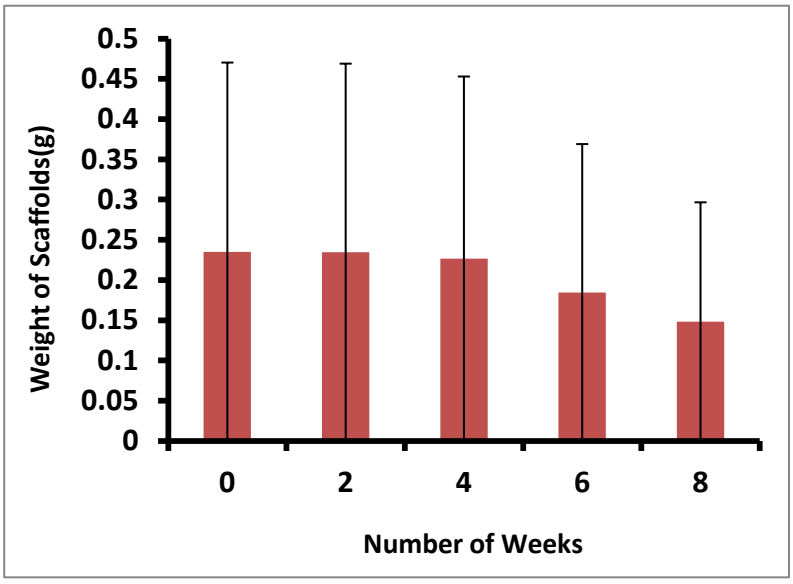


Figure 4.5: weight of scaffolds against number of Weeks

4.2.7 Mechanical Properties of 3D Scaffolds

The tensile strengths of the PLA scaffolds were 3.42 and 6.10 MPa for days 0 and 7, respectively, while the Young's Moduli were 234.67 and 337.33 MPa, for days 0 and 7, respectively.

4.2.8 Dependence of Tensile Strength on the Number of Weeks in SBF

The bar chart below represents the mechanical properties of scaffold immersed in SBF during degradation period of 8 weeks. This shows the tensile strength against number of weeks and the young's modulus against number of weeks for the samples. In general, the strengths and the Young's moduli increased with increasing duration of exposure to SBF (Figs 4.6a and 4.6b).

(a) (b)

