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DEVELOPMENT OF SCAFFOLDS FOR TISSUE ENGINEERING USING SELECTIVE LASER SINTERING

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ABSTRACT

Tissue Engineering (TE) is an important field in biomedical engineering that has raised interests among clinicians and engineers in the quest for alternative replacements for tissues, implants and organs. TE strategies for creating natural tissues or organs for anchoring dependent cell types rely on the application of temporary three-dimensional scaffolds to guide the proliferation. The characteristics required of TE scaffolds are major concerns in the pursuit to design and fabricate ideal scaffolds. Rapid Prototyping (RP) technologies are fast becoming the techniques of choice for the fabrication of TE scaffolds that can overcome the setbacks and limitations of conventional manual-based techniques.

In this project, the capability of fabricating reproducible porous structures directly without the use of organic solvents was studied. Biomaterials, such as Polyetheretherketone (PEEK), Polyvinyl alcohol (PVA) and Hydroxyapatite (HA) were experimentally used to build TE scaffolds using a commercial RP process, Selective Laser Sintering (SLS). The ability to control the parameters of the process to obtain desired TE features strengthens the usage of SLS to build such TE scaffolds. Pure PVA and different weight compositions of the polymer blend of PEEK/HA were laser sintered to assess the build potential of this polymer blend for bone scaffolds. Disc specimens built on SLS were investigated on the Scanning Electron Microscopy (SEM). The results obtained from the micrographs exhibited promising results as HA particles were observed to be embedded in the discs which were held together by the PEEK powders that had been successfully sintered. Characterisation analyses

including thermogravimetric analysis, porosity, microstructure, composition of the scaffolds, bioactivity and in vitro cell viability of the scaffolds were conducted. The results demonstrated a novel approach to fabricating scaffolds which can achieve controlled micro-architecture at high consistency. The research has established a viable process to fabricate reproducible bioactive TE scaffolds using SLS without the use of organic solvents.

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Chapter One:

Introduction

In This Chapter

- 1.1 Background
- 1.2 Objectives
- 1.3 Scope
- 1.4 Organisation of Report

Chapter One

Introduction

1.1 Background

The emergence of tissue engineering (TE) has garnered vast interest among clinicians as well as engineers seeking alternative replacements for tissues, implants and organs. TE involves the application of engineering principles towards the understanding of complex biological processes that occur in tissue development and repair [1]. Generally, TE can be classified into two main categories: *in vitro*, such as construction of biological replacements that facilitates the restoration of missing or damaged tissues from cells cultivated in a “test-tube” environment or *in vivo*, such as modification of the growth and function of host tissues [2-3].

Many challenges lie ahead in the first category as biological replicas have to be constructed from a basic cell line in a controlled environment for the implantation of the tissues or cells *in vitro*. In order for this to be successful, there is a need to source and create a temporary template for the purpose of cell cultivation. TE scaffolds used in regeneration of tissues or organs serve the purpose of providing cells with a suitable environment for cell seeding and proliferation. The populated TE scaffolds are eventually implanted into the human body for the recovery of the diseased organs. Research has been extensive in the field of tissue engineering in the quest for suitable tissue engineered organs or tissues such as skin [4], liver [5-7], cartilage [8-9] and bones [10-12].

As TE scaffolds are often used as base materials for cells to grow in the replacement of tissues or organs, the structure of tissue scaffolds should enhance cell growth and proliferation. In addition, the scaffold should provide a suitable substrate for cell adhesion and in certain circumstances, cell migration. Depending on the site where scaffolds are required, various unique three-dimensional shapes with interconnected pore network are needed for cell proliferation. This explains the necessity to fabricate easily reproducible scaffolds of unique geometry that are structured to hold cells close together so that cells are able to organise themselves. Conventional techniques such as fiber bonding, gas foaming, emulsion freeze drying, melt moulding, membrane lamination and solvent casting have been used in fabricating TE scaffolds [13-17]. Despite being able to fabricate scaffolds, these conventional techniques lack the consistency to produce scaffolds of uniform pore size and repeatability. In addition, organic solvents are often used in these techniques and are not desirable as residues from the processing techniques often induce toxic effects *in vitro* and elicit inflammatory responses *in vivo* [14].

The application of Rapid Prototyping (RP) techniques [18] seem positive as they are able to fabricate intricate reproducible structures without the use of organic solvent. In addition, the ability to control the parameters of the fabrication process further strengthens the usage of RP for TE scaffolds.

1.2 Objectives

The objectives of the project are as follows:

- i. To analyse the usage of Selective Laser Sintering (SLS) as a systematic approach in the fabrication of 3D porous structure for TE.
- ii. To identify and incorporate the processing of biomaterials in SLS for the fabrication of TE scaffolds for both hard and soft tissues.
- iii. To consider various criteria such as porosity and biocompatibility required for the fabrication of 3D TE scaffolds.
- iv. To verify the suitability of the chosen biomaterials as TE scaffolds.

1.3 Scope

The scope of the project is defined as follows:

- i. Assess the capability of **SLS** in building TE scaffolds on various aspects such as dimensional accuracy and physical properties.
- ii. Evaluate and optimise the microstructure of the scaffolds built using such biomaterials.
- iii. Investigate the suitability of the chosen biomaterials for TE scaffolds through various characterisation analysis.
- iv. Determine the effect of variation of parameter settings such as scan speed and laser power on the model built by SLS.
- v. Carry out *in vitro* cell culture studies on the TE scaffolds fabricated to study its bioactive properties such as biocompatibility and tissue regeneration.

1.4 Organisation of Report

Chapter One of the report presents the background and the motivation for this research. Consequently, the objectives and scope of the project are established in the chapter.

Chapter Two presents the literature review in which the requirements for TE scaffolds are discussed. In addition, biomaterials that have been used in TE as well as biomaterials that would be used in the research are described. Conventional processing techniques for TE scaffolds are briefly mentioned in the chapter as well. The last section of the chapter deals with a comprehensive review on the applications of RP in TE.

Experimental details are described in Chapter Three whereby the methodology undertaken in the research is presented. Various types of analysis and characterisation are also outlined.

Chapter Four provides the results of the experiments and an analysis and discussion of the results obtained. Conclusion and future works are given in Chapter Five.

Chapter Two:

Literature Review

In This Chapter

- 2.1 Requirements for Tissue Engineering Scaffolds
- 2.2 Biomaterials for Tissue Engineering
 - 2.2.1 Polyglycolic acid (PGA)/ Polylactic acid (PLA)/ Poly(lactic-glycolic) acid (PLGA)
 - 2.2.2 Bioceramics: Hydroxyapatite (HA)
 - 2.2.3 Polyethylene (PE)
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 - 2.3.1 Fiber Bonding
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- 2.4 Applications of Rapid Prototyping in Tissue Engineering Scaffolds
 - 2.4.1 Fused Deposition Modelling (FDM)
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 - 2.4.3 StereolithographyApparatus (SLA)
 - 2.4.4 Three-dimensional Printing (3DP)
 - 2.4.5 Selective Laser Sintering (SLS)
 - 2.4.6 Rapid Freeze Prototyping (RFP)
 - 2.4.7 ModelMaker II (MMII)
- 2.5 *Summary*

Chapter Two

Literature Review

2.1 Requirements for Tissue Engineering (TE) Scaffolds

As TE scaffolds are often used as base materials to guide cell growth in the replacement of tissues and organs, the structure of TE scaffolds should enhance cell proliferation. In addition, the scaffold should provide a suitable substrate for cell adhesion and in certain circumstances, cell migration. Features such as high porosity, large surface to volume ratio, pore size, structural strength, geometrical shapes and biocompatibility [19,20] are important determining factors for fabricating quality TE scaffolds.

Scaffolds of high porosity facilitate nutrient and oxygen supply to transplanted and regenerated cells [21]. By having a large surface area to volume ratio, the scaffolds provide adequate spaces for cells to grow and multiply and hence speed up the process for restoring the function of the ailing organs. The selection of an ideal pore size has always been a concern in the fabrication of TE scaffolds. For bone regeneration purposes, the preferred pore sizes are between 100-600 μm diameter [22] to provide adequate space for cell seeding, growth, extra-cellular matrix (ECM) production and vascularization. The inability to produce TE scaffolds of a desired pore size using conventional fabrication methods explain the need to find alternative methods of fabrication that are able to produce scaffolds of the desired pore size.

In addition, TE scaffolds should be able to withstand physiological stresses [23] while implanted in the body. This is to ensure that the rate of degradation be comparable to the rate of tissue generation. That is to say, the scaffolds should last long enough for full colonisation of cells.

Depending on the site where TE scaffolds are required, various unique three-dimensional shapes with interconnected pores network are needed for cell proliferation. This explains the necessity to fabricate easily reproducible scaffolds of unique geometry that are structured to hold cells close together so that they are able to organise themselves. For any scaffolds to be implanted into the body, the materials used have to be biocompatible and ideally biodegradable. Many natural and synthetic biodegradable polymers, such as collagen, poly (α -hydroxyesters) and poly (anhydrides), have been successfully used as scaffold materials because of their versatility and ease of processing [24]. The usage of biodegradable materials would ensure that the scaffolds fabricated would eventually degrade and hence reduce the risk of complications.

2.2 Biomaterials for Tissue Engineering

The continuous need to search for suitable biomaterials makes research in the field of TE a challenging process. As in all biomedical applications, apart from the need to find materials that are chemically inert and biocompatible, other factors such as biodegradability, non-toxicity and adequate mechanical strength amongst others, have to be considered.

Biomaterials can be basically classified into three main groups, namely, metallic, ceramic and polymeric materials. While metallic implants such as titanium-based alloy have been used commonly in both oral and maxillofacial surgery, they faced the problem of stress shielding which is undesirable in load bearing scaffolds. Unlike metallic materials, polymeric materials do not faced the problem of corrosion or stress shielding. In addition, the increasing interest of polymers in TE may be due to its ability for some polymers, such as poly (lactic-glycolic) acid (PLGA) and poly-L-lactic acid (PLLA), to regenerate tissues through interaction with immunologic cells like macrophages [25] and wide range of processing capability. Research has also shown that the introduction of bioactive materials into polymeric materials has enhanced cell proliferation in TE [26]. With these reasons, the emphasis of this research would focus on the feasibility of introducing bioactive materials to promote cell seeding and growth into polymeric materials.

The following sections would discuss various polymeric and ceramic materials such as polyglycolic acid (PGA), polylactic acid (PLA), PLGA, hydroxyapatite (HA), polyvinyl alcohol (PVA) and polyetheretherketone(PEEK).

2.2.1 Polyglycolic acid (PGA)/ Polylactic acid (PLA) / Poly (lactic-glycolic) acid (PLGA)

Synthetic biodegradable polymers, such as PGA and PLA, have been used widely in TE applications. PGA has been one of the earliest and most successful polymeric biomaterials used in wound closure. The structure of PGA, PLA and their copolymer PLGA is shown in Fig 2-1.

Figure 2-1 : Structure of PLA, PGA and PLGA [24]

PGA can be polymerised directly from glycolic acid through bulk polymerisation at 130° C in vacuum with stannous octoate (50-500ppm) as catalyst [27] and is the simplest linear aliphatic polyester. PGA is a hard and tough crystalline (in the range of 46 – 52%) polymer with a melting point of about 225 ° C and glass transition temperature, T_g , of about 35 ° C [28]. Unlike closely related polyesters, PGA is insoluble in most organic solvents and is first developed as a synthetic absorbable suture, Dexon [28]. However, Dexon tends to lose its mechanical strength (due to its

hydrophilic nature) by up to half its initial strength within 2 weeks of application and is absorbed in about 4 weeks.

Films of PLA, a more hydrophobic polymer than PGA, limit the intake of water to approximately 2% and hence lower the rate of backbone hydrolysis as compared to PGA. Furthermore, the presence of an ester bond in PLA makes it less liable to hydrolysis due to steric hindrance to the methyl group. The longer time needed for PLA to degrade makes it more applicable in the TE work when compared to PGA. PLA, being semi-crystalline(37%), is often preferred in cases where high mechanical strength and toughness are required.

The ability to incorporate two chemically different monomers in the same polymer chain gives rise to copolymers of glycolic and lactic acid. The rate of biodegradation of polylactide is enhanced by incorporating glycolide units into the polymer chain while the solubility of the polyglycolide is increased by incorporating lactide units into the chain structure. Copolymers of PLA with PGA have been developed extensively in both medical and drug delivery applications [28]. Depending on the ratio of glycolide and lactide, PLGAs of different composition with varied applications can be obtained. However, it should be noted that there is no linear relationship between the copolymer ratio and the mechanical and degradation properties of the materials. That is to say a copolymer of 50:50 of glycolide and lactide degrades faster than either polymer as illustrated by Fig 2-2 [25].

Figure 2-2: Degradation time of different weight composition of PLA and PGA when implanted in rat [25]

Copolymer of 10% lactide and 90% glycolide yields a crystallinity of about 40% with T_m and T_g at 210°C and 37°C respectively. It is also worthwhile to note that such composition gives a slightly longer strength retention time. However, PLGA is not ideal for growing tissues like bones because it is relatively hydrophobic and hence during hydrolytic degradation, lactic and glycolic acids are released. The increase in localised acid concentrations can result in tissue damage.

2.2.2 Bioceramics: Hydroxyapatite (HA)

Bioceramics materials, especially calcium phosphate based ceramics, have been used widely in the field of orthopaedic surgery such as replacement and reconstruction of bones. Bioceramics materials have been used for the replacement of hips, knees, teeth, tendons and ligaments, repair for periodontal disease, maxillofacial reconstruction, augmentation and stabilization of jawbone, spinal fusion and as bone filler [29]. The interest in one group of the calcium phosphate based bioceramics, hydroxyapatite, arises from its similarity to bone apatite (Refer to Table 2-1 [30] for the comparison of properties). As shown in Table 2-1, the calcium and phosphorous contents of bone and HA are very similar. The similar Ca/P ratio makes HA a suitable replacement material for bones. In addition, the tensile strength of bones and HA are similar as well.

