THE EFFECT OF 3D PRINTING PARAMETERS ON THE PROPERTIES OF THERMOPLASTIC POLYURETHANE SCAFFOLD

CHAI PEI YING

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By

CHAI PEI YING

Supervisor: Dr Syazana Ahmad Zubir

Co-Supervisor: Dr Shah Rizal Kasim

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DECLARATION

I hereby declared that I have conducted, completed the research work and written the dissertation entitled: "**The Effects of 3D Printing Parameters on the Properties of Thermoplastic Polyurethane Scaffold**". I also declared that it has not been previously submitted for the award for any degree or diploma or other similar title of this for any other examining body or University.

Name of Student: Chai Pei Ying

Signature:

Date: 19th August 2022

Witness by

Supervisor: Dr Syazana bt. Ahmad Zubir

Signature:

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LIST OF ABBREVATIONS

3D	Three-Dimensional
AM	Additive Manufacturing
CAD	Computer-aided Design
BTE	Bone Tissue Engineering
FDA	Food and Drug Administration
FDM	Fused Deposition Modelling
HS	Hard Segment
PCL	Polycaprolactone
PGA	Polyglycolic-acid
PLA	Polylactic-acid
PU	Polyurethane
PVC	Polyvinyl Chloride
SS	Soft Segment
TE	Tissue Engineering
TPU	Thermoplastic Polyurethane
SLA	Stereolithography
SLS	Selective Laser Sintering

LIST OF SYMBOLS

°C	Degree Celsius
ΔH	Enthalpy
mm	Millimetre
μm	Micrometre
g	Gram

KESAN PARAMETER CETAKAN 3D TERHADAP SIFAT-SIFAT PERANCAH POLIURETANA TERMOPLASTIK

ABSTRAK

Dalam bidang kejuruteraan tisu, perancah adalah perlu untuk menyediakan persekitaran yang menggalakkan untuk penjanaan semula tisu. Perancah harus meniru ciri mekanikal tisu sedekat mungkin. Salah satu proses pembuatan aditif paling popular yang boleh digunakan untuk mencipta perancah ialah pemodelan pemendapan lakur (FDM). Parameter pencetakan mempunyai kesan yang ketara ke atas morfologi dan sifat mekanikal perancah yang dicetak. Objektif pertama projek ini ialah pencirian filamen TPU-72D dari segi kekuatan tegangan, sifat terma, kadar aliran cair dan kekerasan. Objektif kedua ialah untuk menyiasat kesan parameter pencetakan 3D pada morfologi dan sifat mampatan perancah TPU. Parameter pencetakan 3D penting suhu katil, suhu muncung, kelajuan pencetakan dan ketumpatan menyulam telah dipilih. Perancah telah dibina menggunakan 72 *shore* D poliuretana termoplastik (TPU). DSC menunjukkan kehadiran SS dan HS dalam struktur tetapi kekerasan berkurangan kepada 50 *shore* D menurunkan kadar aliran. Peningkatan dalam T_B, T_N dan I_D boleh meningkatkan kekuatan perancah, manakala peningkatan dalam S_p mengurangkan kekuatan perancah. Tetapan terbaik parameter input untuk modulus mampatan dan kekuatan mampatan ialah T_B 40°C, T_N 235°C, S_P 35mm/s, dan I_D 100%. Gabungan parameter pencetakan 30-225-50-30 memberikan dimensi yang lebih hampir kepada reka bentuk asal daripada parameter pencetakan 40-235-35-100. 4T ialah bahagian kemasan permukaan terbaik di antara perancah TE ini.

THE EFFECT OF 3D PRINTING PARAMETERS ON THE PROPERTIES OF THERMOPLASTIC POLYURETHANE SCAFFOLD

ABSTRACT

In the field of tissue engineering, the scaffold is necessary to provide a favorable environment for tissue regeneration. The scaffold should replicate the mechanical characteristics of the tissue as closely as possible. One of the most popular additive manufacturing processes that can be utilized to create scaffolds is fused deposition modelling (FDM). The printing parameters have a significant impact on the morphology and mechanical properties of the printed scaffold. The first objective of this project is the characterization of TPU-72D filament in terms of tensile strength, thermal properties, melt flow rate and hardness. The second objective is to investigate the effect of 3D printing parameters on the morphology and the compression properties of TPU scaffold. The important 3D printing parameters of bed temperature, nozzle temperature, printing speed, and infill density were chosen. The scaffold has been constructed by 72 shore D thermoplastic polyurethane (TPU). Differential scanning calorimetry shows the presence of soft segment (SS) and hard segment (HS) in the structure but the hardness decreases to 50 shore D lower the flow rate. Increase in the T_B, T_N and I_D could increase the strength of the scaffold, while increase in S_p decrease the strength of the scaffold. The best setting of input parameters for compressive modulus and compressive strength are T_B 40°C, T_N 235°C, S_P 35mm/s, and I_D 100%. Combination of 30-225-50-30 printing parameter gives a closer dimension to the original design than 40-235-35-100 printing parameter. 4T is the best surface finish part among these tissue engineering (TE) scaffolds.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Tissue engineering (TE), an interdisciplinary discipline uses engineering and life science ideas to create biological replacements that restore, maintain, or enhance tissue function (Walles et al., 2011). TE also aims to replace or regenerate the damaged tissues, cells, or organs. The potential effect of TE technologies would be greatly increased by the creation of treatments for people suffering from severe chronic diseases that impair vital organs like the heart, kidney, and liver but who are not yet on transplant waiting lists (Furth and Atala, 2013). In fact, a significant clinical challenge is still regenerating functional bone tissue in critical-size defects(Entezari *et al.*, 2019a). This is due to half of all chronic diseases in adults over 50 years old are related to the bones and their associated disorders (Qu et al., 2019). To overcome such challenge, a proper biomaterial scaffold in created to serve as the template for cell interaction and new tissue ingrowth in order to promote the bone regeneration.