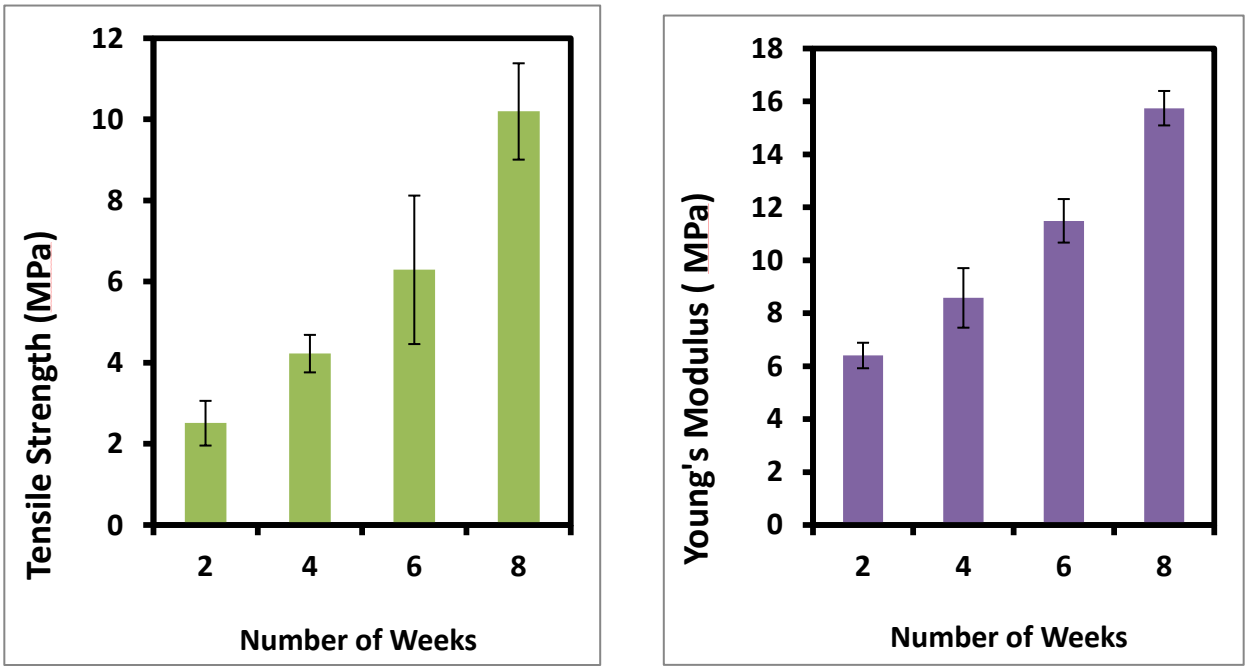


Figure 4.6: (a) Tensile strength against number of weeks, (b) Young's Modulus against number of weeks

4.2.9 Tensile Strength Dependence on the Number of Days of Cell Culturing

The mechanical properties of scaffold seeded with the cells for days 0 and 7 are illustrated below (Fig. 4.a and 4.7b). This represents the tensile strength against number of days and the Young's modulus versus number of days of cell culturing.

(c) (d)

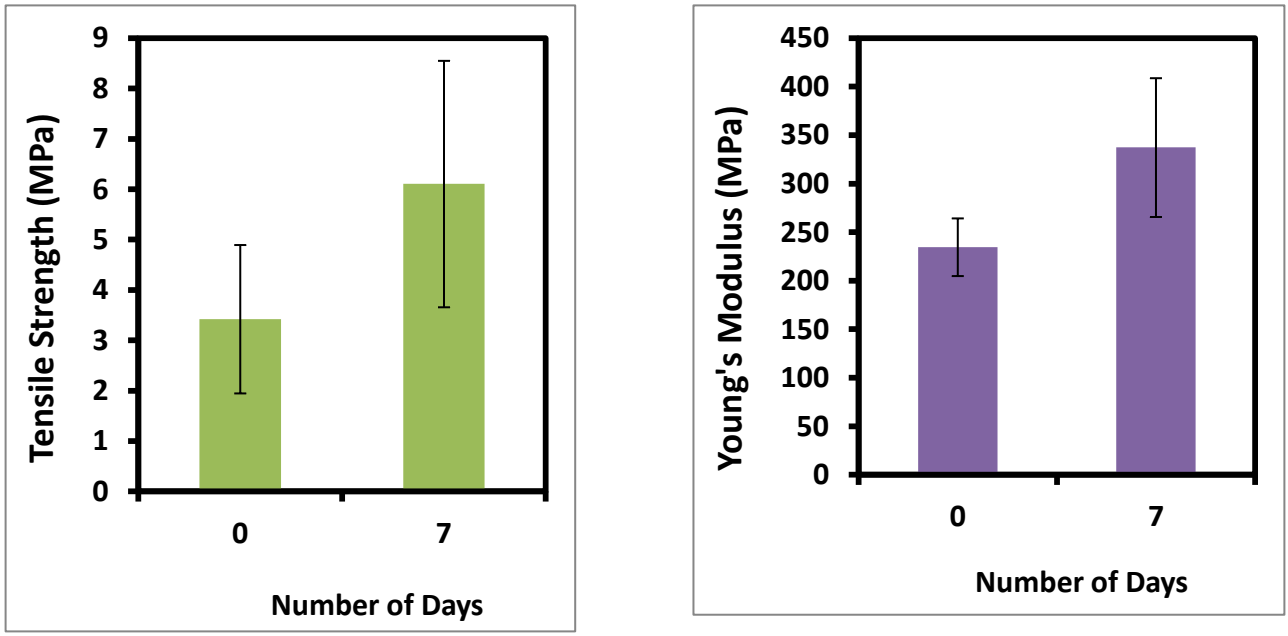


Figure 4.7: (c) Tensile strength against number of Days, (d) Young's Modulus against number of Days