Table 2-1 : Mechanical Properties and Chemical Composition of Human Enamel,
Bone and Hydroxyapatite [30]

Composition (wt%)	Enamel	Bone	HA
Calcium, Ca^{2+}	36.0	24.5	39.6
Phosphorous, P	17.7	11.5	18.5
(Ca/P) ratio	1.62	1.65	1.67
Sodium, Na^+	0.5	0.7	Trace
Potassium, K^+	0.08	0.03	Trace
Carbonate, CO_3^{2-}	3.2	5.8	-
Fluoride, F^-	0.01	0.02	-
Chloride, Cl^-	0.3	0.1	-
Ash (total organic)	97.0	65.0	100
Total Organic	1	25	-
Absorbed H_2O	1.5	9.7	-
Crystallographic properties			
Crystallinity index	70-75	33-37	100
Products after sintering @950°C	HA+TCP (Tri-calcium phosphate)	HA+CaO (Calcium Oxide)	HA
Mechanical Properties			
Elastic modulus (10^6 MPa)	0.014	0.020*	0.01
Tensile strength (MPa)	70	150*	100

Note: * - indicates properties for cortical bones

HA is a compound of definite composition, $\text{Ca}_{10}(\text{PO}_4)(\text{OH})_2$, and can be obtained via a precipitation method through reactions between calcium hydroxide and phosphoric acid. The ability to form direct chemical bonding with hard tissue and improve bone remodelling makes HA a suitable material as a biological interface. Being a bioactive material, it encourages the deposition of osteocytes on its surface and forms contiguous tissue matrix, hence providing a biologically acceptable matrix for cell seeding.

The compression and sintering conditions used to prepare dense HA, which is often required for novel processing techniques, often influence the mechanical properties of dense HA. Several mechanical properties, such as compressive strength, decrease with an increase in microporosity [30]. It is worth noting that HA is highly compatible to human tissues and appears to give rise to close bonding between the ceramic materials and tissues. Research has shown that HA produces an osteotropic effect which exerts a positive influence on the formation of new bone near the implant area that is evident in the formation of a characteristic growth in the open pore [26]. The release of calcium and phosphate ions in the process leads to bone-induced osteogenesis and results in the ceramic implant linking to the bone. The ability of dense HA to allow for the formation of bone on its surface by serving as a scaffold shows its potential in TE applications.

However, due to its relatively low strength, (as shown in Table 2-1), dense HA is primarily viewed as a material used to fill bone defects but not as load bearing

material. **HA** is used often with polymeric materials to form composites so as to widen its scope of applications.

2.2.3 Polyvinyl alcohol (PVA)

Polyvinyl alcohol is a biodegradable, biocompatible and bioinert semi-crystalline copolymer of vinyl alcohol and vinyl acetate. The melting temperature, T_m , and glass-transition temperature, T_g , of PVA depends on the degree of cross-linking. Its T_m ranges from **220** to **240** °C while its T_g ranges from 58 to 85 °C for partially and fully hydrolysed PVA, respectively; but T_g decreases in the presence of water [31]. Furthermore, the degradation period of PVA can be altered accordingly to suit the various TE applications making PVA an ideal material in the fabrication of TE scaffolds [32-34].

In TE applications, PVA is mostly used for its characteristics as hydrogels. Experiments have been done to fabricate scaffolds for heart valves and soft tissue applications. Apart from its hydrogel characteristics, PVA has high binding strength. High molecular weight and partially hydrolysed grade PVA has been used to formulate polymer-bound alumina body resulting in high strength of the green part. PVA has also been used extensively in the treatment of defects in load-bearing joints such as cartilages due to its relatively similar tensile strength to human articular cartilage and its good lubrication property [35,36]. In addition, the ability of PVA to form complex shapes with suitable adhesion makes it one of the ideal materials to treat complex craniofacial defects.

2.2.4 Polyetheretherketones (PEEK)

Ketone-based polymer such as polyetheretherketone is a semi-crystalline material with pairs of ether linkages -O- in the chain backbone (Fig 2-3).

Figure 2-3: Structure of Polyetheretherketone [37]

The relatively high melting point (T_m) and glass transition temperature (T_g) makes it a suitable material to be processed at relatively high temperature. PEEK is able to retain its mechanical properties at high temperature of about 200°C [37] for prolonged periods of time thus making PEEK a desirable material to be used in Selective Laser Sintering (SLS) where a carbon dioxide laser is used for localised sintering. PEEK exhibits excellent chemical resistance and is insoluble in most solvents. The added advantage of PEEK in biomedical application is the extremely good resistance to radiation making it suitable to be implanted in the human body. Furthermore, it can be repeatedly sterilised without any significant degradation of its inherent mechanical or electrical insulation properties.

23 Conventional Processing Techniques for Tissue Engineering

Scaffolds

Porous three-dimensional temporary scaffolds play a critical role in guiding cell functions and hence there is a need to fabricate TE scaffolds that act as support structures for organs to grow. This section deals with some of the more common conventional techniques currently in use to fabricate tissue scaffolds. The various advantages and disadvantages of the existing methods are presented with the emphasis on the need to source for more precise and efficient method of fabrication as conventional manual based fabrication techniques lack consistency and reproducibility.

2.3.1 Fiber Bonding

Fibers provide a large surface area to volume ratio and therefore are suitable as scaffold materials. PGA fibers in the form of tassels and felts were utilised as scaffolds in organ regeneration feasibility studies [24]. PLLA is dissolved in methylene chloride (see Figure 2-4 for a schematic diagram of fiber bonding process [14]), a non-solvent for PGA, and the resulting polymer solution is cast over a nonwoven mesh of PGA fibers in a glass container.

Figure 2-4: Schematic diagram of fiber bonding process [14]

The solvent is then allowed to evaporate and any residual amounts are removed by vacuum drying. The composite material obtained is subsequently heated to a temperature above the melting point of PGA thus allowing the PGA fibers to join at the cross points as melting occurs. However, both the polymers do not join together due to their immiscibility in the melt state. After heat treatment, the PLLA matrix of a

PLLA-PGA composite membrane is selectively dissolved in methylene chloride and the resulting PGA fibers are vacuum dried. Fiber bonding allows the fibers to be joined together without any surface modification and hence is able to retain its initial diameter. The PLLA matrix is required to prevent collapse of PGA mesh and to confine the melted PGA to fiber-like shape.

However, this technique to produce TE scaffold does have many limitations. Concerns over the choice of solvent, immiscibility of the two polymers and their relative melting temperatures restrict this application to other polymers. Furthermore, the inability to control the desired pore size and porosity hinders its application. The method of fiber bonding can produce films but yet to yield scaffolds of complex three-dimensional shapes. The resultant scaffold also lacks structural stability which is often required in TE to cater to the various ailing organs needed for replacement.

2.3.2 Gas Foaming

The usage of organic solvent in the fabrication of scaffolds for TE applications has been of concern. Organic solvent residues left behind from the processing can have toxic effects *in vitro* and elicit an inflammatory response *in vivo* [14]. Gas foaming has been developed to create porous matrices without using any organic solvents. PLGA pellets are compressed into solid discs and exposed to high-pressure carbon dioxide to saturate the polymer. The subsequent reduction in pressure to ambient levels causes the nucleation and formation of pores in the polymer matrix from carbon dioxide gas. However, the matrixes formed have a closed morphology that does not allow for cell seeding to occur and hence the cells cannot proliferate. Since the

porosity and pore structure are very much dependent on the amount of gas dissolved in the polymer as well as the diffusion rate of gas molecules through the polymer to the pore nuclei [15], it is difficult to fabricate scaffolds with the desired characteristics.

2.3.3 Emulsion Freeze Drying

Freeze drying consists of creating an emulsion by the homogenisation of a polymer solvent solution and water. The mixture is then rapidly cooled to lock in the liquid state structure and subsequently the solvent and water are removed by freeze drying [16]. A schematic diagram of the fabrication process is shown in Figure 2-5.

Figure 2-5: Schematic representation of emulsion freeze-drying method [16]

PLGA is first dissolved in methylene chloride to obtain a solution in which ultra pure water was added to form two immiscible layers. These layers are homogenised to form an emulsion with water as the dispersed phase. The emulsion obtained is poured into a copper mould and quenched by placing it into a copper container that is maintained near liquid nitrogen temperature of about -196°C . The quenched scaffolds are then freeze dried at about -55°C and placed in a vacuum desiccators at room temperature for at least 7 days to remove any residual solvent.

Scaffolds with porosity **up** to 90% and median pore sizes in the range of 15 - $35\mu\text{m}$ can be connected with interconnected pore structure [24]. The pore size obtained is inadequate for successful bone regeneration that requires a median pore size of between 100 - $600\mu\text{m}$ [22]. This inadequacy in pore sizes hinders the exchange of nutrients and oxygen to the seeded cells within the scaffolds.

2.3.4 Melt Moulding

Three-dimensional scaffolds can be fabricated by melt moulding that utilises PLGA as the starting block. PLGA scaffolds have been produced by leaching PLGA or gelatin microsphere composite formed using moulding. The composite is subsequently removed from the mould and placed in distilled-deionised water [24]. Since gelatin is soluble in water, it can be leached out leaving behind a porous PLGA scaffold with geometry identical to the shape of the mould. The advantage of this method is that scaffolds of any shape can be obtained by altering the design of the mould.

PLGA is commonly used for this technique as it requires a slightly lower temperature when compared to polymers like **PLLA** and **PGA** as being semi-crystalline polymers; they need to be heated to above their melting temperature. This inevitably limits the application of melt moulding in fabrication of scaffolds.

2.3.5 Membrane Lamination

Membrane lamination allows the construction of scaffolds with three-dimensional anatomical structure that is required for regeneration of hard tissues. The method involves the construction of a contour plot of the particular three-dimensional shape in which the shapes of the contours are cut from highly porous biodegradable membranes prepared using solvent casting technique [24]. Thereafter, a small amount of chloroform is applied onto the contacting surfaces of adjacent membranes resulting in a bond to be formed. The layer-by-layer process is repeated till a desired three-dimensional tissue scaffold is obtained.

The necessity to combine two different processes in the fabrication of the scaffolds would mean a longer processing time for the scaffolds. The usage of organic solvent is also not desired in TE.

2.4 Applications of RP in Tissue Engineering Scaffolds

Conventional techniques such as fiber bonding, gas foaming, emulsion freeze drying, melt moulding, membrane lamination and solvent casting have been used to fabricate TE scaffolds. Despite the availability of conventional methods to fabricate scaffolds, most conventionally manual-based scaffolds produced lack the consistencies in terms of pore morphology and are not able to provide sufficient structural strength that are generally required in TE applications [38]. In addition, organic solvents, often used extensively in most of these techniques, are not desirable as residual traces left after processing can create adverse toxic effects *in vitro* and elicit inflammatory responses *in vivo*. Hence the potential in using RP [38,39] seems viable as most RP techniques are able to fabricate intricate reproducible structures without the use of organic solvent.

The manufacturing flexibility and capabilities of RP systems allow for complex irregular three-dimensional scaffolds with pre-determined internal micro-architectures and external macro-architectures to be fabricated. As each tissue or organ in the human body has its unique geometries, the ability to have customised scaffolds for different TE applications makes RP techniques suitable for the fabrication of TE scaffolds.

2.4.1 Fused Deposition Modelling (FDM)

FDM, a solid based RP technique developed by Stratasys [40], employs the concept of material extrusion and involves the deposition of two materials, one being the

modelling material and the other, the support material. These materials come in the form of thin filaments that are wound in spools. The filament is fed into an extrusion head and heated to a specified temperature where it becomes semi-liquid. The semi-liquid material is then extruded through the FDM head and deposited layer by layer to build the model. A primary modelling material is used to build the actual model while a secondary material is used to build the support structures.

I.A Cornejo et al. [41], D.W Hutmacher [19] and I. Zein et al. [42] investigated the application of FDM in TE scaffolds fabrication. I.A Cornejo et al. developed a method similar to FDM which used bioceramic as the modelling materials. A 52 vol. % β -tricalcium phosphate (TCP) or polymer compound was made as the feedstock for the process. The compounded material was then granulated and extruded in the form of filaments for modelling.

The advantage of this process lies in the elimination of using the support material that is required in FDM. By doing so, the necessity to do a post-processing is eliminated hence reducing time of fabrication. On the other hand, it is also a disadvantage as well because scaffolds with overhanging features cannot be fabricated using this process. This is a major weakness as TE scaffolds often require complex shapes and design to cater to different sites where they are to be implanted. Furthermore, this process is only limited to simple geometrical shapes. Another disadvantage is the usage of toluene (a colourless and flammable liquid from petroleum) during the coating process which is eventually removed during the burnt out cycle producing toxic fumes. In addition, the process involves a burnout cycle for the binder in which the

green β -TCP structures is subjected to heating in a furnace followed by sintering at 1,000°C to 1,250°C and eventual cooling to room temperature. The adverse temperature changes involved inevitably increase the cost of fabricating the scaffolds and shrinkage of parts. Another problem faced in the research is that the as-received powder contains large agglomeration of powders and often requires additional pre-processing step in which the powders are calcined before it can be made as the feedstock material.

D.W Hutmacher in his work [19], explored the possibility of using non-conventional materials to fabricate scaffolds via FDM. His group evaluated the various parameters of FDM to process potential scaffold materials such as polycaprolactone (PCL), a biodegradable polymer. The usage of such materials is desirable because very often TE scaffolds are meant to be a temporary substrate for organs to be cultivated from the cells. I. Zein et al. [43] in their other related works managed to produce scaffolds of porosity between 50-80% by setting different ratios of wall thickness to channel width for every layer in a manner similar to work done by the research team from Nanyang Technological University as shown in Figure 2-6 [53].

Figure 2-6: Micrographs of Polyethylene-based Scaffolds using FDM [53]

I. Zein et al. [42] in his work has also used PCL in FDM to fabricate TE scaffolds. The research focused on the investigation of anisotropy with different internal architecture and a study on the effect of different porosity and channel size on the scaffold mechanical properties. The scaffolds were modelled after the honeycomb of a bee with regular array of identical pores when viewed in the Z-direction. Limitation in conventional methods such as the lack of interconnectivity within the pore network is eliminated in this technique as micrographs shown revealed a network of interconnected channels of high regularity. D.W Hutmacher et al. took a step further in their work with PCL by investigating the cell culture response on the scaffolds [44]. Results indicated that the fully TE interconnected scaffolds were completely filled with cellular tissues in 3-4 weeks after cell culture has been done.