Scaffolds are synthetic or natural porous materials structures on which new tissue can be generated to replace injured tissue. Biopolymer scaffolds for tissue engineering serve as a scaffolding to support three-dimensional (3D) tissue synthesis. Scaffolds give neo-tissue development mechanical support and structure in vitro and during the initial stage of the implantation while cells grow, differentiate, and organize (Furth et al., 2013). They enable cellular organization into tissue replacement and have the ability to control the formation of connective tissue to minimize scarring (Powell et al., 2009). Ideally, the scaffold will aid in stimulate the natural regenerative mechanisms of the human body so that the self-healing ability of the injury body part can be improved.

To design an ideally good performance bone TE scaffold, there are several critical should be considered. Firstly, the scaffold should be able to deliver cells. Additionally, the scaffold must be osteoconductivity, and it is ideal if the material promotes osteoconduction with the host bone. Although osteoconductivity does not completely prevent the development of fibrous tissue encapsulation, it does result in a solid link between the scaffold and the host bone (Chen *et al.*, 2008). The scaffold should also be sterilisable without losing bioactivity, regulated deliverability, acceptable mechanical qualities in terms of Young's modulus, adequate architectural design in terms of porosity and pore size, and biocompatibility without loss of bioactivity(Ghassemi *et al.*, 2018).

It is important for the scaffold's mechanical qualities to be compatible with the natural human tissue. For cell penetration, tissue in development and vascularization, and nutrient supply, the scaffold must be porous and have interconnected porous structures with porosity more than 90% and diameters between 100 and 400 μm. (Zhang *et al.*, 2022). In addition, the pores must be both large enough to permit cell migration inside the structure and tiny enough to establish a sufficiently high specific surface, which results in a minimum ligand density, to enable effective binding of a crucial number of cells to the scaffold.

Different materials have been used to fabricate the scaffolds for the purpose of bone tissue engineering applications. In general, the materials of scaffolds for bone tissue engineering can be categorised as polymeric, ceramic, metallic, and composite scaffolds. Biopolymer scaffold will be the mostly used TE scaffolds for bone regeneration due to its controllability over physiochemical features, which include pore size, porosity, solubility, biocompatibility, enzymatic reactions, and allergic response (Fuchs et al., 2001). In addition, for surgeons and patients who previously may not have been ideal candidates for dental implants, polymeric scaffolds for bone tissue engineering give up new options (Chen et al.,

2016). As compared with the natural polymers, synthetic polymers are the best choice in generating the bone TE scaffolds due to the excellent mechanical properties. The generally used synthetic polymers include polylactic-acid (PLA), polyglycolic-acid (PGA), polycaprolactone (PCL), polyurethane (PU) and thermoplastic polyurethane (TPU) (Ghassemi *et al.*, 2018).

There are several methods for fabrication of 3D bone tissue engineering scaffolds, including electrospinning, phase-separation, freeze-drying, self-assembly, and additive manufacturing (AM). Additive manufacturing, is the industrial production name of 3D printing, an approach to object construction that involves adding layers one at a time. The advancement of 3D printing technology has given a chance for developments in regenerative medicine. This field of study intends to employ stem cells and engineered biomaterials technology (Diaz-Gomez et al., 2020). The most widely used method for creating biodegradable scaffolds is fused deposition modelling (FDM) of thermoplastics. The concept of FDM is the FDM printer ejects a thermoplastic filament that has been heated to its melting point layer by layer, and produce a 3D object. The fundamental challenge of the FDM technology is the requirement for prepared fibers to be fed through the rollers and nozzle with uniform size and material qualities (de Mulder et al., 2009).

FDM technique offers certain advantages, including the fact that there is no unbound loose powder and no solvent cleanup necessary. It means that by using FDM printer, it is unable to remove the excessive polymer powder due to no organic solvent is used. It also gives the material more processing and handling flexibility (Walker et al., 2017; Altuntaş et al., 2017). Other advantage of 3D printing a bone TE scaffold is that, in the situations of severe bone abnormalities where alternative therapies are not appropriate, 3D printing a bone scaffold enables surgeons to tailor bone transplants to the patient (Chen et al., 2016).

The 3D printing parameters will affect the performance of 3D bone TE scaffolds. In the research by Baptista et al., the 3D printing parameters tested are temperatures, extrusion speeds, filament offset distances and layer thicknesses to investigate the effect analysed regarding scaffold morphology and mechanical properties. Vary the filament offset distances provide different scaffold porosities. (Baptista *et al.*, 2020) Zhang et al. carry out the research about the impact of 3D printing temperature and speed on the microcellular cell shape of TPU scaffold. (Zhang *et al.*, 2022). Several researchers had varied the 3D parameters in terms of temperatures, speeds, and porosities. In this project, temperatures of nozzle and bed platform, infill density, and printing speeds (filament extrusion speeds) are varied to analyzed the effect on the compression properties and morphology of scaffold.