In regenerative medicine, tissue engineering must have the mechanical strength needed for the fabrication of macro porous scaffolds that will retain its orientation and structure during and after implantation, particularly in the human tissue. In order to achieve a functionally successful and satisfactory implant, the mechanical properties must be considered for practical application in the course of its design. All soft tissues of the breast can be assumed to be nearly incompressible. Porous scaffold are therefore suitable for tissue regeneration and organs repairs. In this study, the tensile strength and modulus of the 3D PLA scaffolds conforms the basics requirements of mechanical properties in tissue engineering.

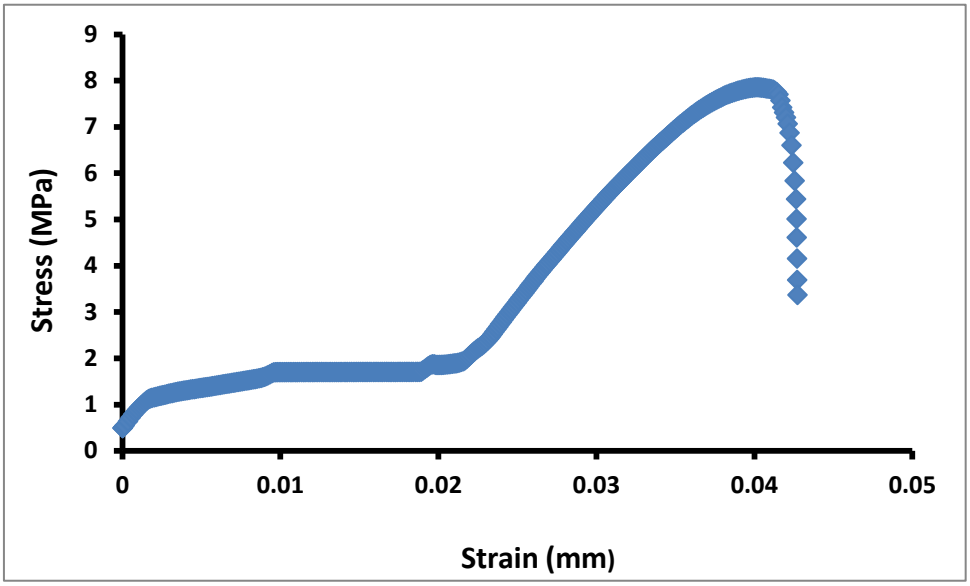


Figure 4.8: Stress-Strain curves obtained for PLA Scaffolds seeded with the mammary breast cells

CHAPTER FIVE

5.0 Conclusion

A 3D printed PLA scaffold has been developed in this study. The scaffold has been shown to degrade during initial exposure to simulated body fluid. The initial stages of cell spreading and proliferation have also been studied using cell culture techniques. The studies reveal relatively slow cell spreading during the first 7 days of cell spreading. This suggests that surface coatings may be needed to improve the initial stages of cell spreading and integration to the PLA scaffolds.

The basic mechanical properties of the PLA scaffold increase with increasing duration of exposure. Furthermore, the tensile strength and Young's Modulus increase progressively as the number of days increases. However, after cell culture for 7 days, there is no significant difference between the mechanical properties of the scaffolds. Also, the increase in % Alamar Blue Reduction and Protein Concentration confirms the growth inhibition of the cells with large fluorescence enhancement during the cell culture experiments.

5.1 Future Work

- There is need to explore the effects of longer durations of exposure on PLA scaffold degradation and cell spreading.
- There is also a need to explore the cell spreading and proliferation, as well as the formation of tissue around the PLA scaffolds under in-vitro and in-vivo conditions.
- Explore possible tissue regeneration strategies for breast tissue repair.

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