S. Bose et al. [45] fabricated porous alumina scaffolds by casting alumina into a mould fabricated by FDM. Using thermoplastic wax as the filament material,

cylindrical moulds were designed and fabricated in alternate direction by varying the raster width and gap from **0.62** to **1.10 mm**. The scaffolds were then cast using water based ceramic slurry by means of lost mould technique. The infiltrated mould was dried at room temperature for a couple of days and subsequently subjected to binder removal and sintering cycles. Upon casting, the TE scaffolds produced had pore sizes ranging from **200** to **600** μm . Thereafter, the scaffolds were dipped into a water-based **HA** slurry so as to ensure that the scaffolds fabricated were coated with **HA** to improve its bioactivity. Subsequently, the cytotoxicity behaviour of the TE scaffolds was studied by seeding it with rat pituitary cells (PR1) cells. Results indicated that cell proliferation was more obvious in the scaffolds coated with **HA** when compared to pure alumina scaffolds. One of the limitations of this method is that as lost mould technique is applied, the usage is limited to one scaffold from each mould and hence the wastage of materials is high. Similar research works with respect to FDM were carried out to produce scaffolds with 3D microstructures with consistent pore sizes and arrangement [46-49].

Z. Xiong et al. [50] developed a new system using the concept of extrusion to fabricate controlled porous scaffolds made of **PLLA** that was of 200 to 500 μm in size. **PLLA** was melted in the liquefier before being extruded in a custom made sprayer thus eliminating the need to use organic solvent. The porosity of the scaffolds (**60.3%**) was measured by liquid displacement using ethanol as it did not penetrate easily into the pores and did not induce swelling or shrinkage. As the extruder consisted of only a single head, the supports had to be incorporated in the design of the TE scaffolds. In

addition, the removal of the support was done manually using force, hence there was a risk of destroying the scaffolds built.

A new in house machine based on the principle of RP developed by **T.H. Ang** et al. [51] used a mixture of chitosan (a naturally occurring amino-polysaccharide that is biodegradable and non-toxic) and **HA** to fabricate scaffolds, Known as the rapid prototyping robotic dispensing (RPBOD) system, the process deposited continuous strands of material to fabricate 2D layers at different angles to yield 3D scaffolds. The feedstock materials were prepared by dissolving different percentage of **HA**-chitosan which was dissolved in acetic acid to form a hydrogel. Unlike FDM, whereby the material is dispensed in a platform, the material in this case was dispensed into a medium comprising of sodium hydroxide and ethanol to form a hydrated gel-like precipitate. Upon fabrication, the scaffolds were rapidly transferred to a freezer with temperature of -20°C to solidify the solvent. Scaffolds produced exhibited curling at its edges due to the unavoidable shrinkage where the edges dried faster due to the different rates of solvent evaporation.

Working on the principle of FDM, Z. Xiong [52], developed their own machine to fabricate porous TE scaffolds for bone TE. Unlike FDM, the process took place in a low temperature environment (below 0°C) using PLLA and tricalcium phosphate (**TCP**) as the building materials. Known as low-temperature deposition manufacturing (LDM), the technique extruded the slurry comprising of **PLLA**, which was dissolved in dioxane, and **TCP**. As the fabrication was carried out in a sub zero environment, the scaffold froze upon extrusion from the nozzle. Unlike previous fabrication patterns

[41-45], the scanning direction was alternated every three layers instead of for each layer. Upon fabrication, the scaffolds were freeze-dried for **38** hours to remove the solvent. Results indicated a measured porosity of about **89.6%** with average pore size of **5** μm . The composite scaffolds fabricated were loaded with bovine bone morphogenic protein and implanted into dogs to repair 20mm segmental defects. The scaffolds implanted became invisible based on the X-ray taken around the defects indicating successful bone healing. The degradation rate of PLLA had to be taken into consideration as it was too low to match the tissue regeneration after implantation. Furthermore, the hydrophobic surface property of PLLA may not enhance cell attachment.

Due to the processing parameters such as diameter of filaments, raster gap, road width and slice thickness [53], scaffolds built using FDM are limited to simple geometrical shapes. Simple geometrical shapes are not applicable in surgical practice as often the organs to be cultivated are of complex shapes. Additional post-processing steps are often required for parts built using FDM, as support material used in the building process needs to be removed. It is interesting to note that several research work reviewed in this report on fabricating tissue scaffolds using FDM requires the usage of organic solvents which are not desirable as they can kill the cultured cells.

2.4.2 Laminated Object Manufacturing (LOM)

The technique developed by Helisys Inc. (now distributed by Cubic Technologies Inc.) [54] uses thin sheets of stock such as paper as its building materials. The stock may be paper, plastic or polymer composite depending on the applications. As the part is built, each sheet is adhered together with adhesive with the aid of a hot roller to press the heat sensitive adhesive bonding sheets. The contour of each layer is then trimmed with a carbon dioxide laser that penetrates exactly one layer thickness. The region that does not form the functional part is then trimmed into rectangles to facilitate the later removal of these unwanted regions but is kept during the building stage to act as support.

Past researches on LOM using ceramic materials [55-56] prompted C. Steidle et al. [57] to explore on the possibility of making bone implants using LOM. Though not exactly considered as scaffolds, the custom-made bioceramic bone implants seek to replace the large voids present during bone reconstructions. A hydroxyapatite (HA)/phosphate glass powder mixture was chosen as the base material for fabrication. The role of calcium phosphate was to act as a binding agent in which upon sintering, the glass would melt and fuse the HA particles together. In addition, the addition of glass lowered the sintering temperature hence reducing the tendency of HA to lose the hydroxyl groups. As in all parts fabricated by LOM, the implants faced the problems of delamination and the possibility of void formation that is eliminated by using a slow heating cycle. This would mean a longer processing time that is not desirable especially for LOM which often requires post processing. The removal of the

entrapped material, which has acted as the support, is another disadvantage faced by this technique especially for scaffolds that are very small. Concerns are raised over the need to use organic solvents as well as the sterility of the end product. The most critical limitation with this process is perhaps that pore sizes are too small to accommodate sufficient bone in growth. For bone regeneration purposes, the preferred pore size should be around 100 - 600 μm diameter [22] to provide adequate space for cell seeding, growth, extra-cellular matrix (ECM) production and vascularisation.

Generally, the application of LOM to fabricate TE scaffolds is considered very limited mainly because of the need to obtain suitable materials that can be processed into thin sheets as well as to eliminate the difficulties faced in removing the entrapped materials. Other problems such as delamination and pore sizes also hinder the application of LOM for tissue scaffolds.

2.4.3 Stereolithography Apparatus (SLA)

The SLA process, a liquid based rapid prototyping system, builds parts in a vat of photo curable liquid resin that solidifies upon exposure to laser radiation usually in the UV range [18]. The process begins with the vat filled with the photo curable liquid resin and the elevator table set just below the surface of the liquid resin. The computer-controlled optical scanning system then directs the focused laser beam so that it solidifies the two dimensional cross section corresponding to the slice data on the surface of the resin to a depth greater than one layer thickness with the elevator descending with each layered scanned. The elevator table then descends enough to

cover the solid part that is just fabricated with another layer of resin on the surface. The laser proceeds to draw the next layer and the process is repeated until the whole part is fabricated.

Research on SLA using ceramic materials [58-61] shows the feasibility of using ceramic in SLA systems. G.A. Brady et al. [58] and C. Hinczewski et al. [59] in their research used a ceramic suspension containing alumina powder, W curable monomer, diluent, photoinitiator and dispersant in a process illustrated in Figure 2-7 to produce three-dimensional ceramic parts. Upon polymerisation, the photopolymer was subjected to heating to remove the organic phase. Subsequent sintering (by means of heating) was carried out to obtain the final dense ceramic piece. The key requirement in these researches is that the suspension must be UV curable that is required for all stereolithography (SL) processes. Other concerns include the ability to obtain a resin with a low viscosity (1,200 cps or less [62]) and a curing depth sufficient for typical layer thickness of $100\mu\text{m}$ [18].

Figure 2-7: Stereolithography process for dense ceramic parts

In the need to seek for alternative materials to be used on SLA, various research teams looked into the possibility of using HA [62-64] and polymers such as poly (propylene fumarate), PPF [65]. T.M. Chu et al. [62,63] in their research explored the possibility of using an aqueous HA suspension in acrylamides to fabricate custom made ceramic implants by SL. By using different volume percentage of HA powder and different weight percentage of dispersant, different varying viscosities of the resin were obtained. A higher volume percentage of HA powder would result in a higher viscosity making it impossible to be used for SL. Parts fabricated using ceramic suspensions tended to be non-porous which are not desirable for tissue scaffolds because a porous structure is required for cell proliferation.

S.J. Hollister et al. [64] combined an image-based approach (based on Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) data) with SLA to fabricate custom designed TE scaffolds for application in craniofacial surgery. Computer software was used to read the CT data of the area of defect that was to be reconstructed and the data was regenerated to form the external shape. With the shape of the defect obtained, internal pore architecture based on the defect was generated taking into consideration crucial factors such as tissue infiltration and mechanical behaviour and was combined via Boolean combination to obtain the image of the scaffold. The image of the scaffold was exported to a format readable by **SLA** (in this case .stl) and was ready to be fabricated using the two techniques proposed by the team. In the first technique, casting was used as HA slurry was poured into the moulds that are fabricated by SL and cured. The second technique used by the team is similar to the one carried out by T.M. Chu who is also a member of this research team. Both techniques are capable of fabricating scaffolds that allows control over the external shape as well as internal pore architecture.

The presence of double bonds in PPF allows the polymer to be cross-linked into a solid using a thermal initiator making it a suitable material for use in **SL** for the work carried out by J.P. Fisher et al. [65]. Soft and hard tissue response to the scaffolds was investigated upon in a rabbit model and results showed that such scaffolds elicited a mild tissue response in both soft and hard tissue making PPF a viable material for use in TE.

Due to the need to obtain resins that are *UV* curable, the application of SL on TE is limited. Furthermore, the process involves various other sub-components (such as diluent and dispersant) making it a tedious and complex process if **HA** powders are to be introduced. This inevitably prolongs the fabrication time because parts fabricated by SL process needs further curing in *UV* chamber. The usage of organic solvent also hinders its application for TE scaffolds. Like FDM, SL process would include the need to allocate supports in parts with overhang that need to be removed during the post processing stage.

2.4.4 Three-dimensional Printing (3DP)

A powder-based technology first developed by Massachusetts Institute of Technology (MIT) [18,66] uses the same principle like all RP processes whereby the part is built layer by layer. The main difference as the name implies is the usage of printing technology similar to inkjet printing. A thin layer of powder is first spread over the powder bed and thereafter a slicing algorithm computes information for the layer. A binder material is then injected onto the powder where the object is to be formed resulting in the formation of the first layer which is followed by the lowering of the powder bed by a layer thickness. The process is repeated till the part is formed and any unbound powder is removed leaving the fabricated part. M.J Cima et al. [67] spray-dried granules of fine ceramic powder (in this case alumina) with a latex binder to fabricate structural ceramic components. Dense ceramic parts built using this process are not desirable in scaffolds where porous structures are required. Though alumina powders have no usage in TE scaffolds, it opens up the possibility of using

other biomaterials such as PLA and HA powders or biocompatible materials such as titanium in future research.

Research carried out by S.B. Hong et al. [68] used spherical atomised pure titanium powder with silver carbonate and silver nitrate as the binding materials. The usage of silver is due to its high solubility and diffusivity in titanium. Full densification of parts is obtained through further sintering at a higher temperature of about 1150 °C in addition to the high part bed temperature required during the building process via printing at 450 °C. 3DP has also extended its application to PLLA in the work done by R.A. Giodarno et al. [69] The usage of PLLA eliminated problems such as high elastic modulus of metals which may lead to stress shielding and insufficient transfer of stress to the bones which may result in delayed healing. The usage of 100% chloroform as a binding material was undesirable as organic solvents were harmful to the human body and the inability to completely remove the solvent posed a barrier in its application for TE scaffolds. Results from this work revealed that traces of chloroform (0.5 wt %) remained after one week of drying period. Furthermore, channels with less than 1000 µm could not be successfully fabricated.

By using an indirect method of fabricating TE scaffolds, A. Curodeau et al. used 3DP to fabricate a ceramic mould comprising of alumina and silica binder to cast the scaffolds using Co-Cr (ASTM F75) alloy [70]. The research aimed to cast a part with intricate surface texture via a single step so as to minimise production cost as well as to improve the mechanical characteristics of the final **part**. Dimensional accuracy of

the 3DP ceramic mould deviated within $\pm 5\text{-}10\mu\text{m}$ in all three axes and an overall deviation in dimension on the X-axis of the tissue-engineering scaffold to be around $(350 \pm 50)\mu\text{m}$ as deviations in Y and Z axes were neglected. One of the advantages of using powder-based system such as 3DP is that it allows geometric flexibility which is desired in TE scaffolds. However, the minimum printed feature size is very much determined by the particle size of the powder. In addition, the process of casting is hindered by the tedious cleaning procedures for the mould as voids created in the metal casting by small printed ceramic features need to be cleaned before filling it up with the metal alloy so as to minimise infections inside the human body. The application of sand blasting is not recommended as it can ingrain alumina particles in the casting surfaces. As such, a chemical cleaning procedure involving sodium hydroxide is used.