1.2 Problem Statement

The population of the globe is living longer, which has increased the need for healthcare in terms of accessibility and quality. Researchers' attention has been focused on the creation of new biomaterials, new manufacturing processes, and new technologies for the production of medical devices. The appropriate material must be chosen carefully when developing medical devices since the device's success depends on the material's ability to perform the desired purpose.

The increase prevalence of bone disorders among the citizens aged 45 years and above become a significant issue which the world concerned (Ghassemi *et al.*, 2018). It is predicted that the incidence of bone disorders could double globally by 2022, particularly in the population where ageing is associated with rising obesity and insufficient physical activity. Therefore, there is still a critical clinical need for methods to repair and replace broken bones in severe fractures in the field of orthopaedic surgery. Tissue engineering is one of the most

promising methods for creating artificial replacements for broken bones (Pina *et al.*, 2015). Biodegradable synthetic extracellular matrix (ECM) has been widely used for the tissue engineering field which numeral researchers concerned with. Bone TE scaffold is example of ECM, and has been developed for the future of bone defect treatment. Scaffolds are extremely porous, interconnected structures. They should preferably be osteoconductive, biodegradable, mechanically durable, and biocompatible. (Polo-Corrales et al., 2014; Chen and Liu, 2016; Zhang et al., 2022) PLA, PCL, PU, TPU and PGA are the examples of polymers that have been developed for orthopeadic applications.

TPU is selected as the biomaterial to be used for bone tissue engineering scaffold due to its capability of excellent performance characteristics. TPU is extremely popular for medical applications due of its superior mechanical characteristics, durability, and resistance to oils and chemicals. TPU provides the medical sector with a flexible, ecologically acceptable alternative to polyvinyl chloride (PVC) as it does not include plasticizers. Many researches have investigated the biocompatible and biodegradability of TPU for almost 30 years to ensure that it can be used as scaffolds for TE applications. Numerous interdisciplinary research have been conducted in this TE field, ranging from design and modelling to material processing and post-treatments to in vitro and in vivo biological assessments. (Polo-Corrales, Latorre-Esteves and Ramirez-Vick, 2014; Chen and Liu, 2016; Ghassemi *et al.*, 2018; Baptista *et al.*, 2020)

Various techniques have been carried out for fabrication of 3D bone tissue engineering scaffolds, including salt leaching (Sadiasa *et al.*, 2013), gas foaming (Gentile *et al.*, 2014), freeze casting (Sadeghpour *et al.*, 2014), and electrospinning (Rajzer *et al.*, 2014). However, the majority of these techniques have not been able to totally regulate the scaffolds' structural characteristics or their repeatability. These traditional systems can require significant lead-times, which means that the product can be fabricated in several days or week, depending on

the part complexity and difficulty in ordering materials. Traditional method such as salt leaching, the porosity of the TE scaffold is highly dependents on the porogen size, granule size/density, or consolidation temperature, which these parameters are difficult to be controlled precisely, make the fabrication of scaffold's porosity is out of control.

As a result, AM techniques have received a lot of attention recently. These are sophisticated production processes that allow 3D products to be built layer by layer in an additive fashion directly from data provided by computer-aided design (CAD), computed tomography, and magnetic resonance imaging. Furthermore, fast prototyping approaches have demonstrated the capability of producing pre-defined, customizable, and repeatable scaffolds with customised architecture and porosity (Wu et al., 2014; Fakhruddin et al., 2019; Zhang et al., 2022). Rapid prototyping (RP) allows a part to be made in hours or days, given that a computer model of the part has been generated on a CAD system. Although the CAD model may not be sufficient for the designer to visualise the part adequately, RP can aid for the designer to see and feel the part and assess its merits and shortcoming. CAD model easy to be created and can provide more precise design which require by the designer as compare with the traditional method.

One of the key determinants of a high-quality print may be the filament that was used. Because it typically appears fine from the outside, filament is one of those characteristics that is frequently disregarded. However, items like air pockets and particles could be lurking below the surface, ready to damage the printing process. Too much or too little filament being delivered would pose massive problems. To ensure that it can be used to create parts without any issues, the filament needs to be completely compatible with the 3D printer. When commercial filaments are ordered, it will save money, time, and other resources. To fabricate

a good performance 3D printed tissue engineering scaffold, the quality of the filament needs to be considered to ensure the appearance and the strength of the scaffold will not be affected.

1.3 Research objectives

The main objective of this research is to find out the optimal 3d parameters of printing the TPU tissue-engineering scaffold. To achieve the goal, two objectives are carried out:

1. To characterize the TPU-72D filament in terms of tensile strength, thermal properties, melt flow rate and hardness.
2. To investigate the effect of 3D printing parameters (bed temperature, nozzle temperature, printing speed, and infill density) on the morphology and the compression properties of TPU scaffold.

1.4 Scope of research

The samples of scaffold structure are fabricated via 3D printing technique with different combination of parameters. These parameters include nozzle temperature, bed temperature, printing speed and infill density which will significantly affect the performance of the scaffold structure. Then, these printed samples will be tested under different characterisation technique.