C.X.F Lam et al. [71] understood the limitations faced by using organic solvents and extended the usage of 3DP on a unique blend of starch-based polymer powders comprising of cornstarch (50 wt. %), dextran (30 wt. %) and gelatin (20 wt. %) to produce cylindrical and rectangular scaffolds with pore sizes of $2500\mu\text{m}$. The presence of a water-based binder replaced the necessity of using organic solvent such as chloroform. The advantage of using a water-based binder is that there is a possibility to integrate biological agents or living cells on it which is crucial for TE scaffolds. However, this process still involved the usage of dichloromethane to dissolve PLLA and PCL which was then used to infiltrate the green parts produced so as to increase its mechanical strength such as stiffness and yield. Although no organic

binder was used for this research, as organic solvent was still needed. Results have shown that the copolymer which was dissolved in dichloromethane was able to thoroughly penetrate the microstructure of the scaffolds and hence their postulation that dichloromethane has completely evaporated remained in doubt.

The advantages of 3DP system include the ability to process a wider choice of materials and in some instances lower temperature required for the binding process. However, as 3DP involves the usage of a binder material, no chemical fusing actually occurs and hence the mechanical strength is not comparable to another powder based RP system, the Selective Laser Sintering (SLS).

2.4.5 Selective Laser Sintering (SLS)

SLS, another powder based system, developed by DTM Corporation and bought over by 3D Systems Inc. in 2001 [72], employs a carbon dioxide laser beam to sinter layers of powdered materials together to form solid three-dimensional objects. The object is built layer by layer from CAD data files in the industry-standard .STL file format. The interaction of the laser beam with the powder raises the temperature to the point of melting and as a result, particles are fused to themselves and the previous layer to form a solid. The individual layers of the fabrication process contain the cross-sections of the part to be fabricated. Subsequent layer are built directly on top of the previously sintered layer after an additional layer of powder is deposited via a roller on top of the previous layer.

U. Lakshminarayan et al. [73] proposed the possibility of using ceramic composites on SLS in their research. By weighing different amounts of alumina powder and aluminium phosphate by means of electronic balance and then blending them together, the composite was then subjected to sintering via carbon dioxide laser. As ammonium sulphate has a much lower melting point (190° C) compared to alumina powder (2,035° C), ammonium sulphate was melted and hence bound the alumina powder together to form the green part. Although ammonium sulphate was melted by the laser, micrograph results showed that the coating of alumina was not uniform and hence the green part was further sintered by means of heating in a furnace (850 °C) to form a uniform coating on alumina particles. Although K. Subramanian et al. [74] also used alumina powders, they used a different approach in their work. The alumina powders were spray coated with a polymeric binder such as polymethylmethacrylate (PMMA) to obtain a composite material. The composite which was in powdered form was then subjected to laser sintering via SLS and the green part was further infiltrated with alumina sol at room temperature. The specimens were then placed in a furnace (set at 600° C) to burn off the binder and the furnace was raised to 1050° C to fuse the nano-sized alumina powder, followed by cooling down to room temperature. A schematic diagram for the process is shown in Figure 2-8.

Process Stages

Figure 2-8: Schematic Diagram for SLS of Alumina Powders with Polymeric Binder[74]

The approach used opens up the possibility for future research in the biomedical field using RP systems especially in the quest for suitable biocompatible materials and using the same technique of spray coating onto bioceramics like HA. Biocompatible materials such as titanium nickelide (NiTi) with bioactive HA additives have been investigated by I.V. Shishkovskii et al. [75] using SLS. N.K. Vail et al. [76] fabricated porous ceramic implants using the concept of encapsulation of HA with polymeric powder, a method similar to work by K. Subramanian et al. In the work by N.K. Vail et al., a substrate material was combined with a binder that served to join substrate particles during laser sintering to obtain green parts of maximum strength of 1.4MPa.

G. Lee et al. [77-79] looked into the development of materials that can aid the regeneration of bone defects and injuries. The approach was very much similar to the

work carried out by K. Subramanian et al. except that instead of metallic ceramics, bioceramics was used. **HA** was coated with a polymeric binder by means of spray drying and micrograph results showed that spherical powders were obtained. The polymer-coated powder was then subjected to laser sintering to obtain bone implants. The binder was subsequently burnt out by means of heating in a furnace after the **SLS** green parts had been infiltrated in an aqueous solution of calcium phosphate.

The advantages of using **SLS** for such application lie in the ability to control pore structure for biogenesis through control of polymer content and the ability to construct accurate bone structure from CT data obtained from patient using appropriate **CAD** software. Results from their research work yielded **SLS** processed green parts with fracture strength of about 0.9MPa that was sufficient to allow for rough handling of complex bone shapes [79].

In addition, **SLS** allows parts of any geometry to be formed as the powders that are not sintered act as support during the fabrication process, hence eliminating the need for support materials that are needed for other RP systems such as **SLA** and FDM. The ability to produce parts of complex geometry is critical in TE scaffolds so as to cater to the site where it is to be implanted during cell/organ growth. Furthermore, small dimensions ranging from 100-500µm can be produced with higher precision that further boost the application of this technique in TE scaffolds whereby pore sizes are very critical for successful cell proliferation. Due to its ability to process various materials, the potential of using SLS is further enhanced as biomaterials with respect

to **SLS** can be tested upon to explore its usage in TE. The **SLS** system has the ability to fuse powder particles into a solid part without having to stay in liquid phase for a prolonged duration hence minimising possible distortion that may be caused by the flow of molten material during fusing. Compared to conventional methods and some RP systems, **SLS** does not require the usage of organic solvent, thus eliminating the issue of concern on its harmfulness to human. However, as it is a powder-based system, there are concerns such as the entrapment of powders in small inner holes which may be difficult to remove and possibility of degradation of powders in the chamber during laser sintering. It is interesting to note that research pertaining to TE scaffolds using **SLS** is still very scarce and therefore the potential of using **SLS** can be further explored.

2.4.6 Rapid Freeze Prototyping (RFP)

Rapid Freeze Prototyping (RFP) [80-83], a liquid based RP technique, employs water as its building material. Like most RP techniques, RFP fabricates parts by depositing water droplets layer by layer till the final shape is obtained. The building material is pumped from a reservoir of water to the nozzle and is deposited onto the previously solidified ice surface. The low temperature environment and the previously solidified ice surface allow the deposited water droplets to solidify rapidly and hence sticking to the previous layer. In order to ensure that the flow of water is smooth, the nozzle and pipes are maintained at a temperature just above the freezing point of water. Similar to FDM, RFP also employs a support material which in this case is a solvent with a

melting point that is lower than water hence allowing the easy removal of supports at a sub zero environment.

As RFP is a relatively new RP technique developed in late 1990s in a joint effort between Tsinghua University in China and University of Missouri in Rolla (USA), publications in the field of TE with respect to RFP techniques remain limited. C. Feng [83] in her thesis examined the possibility of building TE scaffolds on RFP by using composite materials comprising of poly (L-lactic) acid (PLLA) and nano-HA-collagen as the materials for scaffolds. In her approach, water was used as supports for creating the scaffolds. Water droplets were deposited onto a flat surface as shown in Figure 2-9 in an alternate direction. Upon forming the first layer of the ice pattern, the composite materials which were dissolved in an organic solvent were extruded into the gaps between each ice pattern and were subsequently milled to form a flat surface before another layer of ice pattern was formed in the opposite direction. Using the same techniques, the process was repeated till a three-dimensional scaffold was obtained. Thereafter, the ice pattern was melted leaving behind a porous three-dimensional structure comprising of PLLA and nano-HA-collagen structure. The thesis reported pore sizes ranging from 500 μm to 1000 μm which are adequate to promote cell proliferation of bone tissues. Furthermore, pore sizes can be varied by changing the width between the ice lines making RFP advantageous as compared to conventional techniques of building scaffolds whereby consistent pore sizes have always been a concern.

Figure 2-9: Schematic Diagram of Fabrication of Scaffolds using RFP [83]

The potential of using RFP in TE is greatly enhanced by the abundant availability of water and the non-toxic effect of water makes it a suitable material in building scaffolds. The low temperature environment where the fabrication process is carried out minimises degradation or decomposition of the materials in use and the bioactivity of the materials is not affected. In addition, RFP does not require additional support materials as water itself is used as the supports. One of the disadvantages of this technique lies in the necessity to operate in a low temperature environment making it costly to maintain such a facility. Furthermore, the set-up is difficult to operate as care has to be taken in ensuring the right ratio of the in-nozzle material flow rate to XY scanning speed [80]. If the ratio is too large, excess materials are extruded resulting in parts being distorted and out of proportion. On the other hand, a low ratio would result in inconsistent extrusion of the materials resulting in incomplete forming of the fabricated parts. Like most current RP techniques in used, RFP requires the usage of organic solvent to dissolve PLLA and nano-HA-collagen to obtain a slurry that is used to filled up the gaps within the ice patterns. The usage of organic solvent remains a concern as the work carried out did not further investigate the effect of the organic

solvent on tissues. As the structure obtained was highly porous, the lack of mechanical strength limited the scaffolds to low load bearing tissues.

2.4.7 ModelMaker II (MM II)

The machine developed by Sanders Prototype, Inc. and is currently distributed by Solidscape, Inc. employs a two ink-jet type printing heads to build models. One head deposits the thermoplastic building materials while the other deposits supporting wax. The liquefied build material cools as it is ejected from the print head and solidifies upon impact on the model. After the layer is completed, a horizontal cutter is employed to flatten the top surface before the process repeats itself till a complete model is obtained.

In recent years, MM II has been employed to fabricate a negative mould to create the TE scaffolds in which biomaterials were cast into the mould to obtain biocompatible scaffolds [84-86]. S. Limpanuphap [84] obtained a suspension comprised of tricalcium phosphate in diacrylate cross-linking monomers to form a gel and cast into the mould. The scaffolds were then removed by selective dissolution of the mould in acetone to obtain porous scaffold. The scaffold was subsequently sintered in an oven to burn out the polymeric binder. One key disadvantage of this method was the significant shrinkage after the polymer removal and sintering with a reported shrinkage of up to **22%**. J.M. Taboas [85] et al. combined conventional techniques such as porogen leaching and phase separation with MM II to create porous scaffolds. In an indirect approach, a negative mould was obtained through MM II and thereafter cast with polymer-ceramic composite. The main difference between this approach and

the one by Limpanuphap was the usage of computer-aided software to create a global porous scaffold with pre-determined internal pore architecture and the use of salt particles to create local pores by means of porogen leaching technique to obtain porous scaffolds. In a similar manner, the polymeric composite comprising of **PLA** and **HA** together with sieved salt particles were cast into the mould. Using a lost mould technique, the mould removal was done via melting or dissolution to obtain the porous scaffolds. Likewise, fabrication through this technique yielded shrinkage of up to 50% in volume. In addition, both works used organic solvents in the fabrication process. Varying internal channels for the moulds were fabricated in the research work by E. Sachlos et al. [86] in similar manner but using different casting materials. The two main disadvantages in these works were the two-step approach in fabricating the scaffolds and the high shrinkage generated by these scaffolds.

Recent researches have explored the potential of MM II further by developing a similar machine that used the concept of dual print heads, if not, multiple print heads, to “print” organs directly circumventing the needs to have a template or scaffold for the cells to grow and proliferate [87-89]. Biomaterials such as collagen and possibly cells can be introduced into the print heads and the direct printing of organs and the seeding of cells can be carried out simultaneously. However, this method needs to be further explored and is only applicable to soft tissues at this stage and hence may not be suitable for anchorage-dependent cells that often rely on three-dimensional scaffolds to guide cell proliferation. However, the author believes that the potential of this method can be extended to such anchorage dependent cell types.

2.5 Summary

The previous sections have shown the requirements for TE scaffolds, various biomaterials used in TE scaffolds, limitations of conventional processing techniques for TE scaffolds and the applications of RP in TE scaffolds. While each RP system has its advantages and disadvantages, most RP system that are currently in used for TE scaffolds required the usage of organic solvent which is undesirable. Table 2-2 presents the advantages and disadvantages of each technique.

Table 2-2: Advantages and disadvantages of RP techniques

Process	Materials	Advantages	Disadvantages
FDM	Thermoplastic Polymers, Ceramics	100% interconnected porous structure; no trapped material problem.	Requires support structures; high heat effect on raw materials; usage of organic solvents; unable to build complex structures.
LOM	Papers, Polymer composite	Materials are easy to process	Trapped materials in the interior may be difficult to remove; pore sizes are too small
SLA	Polymers, Ceramics	100% interconnected porous structure; no trapped material problem; capable of producing very fine features	Requires support structure; limited material choice
3DP	Polymers, Ceramics, Starch, Metals	100% interconnected porous structure; no heating of powders necessary; wide material choice	Trapped materials in the interior may be difficult to remove; lack of mechanical strength as no chemical bonding is involved
SLS	Polymers, Ceramics, metals	100% interconnected porous structure; wide material choice; able to build intricate complex structures.	Trapped materials in the interior may be difficult to remove.
RFP	Water	Able to vary pore sizes	Needs organic solvent; limited material choice; needs to operate in a low temperature environment
MM II	Thermoplastic polymers, biodegradable polymers, collagen	Wide range of material choice, able to fabricate porous structure	High shrinkage, dual step fabrication process

Chapter Three:

Methodology

In This Chapter

- 3.1 Introduction
- 3.2 Materials
 - 3.2.1 PEEK/HA
 - 3.2.2 PVA
- 3.3 Design and Fabrication
- 3.4 Experimental Approach
 - 3.3.1 PEEWHA
 - 3.3.2 Sintering of Pure PEEK
 - 3.3.3 Sintering of Pure PVA
- 3.5 Thermal Analysis
 - 3.5.1 Differential Scanning Calorimetry
 - 3.5.2 Thermogravimetric Analyser
- 3.6 Microscopic Analysis
- 3.7 Porosity Analysis
- 3.8 Bioactivity Analysis
- 3.9 X-Ray Diffraction
- 3.10 Cell Culture

Chapter Three

Methodology

3.1 Introduction

As mentioned in the previous chapter, **SLS** has many capabilities when compared to other RP systems. However, the works carried out by the various researches using biocompatible materials have their limitations. Although in the works using polymeric binders on HA, the polymeric powder was eventually burnt off so as to expose HA for cell culture, the disadvantage lies in the need to go through additional post processing steps. In TE of bone scaffolds, it is very important to expose bioactive materials such as HA as it aids cell growth and proliferation. The research carried out here explored both hard and soft tissues using two different groups of materials for each application. For hard tissues such as bone, a physical blend comprising of PEEK and HA is explored. For soft tissues such as articular cartilage, PVA is explored. The emphasis of this research was placed on the fabrication and characterisation of scaffolds for bones to better understand the viability of the process.

3.2 Materials

3.2.1 PEEK/HA

PEEK 150XF from Victrex USA Inc was used for this research. PEEK 150XF is a low viscosity grade used for powder coating and as filler. The thermal and mechanical properties of PEEK 150XF as received are shown in Table 3-1 [90]. HA powders used were purchased from Cam Implants B.V. which met the ASTM F 1185-88

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specification. Particles had a size distribution with at least 90 **wt** % below 60 microns, as determined by Coulter Counter analysis with density of at least 3.05 g/cm³.

Table 3-1 : Properties of Victrex® PEEK TM [90]

		Method	Units	150XF
General Properties				
Relative Density	(Crystalline)	ISO 1183	g cm ⁻³	1.32
	(Amorphous)	ISO 1183	g cm ⁻³	1.26
Level of Crystallinity			%	35
Water Absorption	24 hr. @23°C	ISO 62	%	0.50
Mechanical Properties				
Tensile Strength	@23°C	ISO 527	MPa	100
Tensile Elongation	@23°C	ISO 527	%	Up to 60
Elongation at Yield	@23°C	ISO 527	%	5
Flexural Modulus	@23°C	ISO 178	GPa	4.0
Flexural Strength	@23°C	ISO 178	MPa	170
Izod Impact Strength	@23°C, notched	ISO 180	KJm ⁻²	6.1
	@23°C, unnotched	ISO 180	KJm ⁻²	No break
Thermal Properties				
Melting Point		DSC	°C	343
Glass Transition Temperature		DSC	°C	143
Heat Distortion Temperature		ISO 75	°C	156

3.2.2 PVA

Pure PVA powder (99% + hydrolysed, average Mw 89,000 – 98,000) were obtained from Aldrich Chemical Company, Inc with melting point of about 300°C and density of 1.269 g/cm³ obtained from its Material Safety Data Sheet (MSDS).

3.3 Design and Fabrication

For the purpose of initial testing, a circular disc was designed using CAD software, ProEngineer, by varying the thickness of the disk beginning with a single powder layer thickness of 0.102mm to 0.508mm with a diameter of 16mm. The disc was designed so as to fit the standard cell culture tray. The designs were exported in a standard industrial format (.stl) readable by most RP systems and uploaded into a commercial SLS system, Sinterstation 2500. For repeatability, ten sets of specimens were fabricated for each configuration.

3.4 Experimental Approach

Two groups of biomaterials were explored in this project. In the first group, PEEK and HA were investigated for application as bone scaffolds and in the second group, PVA was used to establish the feasibility of using PVA as scaffolds for soft tissues. As the research is on bone tissues, the emphasis in the experimental approach would be on PEEWHA.

3.4.1 PEEK/HA

In the first approach, a physical blend of a biocompatible polymer, in this case polyetheretherketone (PEEK), and a bioactive material, in this case HA, by means of weighing a different composition can be used by SLS to fabricate parts. Both powders were mixed by means of an electric mixer to obtain a homogenous blend. Starting with 10% **wt** HA and 90% **wt** PEEK, the composition of HA was gradually increased to 40% **wt** HA in steps of 10% **wt**. The addition of HA was to boost the bioactivity of the specimens. In addition, it was not possible to sinter HA by SLS because of its high melting point. As PEEK has a much lower melting point, it is possible to sinter PEEK near its glass transition temperature to fuse HA together.

3.4.2 Sintering of Pure PEEK

In order to study the optimal processing parameters for PEEK on the usage in SLS, pure PEEK was sintered to obtain circular discs with diameter of 16 mm and thickness ranging from 0.102 mm to 0.508 mm. The thickness is designed to range from 0.102 mm to 0.508mm to accommodate the layer thickness as specified by SLS. Three main parameters would be studied in the project namely laser power, part bed temperature and scan speed as research conducted by K.F. Leong et al. [91] and J.C. Nelson [92] had shown that these three parameters were responsible for the energy density or Andrew Number of the part which measures the energy per unit area received by the specimens built on the SLS. By varying these parameters, test specimens of varying porosity could be obtained.

The machine settings were all at their default values except for laser power (8W) and part bed temperature (110 °C). Pure PEEK was then subjected to sintering via SLS at a different laser power and temperature setting to obtain the optimal settings for the sintering of PEEK. For the laser power, the initial setting was set at 8W as this was the optimal setting for Duraform, standard materials for SLS, based on research carried out by Phung, W.J. [93]. The laser power was gradually increased at an interval of 1W to study the different sintering result due to the variation in laser power. The part bed temperature was set initially at 110 °C and increased at an interval of 10 °C. Table 3-2 provides a summary of all the variables used in the sintering of PEEK.

Table 3-2: Variables used in the sintering of PEEK

	Variables	Remarks
Diameter of disc (mm)	16	
Thickness of disc (mm)	0.102 – 0.508	Interval of 0.102 mm
Laser Power (W)	8 - 28	Interval of 1W
Part Bed Temperature (°C)	110 - 130	
Scan Speed (mm/s)	5080 (200 in/s)	Kept constant

3.4.3 Sintering of Pure PVA

In order to study the optimal processing parameters for PVA on the usage in SLS, pure PVA was sintered to obtain circular discs. Detailed experimental procedures for the laser-sintering of PVA was based on the research carried out by Wiria, F.E. [94].

3.5 Thermal Analysis

3.5.1 Differential Scanning Calorimetry

As the polymeric blend comprising of PEEK and HA, and pure PVA would be subjected to laser sintering, thermal properties of the polymers were critical to the research. Thermal properties such as glass transition temperature and melting point can be determined by differential scanning calorimetry (DSC) using thermal analysis instruments (DSC 7 Differential Scanning Calorimeter from Perkin Elmer) as shown in Figure 3-1.

Figure 3-1: Differential Scanning Calorimeter

The as-received PEEK and PVA powders were placed in a small aluminium pan with sample weight ranging from 0.5 to 10mg [95]. PEEK and PVA sample were scanned from 50°C to 350°C and 30°C to 250°C respectively at a rate of 10°C per minute. The cycle was carried out twice so as to obtain an average result.

3.5.2 Thermogravimetric Analyser

As different weight composition of PEEK and HA were used in the polymeric blend, thermal degradation analysis was carried out on the blend. Thermogravimetric analyser (TGA) allows the measurement of the change in mass of the material as a function of temperature. Hi-Resolution Modulated TGA 2950 (TA Instruments) was used in this research to analyse the weight composition of the blend. A heating rate of 10°C/min and a sample weight of $6.0\text{mg} \pm 0.5\text{mg}$ was used. The degradation analysis was carried out over a temperature range from 30°C to 800°C with ambient air as the flow gas.

3.6 Microscopic Analysis

Microstructural characterisation and particle size analysis for both PEEK and HA powders were carried out using scanning electron microscope (SEM), JEOL JSM-5600 LV. SEM provides useful information of surface topology hence allowing the sintering result to be analysed. All test specimens fabricated by SLS were examined under high vacuum conditions at between 12kV to 15kV at different magnification. The test specimens were coated with a thin layer of metal such as gold or platinum to enhance its conductivity for the electron beam. Image formation in the SEM is obtained when the electron beam hits the surface of the specimens and bounces off the

specimens to reach the cathode ray tube inside the chamber. The larger amount of electrons collected from the specimens at the given spot would result in a greater intensity to be registered and hence a clearer image is obtained. The result would clearly illustrate the surface topology of the specimens because it is this surface topology that determines the number of electrons collected from the various spots on the specimens.

3.7 Porosity Analysis

Microporosity of the circular test specimens was measured to obtain the porosity of the specimens. For the sintered specimens, the microporosity is created by the space between the laser-sintered PEEWHA powder grains. The porosity was calculated by the general formula [53]:

$$\text{Porosity, } P_{\text{cal}} = 1 - \left[\frac{W_{\text{spec}}/V_{\text{spec}}}{P_{\text{blend}}} \right] \quad [3.1]$$

in which W_{spec} = mass of the specimen, V_{spec} = calculated volume of the specimen and ρ_{blend} = density of the blend.

The density of the blend is measured using a gas pycnometer, Ultrapycnometer 1000 (Quantachrome Corporation, USA), with pure helium. Ten specimens for each of the different weight percentage of PEEK and HA were weighed and volume was obtained through actual dimensions of the specimens to obtain the porosity of the specimens.

3.8 Bioactivity Analysis

The bioactivity of a specimen is critical as it determines if the material exhibits chemical bonding to living tissues upon the formation of a bone-like apatite layer on its surface in any simulated body fluid environment [96,97]. Previous research [98-100] have shown that the immersion of specimens in simulated body fluid (SBF), which has the same ions concentration as those of the human blood plasma (as shown in Table 3-2), can detect the *in vitro* surface changes of the specimens. In this research, SBF was prepared according to Kokubo's protocol [101] in which reagents such as calcium chloride (CaCl_2), potassium hydrogen phosphate trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), calcium hydrogencarbonate (CaHCO_3) and sodium sulphate (Na_2SO_4) were dissolved in distilled water.

Specimens with different weight percentages of PEEK/HA were laser-sintered and immersed in 50 ml of SBF for a period of **28** days. As the cation concentration would decrease during the period of the *in vitro* studies due to the changes in chemistry of the samples, the SBF was replaced every week. The morphological analysis and phase changes of the specimens were studied under **SEM** and X-ray diffraction (XRD) respectively after every 7 days after the specimens have been removed.

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Table 3-3: Ionic concentrations of blood plasma and SBF [101]

Ions	Ionic Concentrations (mM)	
	Blood Plasma	SBF
Calcium, Ca^{2+}	2.5	2.2
Hydrogen Phosphate, HPO_4^{2-}	1.0	0.8
Sodium, Na^+	142.0	140.3
Chloride, Cl^-	148.8	148.0
Magnesium, Mg^{2+}	1.5	1.3
Potassium, K^+	5.0	5.3
Hydrogen carbonate, HCO_3^{2-}	4.2	4.0

3.9 X-ray Diffraction (XRD)

X-ray diffraction (XRD), (Philips PW 1830 X-ray diffractometer), was used to identify and determine the various crystalline forms of compounds present in a sample. The presence of crystallites in a specimen ensured that a representative intensity distribution for these crystallites can be obtained when X-ray is directed to a specimen [102]. The atomic planes of a crystal caused an incident beam of X-rays to interfere with one another as they leave the crystal allowing the intensity of the diffraction to be recorded in a diffractogram. This diffraction or “reflection” from planes in the lattice was suggested by W.L. Bragg and as illustrated by Bragg’s law [103]:

$$n\lambda = 2d \sin\theta \quad [3.2]$$

in which λ is the wavelength of the X-ray beam, d is the spacing between polycrystalline spacing, θ is the angle the X-ray made with the planes and n is the number of complete wavelength.

Figure 3-2 shows an illustration of Bragg's Law in which an X-ray beam with a wavelength, λ , is projected onto a crystalline specimen at an angle, θ . Diffraction occurs when the distance travelled by rays reflected from successive plane differs by a number of n wavelengths, with d as the distance between crystal lattices. By plotting the angular positions and intensities of the resultant diffracted peaks of radiation produces a pattern that is characteristics of the specimen and is represented on a diffractogram. Identification of the specimen is achieved by comparing the diffractogram obtained from an unknown sample with an internationally recognised database which in this case is the Joint Committee of Powder Diffraction Standard (JCPDS).

Figure 3-2: Monochromatic X-ray beam

XRD analysis were carried out at **30kV** and 20mA using copper X-ray tube. The scanning angle (2θ) was set between 10° to 70° with a step size of 0.02° and time per step of 1s.

3.10 Cell Culture

Cell culture has proven to be an effective method to assess the biocompatibility of the materials to be used for TE scaffolds. For the purpose of this research, the direct contact cell culture assay was used [104]. This proliferation assay enables the change in the morphology of the cells to be observed hence determining its suitability to be used as the processing materials for TE scaffolds. Cell lines that were developed for growth *in vitro* are preferred to primary cells that were freshly harvested from animals because the cell lines improve the reproducibility of the assays and reduce any invariability that may have been introduced. Furthermore, cell lines tend to maintain their genetic and morphological characteristics for a longer period of time. Fibroblast cell lines were chosen because these cells are one of the early cells to populate a healing wound and are often the major cell in the tissues that attach to implanted medical devices.

In direct contact test, the cells were placed directly onto the specimens. Near confluent fibroblast cells were prepared in a cell culture dish. Thereafter, the medium in the cell culture dish was removed and phosphate buffered solution (**PBS**) was added into the dish to wash away the medium. The process was repeated a couple of times to wash away any residual serum based medium in the cell culture dish. Live

cells would strongly adhere to the base of the dish and dead cells lose their adherence to the base of the culture dish. The process of adding PBS to it allowed the dead cells to be washed away. A small amount (1 ml) of trypsin (a protein-digesting enzyme) was added into the culture dish to dislodge the living cells from the base and also to segregate the cells and subsequently placed into a centrifuge tube. Test specimens with various composition of **HA** were pre-soaked in the medium so as to remove any air bubbles trapped within the disc. For the purpose of comparison, a set of controls was used to study the cell proliferation on the test specimens. **4** wells with no test specimens placed in it were filled with 1 ml of the serum (medium). Thereafter, the different compositions of the specimens (10% wt **HA** – 40% wt **HA**) were placed in quadruplets into the **24** wells cell culture tray (as shown in Figure 3-3) and 1 ml of medium was added into each well.

Figure 3-3: **24** Wells Cell Culture Tray

The cell culture tray was then incubated in an incubator with **4%** carbon dioxide for **24** hours. A typical set up for the cell culture is shown in Figure 3-4.

Figure 3-4: Cell Culture Set Up

For the studies on the cell proliferation on the specimens built using the mixture comprising of **PEEWHA** powders, qualitative analysis were carried out to study the cell viability of laser-sintered **PEEWHA** specimens. The cell culture tray was removed 12 hours after seeding to view the cell proliferation and growth using a microscope that allowed the qualitative analysis of the cells to be carried out. The medium in the cell culture tray was changed every **48** hours so as to ensure that the specimens were cultivated in a nutrient rich environment. The duration of cell culture was conducted over a period of **7** days.