This thesis is divided into five chapters. Chapter 1 provides a brief description of the study, including a problem statement and project objectives. In Chapter 2, a comprehensive review on the tissue-engineering scaffold, 3D printing techniques, TPU and the 3D printing parameters are present. The influence of nozzle temperature, bed temperature, printing speed and infill density on the formation of TPU scaffold are discussed in Chapter 2. Chapter 3 describes the methodologies used in this project, including sample preparation with filament

and sample characterization techniques. Chapter 4 presents the experimental results of the effect of 3D printing parameters on the morphology, thermal and mechanical properties of scaffold. Finally, Chapter 5 summarizes the findings of the research and makes recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 Bone

Bone is a dense, hard intercellular matrix that surrounds cells to form a solid bodily structure. Collagen and calcium phosphate, the two main components of this substance, set bone apart from other hard tissues like chitin, enamel, and shell. 99% of the inorganic components of bone's structure are made of hydroxyapatite, while 22% are made mostly of collagen in the organic portion with 90%. The individual bones that make up the skeletons of other animals and the human skeletal system are composed of bone tissue (Datta et al., 2008; Heaney et al., 2022).

The human skeleton is one of the most essential systems in the body. The most crucial of its various functions is to maintain the body's weight structurally and mechanically while safeguarding the nearby delicate tissues. The optimum structural configuration of bone tissue enables it to meet the mechanical requirements of its environment (Guo et al., 2020). The inorganic components are in charge of stiffness and compression strength, whereas the organic components are primarily in charge of the tension qualities. However, conditions like osteoporosis and cancer, as well as sex, age, and species, have an impact on bone composition.

The structure of bone is hierarchical, porous, interconnected, non-homogeneous, and anisotropic. As seen in Figure 2.1, there are two different forms of bone in terms of porosity. Cortical or compact bone, the first kind, is the dense portion of the outer layer of bone with 5-10% porosity (Kim, 2005). The other is cancellous or trabecular, with 50–95 percent porosity,

and is found near the ends of long bones, flat bones, and cuboidal bones. The densely linked holes are filled with marrow that has many cell types and blood arteries (Spears *et al.*, 2000).

In addition to supporting the body's architecture and safeguarding key organs, bone can regenerate and mend itself (fracture healing). Bone is a dynamic tissue that goes through a continual cycle of new tissue synthesis and resorption of older tissues. In actuality, there are four different categories of bone cells based on how they operate in the processes of development, modelling, remodelling, and fracture healing (Moore et al., 2015).

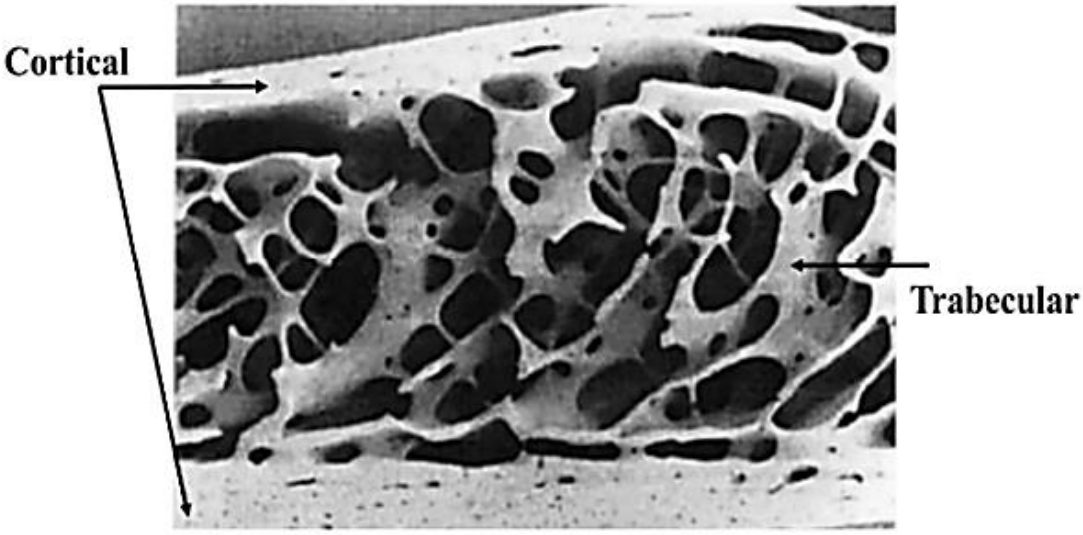


Figure 2.1.1 Bone section showing cortical and trabecular bone (Yuan et al., 2000).

2.2 Background of Tissue Engineering

For the purpose of replacing or repairing tissue that has been harmed by illness or trauma, hundreds of surgical procedures are carried out. The four essential components of tissue engineering (TE) are live cells, growth factor regulation, culturing, and scaffold. By merging body cells with extremely porous scaffold biomaterials, which aid in the creation of new tissue,

the scaffold has been produced to restore the injured tissue. With the trio of signals for tissue reacting to stem cells, scaffold ECM serves as the foundation for tissue engineering. Figure 2.2.1 shows the schematic diagram of tissue engineering process (Babak et al., 2020).

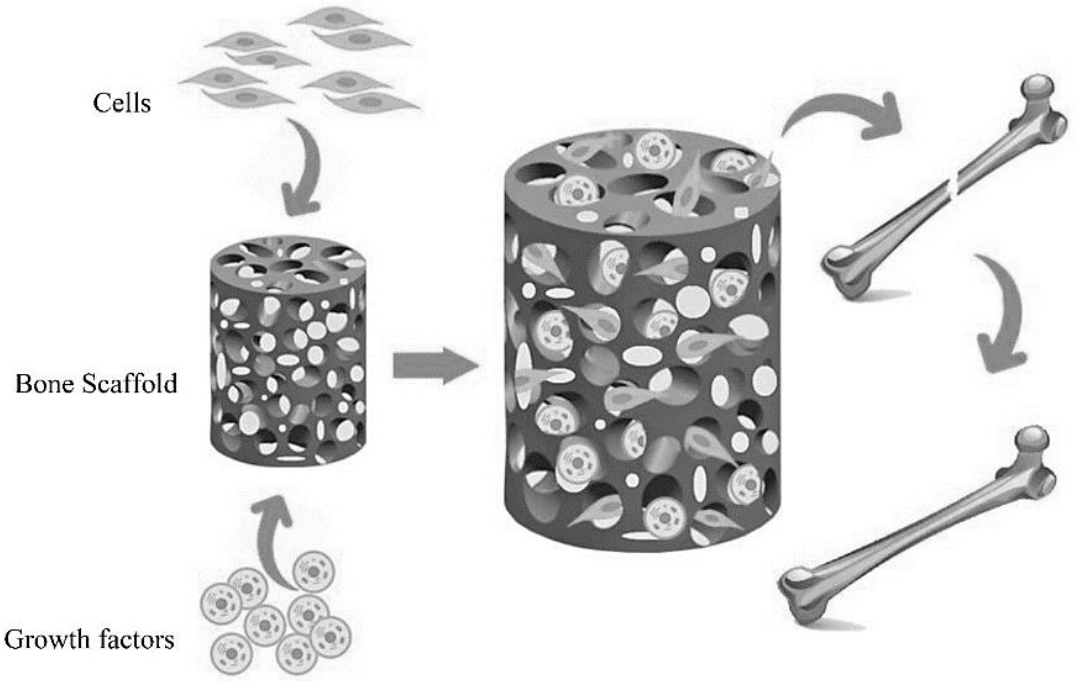


Figure 2.2.1 Schematic bone tissue engineering process (Babak *et al.*, 2020).

TE is a method that combines scaffolds, cells, and physiologically active substances to produce functional tissues. It has its roots in the field of biomaterials development. Building structures that can replace, maintain, or improve damaged tissues or whole organs is the goal of tissue engineering. Artificial skin and cartilage are two examples of synthetic tissues that have received Food and Drug Administration (FDA) approval; nevertheless, their use in human patients is still rather limited (Eltom et al., 2019). Even though some therapies have FDA approval or clearance and are currently available on the market, many problems still need to be resolved in order to effectively meet the diverse range of patient needs (Babak et al., 2020).

Table 2.2.1 lists some of the FDA-approved tissue engineering products available in the clinical market.

Table 2.2.1 Several Tissue Engineering Therapies with FDA Approval (Babak *et al.*, 2020).

Human Organ	*Product*	*Company*	*Application*
Skin	Apligraf	Organogenesis	• For treatment of non-infected partial • Full-thickness skin ulcers
	Composite Cultured Skin	Ortec International	• Covering wounds and donor sites • After surgery
	Dermagraft	Smith & Nephew	• Treatment of wounds
	laViv	Fibrocell Science	• Improving nasolabial fold appearance
Bone	Carticel	Genzyme	• Repair of femoral condyle

OP-1 Implant	Stryker Biotech	• Alternative to autograft in recalcitrant long bone nonunions
InFUSE Bone Graft	Medtronic	• Spinal fusion for degenerative disc disease
GEM 21S	Biomimetic Pharmaceuticals	• Treatment for periodontally related defects

Currently, artificial scaffolds are used as a supportive platform for cell cultures for cell growth supremacy in the repair of injured tissues or organs. The scaffold assists in cell regeneration for a short period of time before gradually degrading either during or after the healing process, leading to the development of a new tissue with the necessary characteristics (Aldana et al., 2017). It is predicted that scaffolds for TE applications would have particular qualities that promote bone repair. In fact, many professionals agree that the development of BTE appears to be related to advancements in scaffold technology (Burg et al., 2000; Guo et al., 2015).

Additionally, scaffold engineering places great demands on design and materials from a technical standpoint. Porosity, permeability, and mechanical strength are linked key factors

that characterize a scaffold's performance in addition to chemistry (Bose et al., 2012; Polo-Corrales et al., 2014). Through standard manufacturing procedures, these parameters are not perfectly controllable (Bose et al., 2012). There have therefore been many interdisciplinary investigations in this area, including design and modelling, material processing and post-treatments, as well as in vitro and in vivo biological assessments.(Sadiasa et al., 2014; Babak et al., 2020) In addition, a few crucial factors must be taken into account while developing or deciding if a scaffold is appropriate for BTE.

2.2.1 Scaffold

Scaffolds can serve as cellular systems or as delivery systems for cells and medications in the regeneration of cells and tissues; as a result, the cellular material must be able to colonise the host cell sufficiently to meet the needs of regeneration and repair. Another possibility is to combine the scaffolds with various cell types that can promote tissue synthesis (Roseti *et al.*, 2017). Whether the tissues that need repair are soft, like neural tissues, or hard, like bones, determines the properties of the constructed scaffold. For example, in the engineering of hard tissues, biological scaffolds are used to fill bone defects and should be able to withstand loads in addition to directing the development of new bone. In addition to resorption kinetics, porosity, surface morphology, surface chemistry, degradation rate, and mechanical stability, the scaffold pore's size, shape, wall thickness, interconnectivity, and wall surface also have an impact on bone healing (Yee Foong et al., 2017). Many materials have been created as scaffolds for tissue engineering purposes.