Chapter Four:

Results and Discussion

In This Chapter

- 4.1 Thermal Properties of PEEK
 - 4.1.1 Thermal Properties of PEEK
 - 4.1.2 Thermal Properties of PVA
 - 4.1.3 Thermal Degradation Analysis of PEEK/HA Blend
 -
- 4.2 Microscopic Examination of Different Composition of PEEK/HA
 - 4.2.1 PEEWHA before Mixing
 - 4.2.2 PEEWHA Mixture before Sintering
 - 4.2.3 Sintering of Pure PEEK
 - 4.2.4 PEEWHA Mixture after Sintering
- 4.3 Microscopic Examination of PVA
 - 4.3.1 As-received PVA before Sintering
 - 4.3.2 Sintering of Pure PVA
- 4.4 Porosity Analysis for PEEK/HA Scaffolds
- 4.5 Apatite Formation
- 4.6 Phase Analysis of PEEK/HA Scaffolds using XRD
- 4.7 Biocompatibility Analysis by Cell Culture

Chapter Four

Results and Discussion

This chapter is divided into 7 sections, namely thermal analysis, microscopic examination of PVA, microscopic examination of PEEWHA, porosity analysis, apatite formation, phase analysis and biocompatibility analysis.

4.1 Thermal Analysis of Polymers

The thermal behaviour of PEEK and PVA are presented in this section to provide a guide for the laser-sintering of both the polymers. In addition, the weight composition of the physical blend comprising of PEEK and HA is verified through thermal analysis.

4.1.1 Thermal Properties of PEEK

The melting behaviour of PEEK studied is presented in this section. Results for measurements conducted on PEEK samples using Differential Scanning Calorimetry are shown in Figure 4-1. From the graph, the highest peak observed at 342 °C indicates the melting point of the polymer. To obtain the glass transition temperature of the polymer, 2 lines were drawn tangentially to the slope of the curve at the juncture whereby there is a change in the slope of the graph. However, the glass transition temperature, T_g , of PEEK is not very distinct as the change of slope is gradual. The change of slope was noticed to occur between 135°C to 145°C. The software would then determine the glass transition temperature automatically. Figure 4-2 gives an exploded view of one section of the graph (between 107.4°C to 155°C)

shown in Figure 4-1 that illustrates the change of slope indicating the glass transition temperature. The glass transition temperature was noted to be 143.3°C.

Figure 4- 1: Thermal properties of PEEK.

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Figure 4-2: Calculation for T_g of PEEK.

The results confirmed the technical specifications supplied by the manufacturers (Vitrex USA **Inc.**) for PEEK 150XF. The purpose of establishing the thermal properties of PEEK was to determine part bed temperature and left and right feed temperature when operating the **SLS**. With the thermal properties of PEEK known, the left and right feed cartridges of **SLS** was set at 110°C and the part bed temperature was set at 110°C.

4.1.2 Thermal Properties of PVA

The thermal behaviour of PVA is presented in this section and the results are shown in Figure 4-3. The glass transition temperature of PVA was obtained in a manner similar to that of PEEK. However, unlike PEEK, the glass transition temperature of PVA is more distinct than PEEK as indicated by the change of slope as shown in Figure 4-3. The glass transition temperature was noted to be 63.54 °C. The purpose of establishing the thermal behaviour properties of PVA was to determine part bed temperature and left and right feed temperature when operating the **SLS**. With the thermal properties of PVA known, the left and right feed cartridges of **SLS** was set at 40°C and the part bed temperature set at 65°C which is close to its T_g .

Figure 4-3 Thermal properties of PVA.

4.1.3 Thermal Degradation Analysis of PEEK/HA Blend

Before subjecting the polymer blend to laser-sintering, the 4 blends comprising of different weight percentage of PEEK and HA were subjected to thermal degradation analysis. Figure 4-4 provides the result for the thermogravimetric analysis of different weight composition of PEEK and HA used in the blend.

Figure 4-4: TGA of different composition of PEEK/HA blend

As seen from the Figure 4-4, 3 out of the 4 blends used in this research showed the correct weight percentage of the materials used in the blend hence verifying that the physical blending of PEEK and HA did yield a mixture with uniform distribution of PEEK and HA particles. However, it should be noted that for the blend comprising of 90% **wt** PEEK and 10% **wt** HA, an average weight percentage of 14% was noted for the blend as compared to other weight composition of the blend which deviate from the actual weight percentage by less than 1% as observed from Figure 4-4. Previous research [105] has shown that the complete burnt off of PEEK did not result in measurable ash content and hence the thermal degradation of 100% **wt** PEEK was not carried out. In addition, since HA will not degrade till at a higher temperature of at least 1200°C, the final weight percentage registered at 800°C would have to be just **HA** alone and not PEEK which would be completely burnt off at about 750°C.

The TGA analysis has shown that the physical blending by means of the electrical roller-mixer have indeed produced blends with the correct weight compositions of both the biomaterials.

4.2 Microscopic Examination of Different Composition of PEEK/HA Blends

Investigations to study the sintering of PEEK/HA powder blends were carried out on the individual component materials prior to mixing, after mixing and composite materials prior to sintering and after sintering. The results are presented in the subsequent sections.

4.2.1 PEEK/HA before Mixing

Figures 4-33 and 4-34 are micrographs from SEM observation of the as-received PEEK and HA powders respectively prior to mixing. As observed in these figures, PEEK powders are irregular in shape with an average particle size of 40 micron whereas HA powders are spherical in shape. The average particle size of PEEK powder is close to that of Duraform (a standard material for SLS), making it suitable for laser-sintering.

Figure 4-33: Micrograph of as-received PEEK powders.

Figure 4-34: Micrograph of as-received CAM-HA powders.

4.2.2 PEEK/HA Mixture before Sintering

Both the HA and PEEK powders were mixed to yield powder blends of different composition by weight before being subjected to laser sintering. Figure 4-35 shows a micrograph taken for the powder blend containing 10% **wt** HA before sintering. The distribution of HA particles in the polymer blend are indicated by the red circles. As observed in Figure 4-35, HA particles can be seen in the mixture with 10 % wt HA and the concentration of HA in the mixture increases gradually with increased amount of HA which is evident from Figures 4-35 to 4-10. Figures 4-35 to 4-10 show the different amounts of HA by weight percentage in the PEEWHA powder blends mixed.

Figure 4-35: Micrograph taken for 10% **wt** HA and 90% **wt** PEEK powder blend before sintering.

Figure 4-36: Micrograph taken for 20 % **wt** HA and 80% **wt** PEEK powder blend before sintering.

Figure 4-37: Micrograph taken for 30 % **wt** HA and 70% **wt** PEEK powder blend before sintering.

Figure 4-10: Micrograph taken for 40 % **wt** HA and 60% **wt** PEEK powder blend before sintering.

It is worthwhile to note that regardless of the different amount of powder mixtures taken for analysis in SEM, the correct percentage of HA particles appeared in all samples. In addition, results obtained from the various micrographs indicated a good dispersion and distribution of HA particles in the blend which is consistent with the different percentage composition of HA. The TGA analysis presented in Section 4.1.3 supports this observation. With this assurance, the powder mixture was subjected to laser sintering and the results are discussed in the next section.

4.2.3 Sintering of Pure PEEK

As mentioned in chapter 3, sintering was carried out on pure PEEK to determine the favourable processing parameters on the **SLS** system. Different specimens of various parameters in terms of part bed temperature and laser power based on the **SLS** system

were fabricated and are tabulated in Table 4-1. For this research, the scan speed of the laser system was kept at the default value of 5080 mm/s (200 in/s).

Table 4-1: Different parameter settings for specimens (Pure PEEK) built using SLS.

	110°C	130°C	140°C
8W			
9 w			Specimen 7
10W	Specimen 1	Specimen 5	Specimen 8
12 w	Specimen 2		Specimen 9
14W	Specimen 3	Specimen 6	Specimen 10
16W	Specimen 4		Specimen 11
18W			Specimen 12
20 w			Specimen 13
22 w			Specimen 14
24W			Specimen 15
26W			Specimen 16
28W			Specimen 17

Figures 4-11 to 4-14 show the sintering results for pure PEEK powders subjected to a part bed temperature of 110°C and at different laser power (10W to 16W).

Figure 4-11: Micrograph taken for Specimen 1 (1 10°C/10W).

Figure 4-12: Micrograph taken for Specimen 2 (1 10°C/12W).

Figure 4-13: Micrograph taken for Specimen 3 (110°C/14W).

Figure 4-14: Micrograph taken for Specimen 4 (110°C/16W).

Though the range of laser power used in the experiment was from 8W to 16W, the discs built using 8W and 9W failed as the laser power was too low to cause sintering of PEEK powder. As observed in the micrographs, “necking” appeared in the samples (as indicated by the blue circles) when the laser power was increased to at least 12W as indicated in Figure 4-12. It was observed that “necking” became more evident with

increasing laser power. However, due to the relatively low part bed temperature, delamination on the sintered PEEK specimens was observed. As such, to obtain better structural integrity, a much higher part bed temperature was required. Furthermore, the PEEK powder tended to stick to the roller during the powder recoating process posing a problem for multi-layered disc to be fabricated. After sintering of the first layer, when the roller went over the part bed for recoating of powder, the previously sintered part tended to adhere to the roller making it difficult to fabricate multiple layers.

As PEEK powders were irregular in shape, the high surface area to volume ratio of PEEK powders as compared to spherical powders causes problems with spreading via rollers. Furthermore, when the roller spread the powders during the recoating process, distinct streak of lines appeared on the part bed indicating a possibility of static charges as the powders tended to “chain” together and were dragged along with the roller. This problem was reduced by using an anti-static cloth. The cloth was able to neutralise the charges and prevented the powder from sticking to the roller during the fabrication process. This explained the possibility to fabricate five-layer thickness (0.508mm) of the circular disc for further characterisation analysis presented in the later sections of this chapter.

With the observations made on the effect of varying temperature and laser power for the sintering process, the part bed temperature was raised to 130°C and the same set of values for the laser power was repeated. The results obtained were similar to those

specimens at 110 °C and hence there was a need to further increase the part bed temperature. Figures 4-15 and 4-16 show the micrograph taken for pure PEEK specimens built using 10W and 14W at 130 °C respectively. As observed from the figures, the test specimens sintered showed necking between particles but the degree of necking was not as prevalent.

Figure 4-15: Micrograph taken for Specimen 5 (130 °C/10W).

Figure 4-16: Micrograph taken for Specimen 6 (130 °C/14W).

The part bed temperature was subsequently raised to 140°C which is just below the glass transition temperature of PEEK. A higher range of laser power from 8W to 24W was used. Figures 4-17 and 4-18 show the micrograph for sintered PEEK at 9W and 10W at 140°C respectively.

Figure 4-17: Micrograph taken for Specimen 7 (140 °C/9W).

Figure 4-18: Micrograph taken for Specimen 8 (140 °C/10W).

Though the circular test specimens were obtained for laser power of **9W** and **10W**, the sintering result did not show good “necking” as observed from the micrographs. However it could be deduced that for successful sintering to occur for PEEK, the part bed should be **140°C**. This was because as compared to the previous settings of **110°C** and laser power of **9W**, the circular test specimen cannot be sintered. However, it should be noted that though specimens were obtained at **140°C** for laser power of **9W** and **10W**, the specimens were too flimsy to be handled by hand and the structural integrity of the specimens was weak. Therefore, the laser power was further increased.

When the laser power was increased to at least **12W** with a part bed temperature of **140°C**, the sintering results obtained appeared more promising as observed from Figures **4-19** to **4-22**.

Figure **4-19**: Micrograph taken for Specimen **9** (**140 °C/12W**).

Figure 4-20: Micrograph taken for Specimen 10 (140 °C/14W).

Figure 4-21: Micrograph taken for Specimen 11 (140 °C/16W).

Figure 4-22: Micrograph taken for Specimen 12 (140 °C/18W).

The degree of necking was more prevalent in specimens fabricated at a part bed temperature of 140 °C when compared to those of both 110 °C and 130 °C. Necking is an observation noted on powder particles when two or more powder particles are fused together forming a "neck" between them as shown by the yellow arrows indicated on Figure 4-22.

Figure 4-23 illustrates the build specimen taken near the edge of the disc. As shown in the micrograph taken, delamination (edges opening up) was noted near the edge for specimens built using laser power between 12W to 18W. As such, the laser power was further increased gradually till 28W to study its effect.

Figure 4-23: Micrograph taken at the edge of specimen showing delamination

It was observed that with an increase in laser power, the degree of melting of PEEK was more evident. Figures 4-24 to 4-28 show the micrograph taken for pure PEEK specimens built using laser power between **20W** to **28W** in steps of **1W** with part bed temperature at **140 ° C**. A visual inspection on the specimens indicated that laser power of **28W** would result in a charred specimen and hence was not desirable. As illustrated in Figures 4-24 to 4-27, laser power ranging from **22W – 26W** would result in the formation of non porous part. For the purpose of cell cultivation, porous specimens were fabricated to mimic the porous conditions required for TE scaffolds and the results are presented in the last section of this chapter.

Figure 4-24: Micrograph taken for Specimen 13 (140 °C/20W).

Figure 4-25: Micrograph taken for Specimen 14 (140 °C/22W).

Figure 4-26: Micrograph taken for Specimen 15 (140 °C/24W).

Figure 4-27: Micrograph taken for Specimen 16 (140 °C/26W).

Figure 4-28: Micrograph taken for Specimen 17 (140 °C/28W).

The satisfactory processing condition for PEEK is 140°C for the part bed temperature and the left and right feed cartridge should be set at 110°C. As for the laser power, the range is between 16W to 21W. The micrographs for the rest of the sintering results can be found in Appendix A-1 to A-3.