2.2.2 Scaffold Requirement

Tissue Engineering scaffolds aid in delivering cells or growth factors to the location of injury, which also serve as an ideal template for the creation of new tissue throughout the structure.

(Dawson *et al.*, 2011) TE is goaled that the new bone tissue is intended to completely replace the scaffold that was implanted. As Dawson et al. stated, the fundamental design criteria for TE scaffold should be included the integration of existing bone and equivalent mechanical strength, hence it should have a 3D structure that persists until the growth of new bone. (Dawson *et al.*, 2011)

Several scaffolds manufactured from a variety of biomaterials and created using a variety of fabrication procedures have been used in the field to try to regenerate various tissues and organs in the body. An ideal bone TE scaffold should consist of the requirements in terms of biological, structural, and mechanical properties. These include biodegradability, biocompatibility, scaffold architecture, encourage cellular interactions and tissue development, as well as have appropriate mechanical and physical properties (O'Brien, 2011).

The biocompatibility of the tissue engineering scaffold is its primary and fundamental

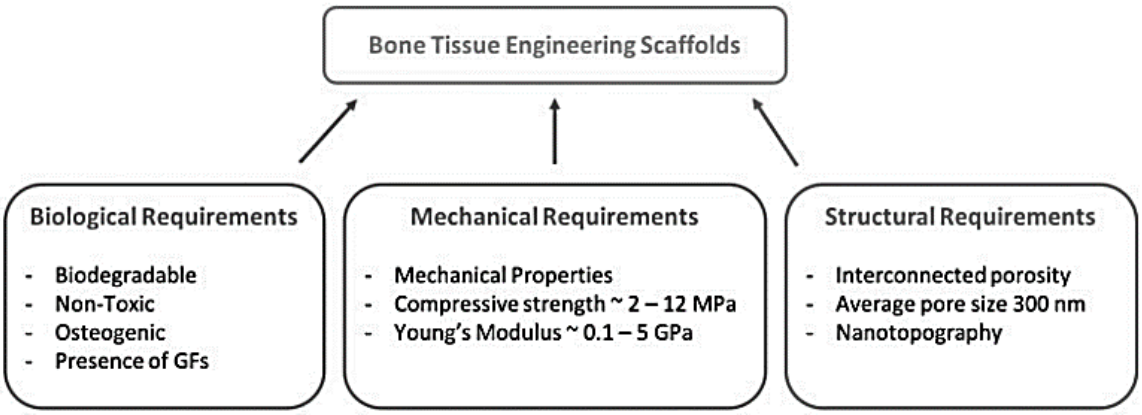


Figure 2.2.2 Biological, mechanical, and structural requirements for an ideal BTE scaffold (de Witte *et al.*, 2018).

requirement. The ability of a scaffold to support normal cellular activity, including molecular signalling systems, without causing any immediate or long-term toxic effects on the host tissue is known as biocompatibility (Maquet et al., 2015). The scaffold must integrate into the host

tissue without causing any toxic effects or triggering an immune response. The TE scaffold was designed with the intention of remaining inert during the initial implantation, remaining unaffected by reactions with the surrounding tissues. To actively support nutrient, oxygen, and waste transport, an ideal scaffold must form blood vessels in or around the implant within a few weeks of implantation (Maquet et al., 2015; O'Brien, 2011; Bose et al., 2012).

Biodegradation, also known as bioresorption, is a crucial requirement of the ideal tissue engineering scaffolds. It means the chemical dissolution or decomposition of biomaterials under physiological environments which the TE scaffolds must be absorbed by the surrounding tissues without the need for surgical removal (Wu et al., 2014). Hence, it is also important to take the controlled resorption rate into account while building TE scaffolds. Due to the inadequate bone integration, early scaffold resorption will also affect bone healing by impairing early bone formation and removing osteoconductive surfaces for future bone apposition (Dawson *et al.*, 2011). Constructions and scaffolds are not meant to be long-term implants. As a result, for cells to produce their own extracellular matrix, the scaffold needs to be biodegradable. The by-products of this degradation ought to be non-toxic and able to leave the body without harming other organs (Brown *et al.*, 2009).

An ideal bone TE scaffold with its mechanical characteristics should be compatible with those of the host bone, and proper load transfer is also crucial. Reconstruction of hard and load-bearing tissues, such as bone tissue, should be taken into account. From cancellous to cortical bone, mechanical properties of bone vary greatly. Cacellous bone with an elastic modulus ranging from 0.1 to 5 GPa and a compressive strength of 2 to 12 MPa. Contrarily, cortical bone has an elastic modulus of 15 to 20 GPa and a compressive strength of 100 to 200 MPa (Maquet et al., 2015; Bose et al., 2012; de Witte et al., 2018).

Internal and external scaffold architectures, such as pore size, shape, and distribution, can influence both in vivo and mechanical performance. Other than that, the scaffold porosity and pore interconnection also should be considered due to their impact on the surface area for cell growth. When it comes to morphologic design, biomechanical modulation that takes into account both structural and bio-fluidic properties is critical (Dias et al., 2014; Dawson et al., 2011). The pore size needs to fall within the acceptable range of sizes. Large pore sizes decrease the available surface area, limiting the ability of cells to attach. On the other hand, the tiny pores slow down cell migration and scaffold permeability (Rajagopalan et al., 2006). Highly porous biomaterial is preferred for the simple diffusion of nutrients and waste products to and from the implant as well as for vascularization, which is a crucial requirement for the regeneration of the highly metabolic organs like the pancreas and liver (Maquet et al., 2015).