4.2.4 PEEK/HA Mixture after Sintering

Using the parameters determined in the Chapter 3, thin discs of pure PEEK as shown in Figure 4-21 were laser sintered for the purpose of cell culture and to be used as a comparison between pure PEEK and powder blends comprising of PEEK and HA powder. Promising results as illustrated by the micrographs in Figure 4-21 showed evidence of fusion as indicated by the necking between PEEK particles. All specimens were fabricated at a part bed temperature of 140°C and laser power of 16W. Table 4-2 shows the breakdown of the different specimens built.

Table 4-2: Different weight composition of HA and PEEK in the powder blend built using SLS

		10% wt	20% wt	30% wt	40% wt
PEEK	90% wt	Specimen 18			
	80% wt		Specimen 19		
	70% wt			Specimen 20	
	60% wt				Specimen 21

Figures 4-29 to Figure 4-32 show the micrographs taken for different weight compositions for powder blend of PEEK and HA after laser sintering. As noted from the figures, the fabricated specimens exhibited promising results as HA particles can be seen embedded in the discs which were held together by the PEEK powders that had been successfully sintered. For the initial purpose of cell culture, laser power of 16W was used for the polymer blend comprising of different weight composition of PEEK and HA. Micrographs of the specimens built based on different composition can be found in Appendix B1 to B-4.

Figure 4-29: Micrograph taken for Specimen 18 (10% **wt** HA/ 90% PEEK).

Figure 4-30: Micrograph taken for Specimen 19 (20% **wt** HA/ 80% PEEK).

Figure 4-31: Micrograph taken for Specimen 20 (30%wt HA/70% wt PEEK).

Figure 4-32: Micrograph taken for Specimen 21 (40%wt HA/60% wt PEEK).

The successful sintering of the test specimens indicated that PEEK could act as a base material for TE scaffolds. In addition, the successful incorporation of **HA** into the polymer matrix has enhanced the bioactivity of the specimens. Unlike previous research works in which the polymeric binder was burnt out after subjecting the

specimen to further sintering by means of firing in a furnace, it is not necessary in the current application. The elimination of the additional step of burning off the polymeric binder would quicken the fabrication process which is critical in TE scaffolds as very often seeding of cells need to be done in a short period of time. However, observations indicate that with a decrease in the percentage of PEEK in the composition of the powder blend, the fabricated disc specimens lacks solidity and can be easily torn. In order to produce a structure with good integrity, it is proposed that the composition of HA in the mixture should be kept at 40% **wt** HA and hence no further increment in the composition of HA in the PEEWHA mixture was carried out.

The possibility to laser-sintered PEEK/HA to obtain porous scaffolds with well defined pore interconnectivity clearly indicated its potential to be used as TE scaffolds.

4.3 Microscopic Examination of PVA

Investigations were carried out on the as-received **PVA** powder and the laser-sintered **PVA** discs. The results obtained are presented in the subsequent sections.

4.3.1 As-received PVA before Sintering

Microscopic examination was carried out on the as-received powder to look at the particle morphology of **PVA** prior to laser-sintering. Figure 4-33 is the micrograph obtained for the as-received **PVA** powder stock.

Figure 4-33: Micrograph of as-received **PVA** powders

As observed from Figure 4-33, pure **PVA** are spherical in shape with a fibrous-like surface with an average particle size of about 100 μm .

4.3.2 Sintering of Pure PVA

Variation on the part bed temperature, laser scan speed and laser power was done to establish a set of favourable processing parameters to laser-sinter PVA powder. Attempts to sinter pure PVA powder at part bed temperature lower than 65°C with a default scan speed of 5080 mm/s (200 in/s) yielded specimens that exhibited limited sintering effect. The resulting specimens were too weak to be handled manually. Furthermore, it was noted that a difference in laser power of 4W and below did not give a significant variance in terms of sintering result and hence the laser power was raised to beyond 20W. However it was noted that at laser powers above 20W, the powder actually burst into flames and hence the maximum laser power used was capped at 15W. With these observations, the scan speed was lowered to 2540 mm/s (100 in/s) and the part bed temperature was set at 65°C with the laser power kept constant. Figures 4-34 and 4-35 show the sintering result of pure PVA sintered at a constant part bed temperature (65°C) and scan speed (2540 mm/s). Efforts to laser-sinter pure PVA powder at laser power lower than 13W did not yield successful specimens. The laser power was too weak to cause “necking” to occur between PVA particles. In addition, the specimens lacked solidity and cannot be held indicating that “necking” has not occurred. “Necking” between particles, a phenomenon of sintering, was noted in the specimens laser-sintered at 13W. Although there was sintering effect at 2540 mm/s (100 in/s) and 15 W, it was observed that the test specimens changed colour from white (colour of the PVA powder) to brownish indicating possible charring. However, the test specimen did not flame during the processing.

Figure 4-34: Micrograph of sintered **PVA** specimens at laser power of 13W.

Figure 4-35: Micrograph of sintered **PVA** specimens at laser power of 15W.

According to the Andrew's Number, the energy density received by the powder in a specimen is directly proportional to the laser power and inversely proportional to the scan speed [91, 92]. Therefore, to obtain the same energy density in the test specimens, the scan speed was decreased and the laser power was increased to study the sintering effect. At lower laser power, the adjacent PVA particles were not successfully sintered to yield significant necking between PVA particles. As a result delamination (edges opening up) occurs. As delamination was observed at laser power lower than 10W, the laser power was gradually increased to 15W for both scan speed of 1270 mm/s (50 ids) and 1778 mm/s (70 in/s). Promising results of structurally stable specimens was observed from 13W at 1778 mm/s (70 in/s) and 10W at 1270 mm/s (50 in/s). Figures 4-36 and 4-37 illustrate the sintering results of pure PVA specimens laser-sintered at different scan speed and laser power. No significant variances in the sintering result between the two micrographs were observed.

Figure 4-36: Micrograph of sintered PVA specimens produced at scan speed of 1778 mm/s (70 ids) with laser power of 13W.

Figure 4-37: Micrograph of sintered PVA specimens produced at scan speed of 1778 mm/s (70 in/s) with laser power of 10W.

Attempts to sinter pure PVA at 1270 mm/s (50 in/s) and 16W resulted in the flaming of the specimens suggesting that the combination of low scan speed and high laser power led to excessive energy density in the test specimens. Therefore, it is concluded that the favourable processing parameters on SLS were 13W to 15W for the laser power and 1270 mm/s (50 in/s) to 1778 mm/s (70 in/s) for the scan speed so that the sintering process was energy efficient.

The feasibility of sintering PVA powders to obtain solid circular specimens exhibited the potential of using this biomaterial in SLS for TE scaffolds, in particular for cartilages. However, as the main focus of the research was in the development of scaffolds for bones, further characterisation analysis of PVA specimens was not

carried out. Nevertheless, the successful laser-sintering of PVA displayed the novelty of processing such biomaterial. The similar tensile strength of PVA to human articular cartilage makes it an ideal processing material for such scaffolds. Its good lubrication and ability of PVA to form complex shapes would allow the scaffolds to conform to the shape of the implants required hence further enhancing its application in the TE field. In terms of structural integrity, PVA produced scaffolds lacked solidity or strength as compared to PEEK scaffolds as discussed in Section 4.2.4 and it is this nature that makes PVA scaffolds suitable for usages in cartilages which is an elastic connective tissues. Upon contact with water, PVA scaffolds will swell and become a gel which may be sufficiently flexible enough to play the role of a connective tissue. The positive sintering result obtained for PVA opens up the potential of this biomaterial for usage in soft tissues. As this research is focussed on hard tissues, further recommendations that this work should be extended would be described as future work provided in the last chapter.

4.4 Porosity Analysis for PEEK/HA Scaffolds

As a dual material system is used for the fabrication of the scaffolds, the density of the different weight composition of PEEK and HA in the blend was obtained. The average densities for 90% **wt** PEEK-10% **wt** HA, 80% **wt** PEEK-20% **wt** HA, 70% **wt** PEEK-30% **wt** HA and 60% **wt** PEEK-40% **wt** HA obtained through gas pycnometer were 1.6994 g/cm³, 1.7559 g/cm³, 1.8312 g/cm³ and 1.9241 g/cm³ respectively. As mentioned in Section 4.3.3, the favourable laser power settings to produce porous specimens were between 16W to 21W. Thus, the laser power used for the investigation of microporosity of laser-sintered PEEK/HA scaffolds was set at 18W, which is the mid point of the range of laser power, for all 10 specimens in each of the different blend used. Figure 4-38 shows the microporosity obtained for the different weight percentage of the composite blend.

Figure 4-38: Microporosity of PEEWHA scaffolds.

For 90% **wt** PEEK-10% **wt** **HA**, the average microporosity was 73.5% with a deviation of 3%. As observed from Figure 4-38, all the microporosity obtained deviated from the average obtained by about 3% except for the blend with 80% **wt** PEEK-20% **wt** **HA** which deviated by about 6%. With increasing amount of **HA** in the blend, the microporosity of the scaffolds decreased to 69.83% for 60% **wt** PEEK-40% **wt** **HA**. The microporosity, the pores between adjacent PEEK/HA particles, is caused by the “gap” between PEEK particles subjected to laser-sintering to produce a porous specimen in which **HA** particles are embedded in the PEEK polymer matrix. Therefore, with increasing amount of **HA**, the “gap” became smaller with the presence of more **HA** particles embedded in the “gap”. This is because **HA** cannot be laser-sintered during the fabrication as sintering of **HA** only occurs beyond 1000°C. Hence, the microporosity of the scaffolds decreased with increasing weight percentage of **HA** in the scaffolds. The availability of micropores would allow for vascularisation which is often a problem for TE scaffolds. In addition, as the laser power used was 18W, the microporosity could be increased if a lower laser power is used. This is because the degree of necking is more significant at a higher laser power resulting in a non-porous network and hence the microporosity is decreased. Therefore if a porous structure is desired, the laser power can be reduced to between 16W to 17W to obtain average porosity of 75% for 90% **wt** PEEK-10% **wt** **HA**.

In addition, based on the designs of the author’s research group [106], scaffolds with predetermined pore sizes were fabricated and the macroporosity, the pores created by the pre-determined architecture of the scaffolds, investigated. The aim of the

experiment is to illustrate that besides, microporosity, the macroporosity has been considered as well in the fabrication of PEEK/HA scaffolds via SLS. Using the blend comprising of 90% wt PEEK-10% wt HA, scaffolds based on the dimension specified in Section 3.4 were fabricated. The only difference was that these scaffolds have a predetermined internal architecture.

Figure 4-39 shows a three-dimensional PEEK/HA scaffold with predetermined internal architecture. As shown in the figure, SLS can produce porous three-dimensional scaffold with predetermined internal architecture and pores distribution.

Figure 4-39: Macro-view of PEEK/HA scaffolds built using SLS.

Figure 4-40 shows the micro-view of another PEEK/HA scaffold [106]. Macroporosity of the scaffold was obtained using the same method as microporosity analysis. The average porosity was about 88% with an average pore size of 700 microns. With the presence of both macro-pores for the supply of nutrients and micro-pores to aid vascularisation, these scaffolds meet the required specifications of

3D TE scaffolds. The results obtained for this set of scaffolds reinforced the viability of using PEEK/HA polymeric blend on SLS for TE scaffolds.

Figure 4-40: Micrograph of PEEK/HA Scaffold with predetermined internal architecture.

4.5 Apatite Formation

Scaffolds with different weight percentage of PEEK and **HA** were immersed in SBF for a period of **28** days. Investigating the biological behaviour of biomaterials in SBF is **an** efficient way to predict their bioactivity in body environment [96,97]. Figure 4-41 shows the micrograph of PEEK/HA scaffold before immersion in SBF. As shown in Figure 4-41, the surface morphology of HA exhibited thin irregular patterns on its surface with its peripheral not clearly defined. Figure 4-42 (a) – (d) shows the micrographs of PEEWHA scaffolds with different weight percentage of HA.

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Figure 4-41: Micrograph of PEEK/HA scaffolds before immersion in SBF.

Figure 4-42: Micrographs of PEEK/HA with (a) 10% **wt** HA; (b) 20% **wt** HA; (c) 30% **wt** HA and (d) 40% **wt** HA, after 7 days immersion in SBF.

After immersion in SBF for a period of 7 days, all PEEWHA scaffolds with different weight composition of HA exhibited morphological changes on the surface of HA. The morphological change was typical of apatite layer formation showing an apparent thickening of the apatite layer observed on all samples as noted in Figure **4-42**. This formation of apatite layer was due to the precipitation of the highly saturated calcium and phosphate ions in the SBF. The surface of the HA powders became more defined and there was the formation of random-sized spherical particles indicating the precipitation of the apatite layer, a phenomenon observed in bioactive specimens [98-100]. However, due to the short duration of immersion, it was observed that there were areas with no clear indication of apatite precipitation. The HA powders remained crystalline as observed in the XRD results though newly precipitated amorphous apatite on the surface of the HA particles was noted from the diffractogram. The thickening of the apatite layers became more prominent and defined for specimens immersed in a longer period of SBF as shown in Figures **4-43** to **4-45**. Figures **4-43** to **4-45** show the micrographs of PEEWHA scaffolds with different weight percentage of HA after immersion in SBF for **14** days, 21 days and **28** days respectively.

Figure 4-43: Micrographs of PEEK/HA with (a) 10% wt HA; (b) 20% wt HA; (c) 30% wt HA and (d) 40% wt HA, after 14 days immersion in SBF.

Figure 4-44: Micrographs of PEEK/HA with (a) 10% **wt** HA; (b) 20% **wt** HA; (c) 30% **wt** HA and (d) 40% **wt** HA, after 21 days immersion in SBF.

Figure 4-45: Micrographs of PEEK/HA with (a) 10% **wt** HA; (b) 20% **wt** HA; (c) 30% **wt** HA and (d) 40% **wt** HA, after 28 days immersion in **SBF**.