The macro-pore size range of 100-1000 µm is typically reported for cell attachment and vascularization through the pores (Voronov *et al.*, 2010), but some other studies have reported different pore size ranges. Cell penetration has been found to require a minimum pore size of 80 µm. (Rose *et al.*, 2004). Besides, the study of Bose et al. demonstrated that scaffolds with a mean pore size of 300 µm and a minimum pore size of 150 µm are the best for forming bone tissues (Bose et al., 2013). Additionally, values greater than 85% in terms of porosity were discovered to improve cell penetration up to 400 µm (Ji *et al.*, 2011), whereas porosities greater than 75% were suggested to ensure cell proliferation (Gomes, et al., 2006). Furthermore, Danilevicius et al. found that scaffolds with 86% porosity were more effective than those with 82% and 90% porosity (Danilevicius *et al.*, 2015). Unfortunately, porosity decreases mechanical qualities like compressive strength and makes it more difficult to manufacture reproducible scaffolds (Bose et al., 2012).

From the medical side of view, the criteria need to be fulfil included (Dawson et al., 2011):

a) radiographically identifiable from freshly created tissue,
b) allow for minimally invasive implantation,
c) allow for cost-effective manufacturing,
d) allow for sterilising,
e) promote easy handling without lengthy preparation processes, and
f) fulfil FDA clearance.

Other than that, to design a BTE scaffold, material selection also be the significant part of the requirement. The choice of material highly important in designing the scaffold as the different materials consist of different mechanical properties and performance.

2.2.3 Materials for Scaffold

The bone tissue-engineering scaffold can be fabricated with several materials, generally in natural form, metallic, ceramic, biocompatible polymeric (natural or synthetic) materials or their combinations. The ideal materials for creating bone scaffolds are those that closely resemble the setting and functionality of the human skeleton. Candidate biomaterials for bone scaffold applications should have one or more of the following features in order to accomplish this, which include the support of mechanical and biological processes by promoting cell adhesion and migration, improving vascularization, and facilitating the diffusion of vital cellular nutrients and secretions (Babak et al., 2020). Some of the ideal materials which have been shown in table 2.2. For tissue engineering applications, metals and ceramics have two significant drawbacks: they are not biodegradable and have very limited processability (Maquet et al., 2015).

Table 2.2.2 Some of biocompatible materials for bone scaffold fabrication (Babak *et al.*, 2020).

Scaffold material	Examples	Advantages	Disadvantages
Metals	NiTi	High young's modulus	Not degradable
	Porous tantalum	High compressive strength	Ion release
Ceramics	TiO_2	Osteogenic, biocompatible	Brittle
	Hydroxyapatite	Can be biodegradable	Prone to fracture and fatigue
Natural Polymers	Collagen	Biocompatible, Biodegradable	Low mechanical strength
	Chitosan	Osteogenic	
Synthetic Polymers	PLGA	Tunable properties	Acidic degradation byproducts
	PCL		Rapid strength degradation in vivo

Metals are the generally used materials applied in the medical devices, such as orthopedic implants, bone fixators, artificial joints and external fixators due to their high strength to act as the hard tissue to support the orthopedics (Hanawa, 2012). Around 70% to 80% of metals are used as the implant medical devices because of their properties of high strength, mechanical dependability, elasticity, excellent wear and corrosion resistance, toughness, and thermal and electrical conductivity (Festas et al., 2019). The most commonly biomaterial-metals include stainless steel, gold, cobalt-chromium alloy and nickel-titanium alloy due to their high strength, corrosion resistance and high durability. These metals do not show any metal ion toxicity due to the chemical reaction of ion dissolution by corrosion or the wear debris generation (Hanawa, 2012).

Cells eventually invade the scaffold pores to form new bone tissues. The fact that metals are generally synthetic materials with no biological activity is a drawback to employing them as biomaterials. Unfortunately, the prolonged presence of metals within the tissue causes health issues like alzheimer's, infertility, neurological, and cardiovascular symptoms (Babak et al., 2020). As a result, before they can be employed as biomaterials, metals need to possess additional qualities (Hanawa, 2012). Other than that, biomaterial-metals low in machinability because they maintain high hardness and strength at elevated temperature. This can decrease the tool life of the metal medical implants rapidly as the metals are wear rapidly (Festas et al., 2019).

Aside from metal, ceramic would be a good bone substitute in bone regeneration. Because of their similar bone composition and high biocompatibility, hydroxyapatite and tricalcium phosphate (TCP) are the most widely used ceramics due to the more adaptable biodegradability. Hydroxyapatite's porous structure allows bone cells to grow along its surface. As time passes, it will degrade, releasing minerals that promote the growth of new bone (Bose

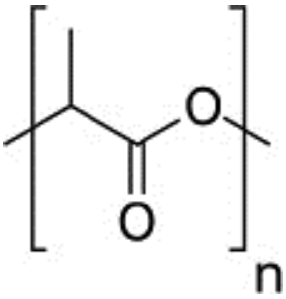
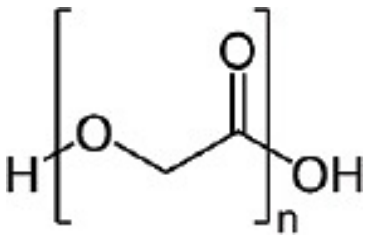
et al., 2012). The main benefit of using ceramic in bone scaffold applications is that it has low immunogenicity and provides less negative feedback to the body. Because of its high compressive strength, the ceramic can be processed into highly interconnected microporous structures that reassemble trabecular bone. This will improve blood vascularization, nutrient delivery, and bone in growth in the body(Kim *et al.*, 2008). Furthermore, Laponite (LAP) is a silicate-based nanoparticle that interacts with polymers to form nanocomposites. The resulting product has improved durability, mechanical strength, thermal stability, gas-barrier properties, surface characteristics, and biocompatibility. It may promote cell proliferation and metabolism activity under certain conditions. Nonetheless, ceramic exhibit poor performance in load-bearing condition and is easier to fracture when too much stress is applied. Ceramic's brittleness had limited its use in loaded bone applications(Fu *et al.*, 2011).

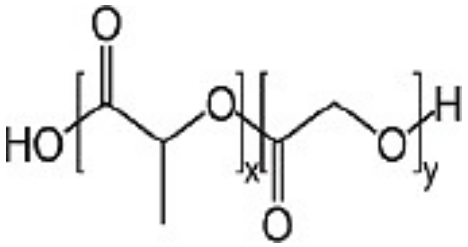
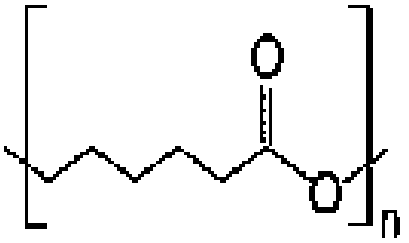
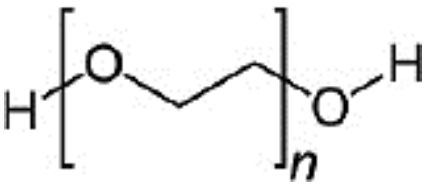
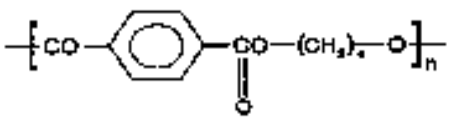
Usually, the polymeric materials are famous to be applied in the medical field due to polymers can be both bioactive and biodegradable. Natural polymers like collagen, fibrin, alginate, silk, hyaluronic acid, and chitosan are frequently employed for bone TE et al., 2007).

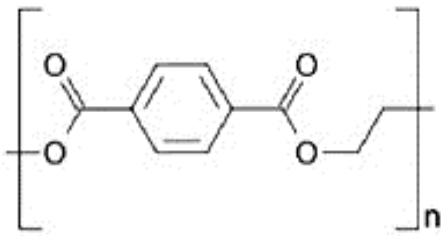
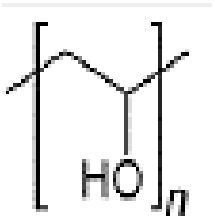
Synthetic polymers like poly(lactic acid), poly(glycolic acid), and polycaprolactone (PCL) break down into monomers that are easily eliminated by the body's physiological system. When it comes to compressive strength, some polymers, including poly(propylene fumarate, or PPF), are equivalent to cortical bone and their breakdown times can be adjusted across a wide range (Yan *et al.*, 2011). Natural polymers may aid in cell adhesion and bone formation. Proteins of natural extracellular matrices have been used in nerve repair, skin, cartilage and bone. However, its mechanical properties, biodegradability, and batch to batch consistency are all uncertain. It is less likely for humans to control those features, making it more expensive. Such a situation can be resolved by introducing synthetic polymers, which have guaranteed batch-to-batch consistency and better control over the parameters and characteristic. Synthetic

polymer has high processability where the microstructure can be well controlled. The ester bond in synthetic polymer degrades easily without the need of enzyme and the resulting by product are nontoxic. Hydroxyapatite (HAP) mimics natural bone mineral and has been shown to have good mechanical and osteoconductive properties.

Table 2.3 Example of synthesis polymeric scaffold for tissue engineering applications (Liu et al., 2004).

Name	Advantages	Toxicity	Chemical structure
Polylactic acid (PLA)	- biocompatible - biodegradable - support cell adhesion	- nontoxic - non-inflammatory - FDA approved	
Poly glycolic acid (PGA)	- biocompatible - biodegradable - support cell adhesion	- nontoxic - non-inflammatory - FDA approved	

Poly (lactic-co-glicolic acid) (PLGA)	- biodegradable - support cell adhesion	-exhibit immunogenicity and contains pathogenic impurities - FDA approved	
Poly ε-caprolactone (PCL)	- biodegradable	- deficiency of toxicity - FDA approved	
Polyethylene glycol (PEG)	- biocompatible - steering cells into scaffolds - osmotic effects in body	- nontoxic - FDA approved	
Polybutylene terephthalate (PBT)	- highly biocompatible -	- nontoxic - FDA approved	

	biodegradable - impact resistance		
Polyethylene terephthalate (PET)	- highly biocompatible - biodegradable - impact resistance	- nontoxic - FDA approved	
Polyvinyl alcohol (PVA)	- non-biodegradable - great resistance against organic solvents	- little toxic effect in oral consume	
Poly propylene fumarate (PPF)	- biocompatible - suitable physical properties and decomposition rate	- nontoxic - FDA approved