The precipitation of apatite layer was localised in the area in which **HA** was present. For porous structures the apatite layer can also be observed inside the pores that have **HA** embedded inside. After immersion in **SBF** for **14** days, the surface of the **HA** particles became rougher by deposition of random-sized spherical particles indicating the precipitation of apatite layers. This phenomenon was observed on all the **PEEK/HA** scaffolds with different weight percentage of **HA**. The newly formed apatite layer has been reported to be poorly crystallized calcium phosphate precipitates which can be clearly seen in Figure 4-44 for scaffolds immersed in **SBF** for **21** days. This observation was further supported by the XRD characterisation discussed in Section 4.6. In addition, the size of the precipitates increased as the soaking duration increased as shown in Figure 4-43 (d) and Figure 4-45 (d) showing sign of thickening of the apatite layers. Small **HA** crystals developed into a continuous **HA** layer formed by coalescence of large crystals after immersion for 28 days.

However, for **PEEK/HA** scaffolds with 10% **wt HA**, the precipitation of the apatite layer remained limited and isolated due to the relatively low amount of **HA** present in the specimens. With increasing weight percentage of **HA** in the **PEEK/HA** scaffolds, the thickening of apatite layer became more evident in the specimens. For **PEEK/HA** scaffolds with 40% **wt HA**, the precipitation of apatite became more pronounced in the specimens. Nevertheless, it remained localised only in area with the presence of **HA**.

Another indication of the precipitation of the apatite layer can be observed from the morphology of the HA particles. As shown in Figure 4-3, the as-received HA particles were spherical in shape. However, after immersion in SBF, a change in its surface morphology was noted. Figure 4-46 shows the micrograph of PEEK/HA scaffolds after immersion in SBF for 14 days. The smooth spherical morphology had changed to an uneven rough surface indicating a layer of precipitate, likely to be apatite as verified by the XRD results.

Figure 4-46: Micrograph showing apatite precipitation on PEEK/HA scaffolds after immersion in **SBF** for 14 days.

The bioactivity analysis also showed that PEEK being bioinert would not participate in this ions exchange reaction as shown in Figure 4-47. Figure 4-47 shows the micrograph of PEEK/HA scaffolds (40% **wt** HA) after immersion in SBF for 7 days. As observed from the micrograph, the interface between PEEK and HA remained unaffected by the ions exchange hence showing that only HA is bioactive and PEEK is not.

Figure 4-47: Micrographs of the interface between PEEK and **HA** showing apatite growth after immersion for **7** days.

The successful precipitation of bone-like apatite layer on the surface of the **HA** particles reiterated the feasibility of PEEWHA scaffolds in TE. The bioactivity of the scaffolds would enhance cell proliferation when osteoblast cells are seeded onto the scaffolds. In addition, the bioactivity analysis has shown the importance of exposing **HA** in the scaffolds as proposed in the earlier chapters.

4.6 Phase Analysis of PEEK/HA Scaffolds using XRD

In order to understand the phase changes of PEEK/HA scaffolds during the immersion in SBF, XRD was carried out on the specimens. Figures 4-48 and 4-49 show the diffractograms for as-received HA and PEEK respectively.

Figure 4-48: XRD for as-received pure HA

As observed from Figure 4-48, the maximum peak occurred at about 31.7° and the diffractogram is typical of the XRD pattern obtained for HA based on JCPDS. However, as XRD pattern was not available for PEEK, XRD was conducted on PEEK to establish its diffractogram.

Figure 4-49: XRD for as-received PEEK.

From Figure 4-49, it was noted the 3 most significant peaks were observed at 19.5° , 21.3° and 23.1° . These values were used as a reference point for subsequent diffractograms comprising of different weight percentage of HA to identify the change in phase for HA in the PEEWHA scaffolds during the period of immersion in SBF. Figures 4-50 to 4-53 show the XRD patterns for different weight percentage of PEEWHA scaffolds after immersion in SBF for a period of 28 days.

Figure 4-50: XRD pattern for PEEK 90% wt-HA 10%**wt** scaffolds after immersion in SBF

The crystallinity of the precipitate formed was investigated using XRD. After immersion for 7 days, the width of the peak broadened and the intensity decreased. These patterns suggested poor crystalline peak, typical in the initial stage of the apatite formation. As observed from the figure, the peak of HA becomes more pronounced and noticeable after immersion of 28 days indicating the precipitation of bone-like apatite.

Figure 4-51: XRD pattern for PEEK 80% wt-HA 20% **wt** scaffolds after immersion in SBF.

As observed in Figure 4-51, the laser-sintering of PEEWHA composite reduced the crystallinity of HA. After immersion for 7 days, the reduction in peak intensity coupled with peak broadening clearly reflects the low crystalline nature of the precipitation. The crystallinity of HA was observed to increase after 21 days as shown in Figure 4-51. After a longer period of immersion, only the diffraction peaks of the apatite were detected as noted in specimens that were immersed in SBF for 28 days.

Figure 4-52: XRD pattern for **PEEK** 70% wt-HA 30% wt scaffolds after immersion in SBF.

As noted from the 3 most significant peaks for HA at about 32.1° , 32.5° and 33.1° , there was a clear indication of phase change for the HA indicating the precipitation of the apatite layer. Before the immersion of the scaffolds in SBF, the scaffolds exhibited crystalline nature which after immersion for 7 days, the intensity reduced indicating the low crystalline nature of the precipitation. This low crystalline nature was further exemplified by the broadening of the peak as observed in specimens that have been immersed for 14 days. After a longer period of immersion, the intensity of the peak increased indicating the change from low crystalline to crystalline structure as shown from Day 21 to Day 28. Similar observations were noted for **PEEK/HA**

scaffolds with 40% wt HA as shown in Figure 4-53. It was noted that a more accentuated decrease in the intensity of the polymer with immersion time was observed indicating that a thicker layer of apatite was growing. Furthermore, the broadening and decreasing intensity corresponding to the low crystalline nature of the precipitation was observed for the first 14 days of immersion. As compared to Figure 4-50, it was observed that the peak for PEEK was more significant in scaffolds with 10% wt HA than for scaffolds with 40% wt HA, hence illustrating the effect of varying the weight percentage of HA in the composite for bioactivity analysis.

Figure 4-53: XRD pattern for PEEK 60% wt-HA 40% wt scaffolds after immersion in SBF.

However, the bioactivity analysis result obtained for varying the different weight percentage of HA in PEEWHA scaffolds was not definitive and further recommendation was proposed in the last chapter.

A few conclusions can be drawn from the series of XRD analysis. Firstly, it can be concluded that for specimens with a higher percentage of the polymer, PEEK, the intensity was more prominent as indicated in Figure 4-50 and less prominent in specimens with lower percentage of PEEK as shown in Figure 4-53. The maximum peak in all the diffraction patterns for 10% wt HA scaffolds was due to PEEK as shown in Figure 4-50 whereas the maximum peak in all diffraction patterns for 40% wt HA scaffolds was due to HA as shown in Figure 4-53. These observations clearly indicated that there was an increase in the amount of HA in the different weight composition of the specimens. The set of XRD diffraction patterns clearly indicated a series of phase changes in the specimens immersed in SBF which was a typical phenomenon for such analysis. That is to say, the processing technique via SLS had not hindered apatite precipitation and HA had not degraded during the sintering process. A decrease in peak intensity was noted in all different weight composition of PEEK/HA scaffolds after immersion for 7 days showing the precipitation of amorphous HA which resembled bone-like apatite. This low crystalline region continued to precipitate and a change in phase was noted. The transitional period occurred after 14 days as shown in Figures 4-50 to 4-53 and crystalline phase was noted in all specimens after immersion for 28 days. The series of bioactivity analysis has clearly indicated that regardless of the weight percentage of HA in the scaffolds,

crystalline HA was precipitated in all specimens. This observation is critical because it indicated the formation of bone-like apatite which is crucial for bone regeneration.

All the PEEWHA scaffolds fabricated in this research exhibited bioactivity as verified by the SEM and XRD results and hence this further iterated the feasibility of fabricating porous structure that exhibited bioactivity.

4.7 Biocompatibility Test by Cell Culture

As the polymer blends will eventually be used for load bearing applications such as bones, fibroblast cell lines were used for this research. Furthermore, fibroblasts are one of the easiest cells to grow and their durability makes them amenable to a wide variety of manipulations. In the cell culture analysis, a few of the important aspects of biocompatibility are being looked into, namely cell adhesion and cell proliferation. If the material is biocompatible, it should not cause toxic effect on the cells and the cells should adhere to the base material and proliferate. Healthy fibroblasts cells should exhibit characteristic spindle morphology that is elongated in shape with fibrous like structure whereas for cells that are depleted of their nutrients, it would be spherical in shape and are clustered together.

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The specimens in the cell culture tray were removed 24 hours after the seeding of cells and placed under a microscope for observation to ascertain its biocompatibility. For the control set up, no specimens were placed into the wells and the cells multiplying are shown in Figure 4-54. As shown in Figure 4-54, healthy fibroblast cells can be easily differentiated and are not shrivelled. Though all the cells are clustered together, individual cells can be differentiated. The control set up in the cell culture is to enable the differentiation between healthy and unhealthy cells. This is to ensure that variants in the cell culture are reduced to only one which in this case, is the specimen itself. Furthermore, the control would allow for the morphology of the cells to be compared to other wells with the specimens placed in it.

Figure 4-54: Control set up with no specimens

Different specimens produced from different composition of PEEK/HA as shown in Table 4-2 were viewed under the microscope. Figures 4-55 to 4-59 show the images taken from the microscope of the various specimens seeded with fibroblast. As

observed from Figures 4-55 to 4-59, all specimens showed positive cell adhesion. The *in vitro* evaluation of toxicological risks by means of cell culture on the test specimens has demonstrated the potential of the polymer blend comprising of PEEK/HA to be used in TE scaffolds.

Figure 4-55: Cell adhesion at the edge of pure PEEK specimen

As shown in Figure 4-55, cell adhesion can be observed near the edge of the pure PEEK specimen that is indicated by the red circle. Varying degrees of cell adhesion were observed on different specimens with different weight composition of HA as shown in Figures 4-56 to 4-59 (indicated by the red circle).

Figure 4-56: Specimen with 10% wt. HA

Figure 4-57: Specimen with 20% wt. HA

Figure 4-58: Specimen with 30% **wt.** HA

Figure 4-59: Specimen with 40% **wt.** HA

Although the cells were observed to attach and grow on the test specimens and the qualitative analysis of the cell culture showed the biocompatibility of the polymer blend, the results obtained cannot fully establish the full viability of the blend. As the cell culture analysis can only show that fibroblast cells can proliferate in the cultured conditions on PEEK/HA scaffolds but it will not participate in the interaction with

HA and hence the role of HA as a major component of in vivo component is not investigated. Since the initial proposition for PEEK/HA scaffolds is to be used as bone scaffolds, there is a need to look into osteoblast cells. However due to inavailability of osteoblast cells at the experimentation, this set of cell culture experiments is not carried out. Further recommendations that this work should be extended would be provided in future work would be provided in the last chapter. Nevertheless, the results obtained from the cell culture have established the biocompatibility of the polymer blend as it did not cause any toxic effects at the cellular level and did not prohibit cell proliferation.

Chapter Five:

Conclusion and Future Work

In This Chapter

- 5.1 Conclusion
- 5.2 Publications
- 5.3 Future Work

Chapter Five Conclusion and Further Work

5.1 Conclusion

The project explored the potential of using RP technology to replace the conventional techniques for fabricating TE scaffolds which are not versatile to meet the various aspects required for TE scaffolds. RP technology, in particular **SLS**, was investigated upon to bridge the gap in conventional techniques.

The advantages of using **SLS** for TE scaffolds lie in the ability to control pore structure for biogenesis through the control of polymer content and the ability to construct complex three-dimensional structure for TE applications. Due to its ability to process various materials, the potential of using **SLS** is further enhanced as unconventional materials such as PEEK and PVA, which were used in this research, were tested upon to explore its usage for TE scaffolds. Three main parameters of **SLS**, namely the part bed temperature, laser power and scan speed were varied to study its effect on the integrity of the test specimens fabricated for the purpose of biocompatibility of the materials used. With the scan speed kept constant and varying part bed temperature and laser power, various circular specimens measuring 16 mm in diameter and 0.508 mm thick were fabricated. The results obtained showed that a low part bed temperature should be complemented by a higher laser power. The research has also shown the promising result of being able to laser sinter a high melting point polymer in a much lower temperature environment. Furthermore, the ability to incorporate different amounts of bioactive material like HA, into the polymer blend

comprising of PEEK and HA reiterated its usage for TE scaffolds, especially bone scaffolds, as apatite is one of the naturally occurring components in human bones.

Characterisation of the PEEK-HA scaffolds such as microscopic analysis, porosity analysis, bioactivity and cell viability analysis yielded positive results. Microscopic observations on the PEEK-HA scaffolds showed pore interconnectivity within the scaffold hence reiterating the potential of SLS on building TE scaffolds. Furthermore, both micro-porosity, pores between adjacent PEEK-HA particles, and macro-porosity, pores created by the pre-determined architecture of the scaffolds, of the scaffolds showed that highly porous PEEK-HA scaffolds can be obtained. The immersion of PEEK-HA scaffolds in **SBF** demonstrated the bioactivity of the specimens with the precipitation of bone-like apatite as supported by XRD and SEM results. Test specimens were successfully fabricated in which cell seeding using fibroblast cell lines were used to assess cytotoxicity. Qualitative analysis of the cell culture **was** carried out in which results obtained demonstrated positive cell adhesion and growth.

5.2 Publications

At the time of submission of this report, three papers have been published [107-109] from this research project. The first, an international journal paper entitled “*Scaffold Development using Selective Laser Sintering of Polyetheretherketone-hydroxyapatite Biocomposite Blends*” has been published in **Biomaterials**, Volume 24, Issue 18 pp. 3115-3123. This paper has also been awarded the **Andrew Fraser Prize** given out by Institution of Mechanical Engineers, United Kingdom, for recognition as the best postgraduate project in the field of mechanical engineering. One conference paper related to this research work has also been presented. The paper entitled “*Selective Laser Sintering of Biocompatible Polymers for Applications in Tissue Engineering*” presented at the Second International Conference on New Biomedical Materials, 05 to 08 April 2003, Cardiff UK. This paper has also been selected by the organising committee of the conference for publication in **Bio-medical Materials and Engineering**.

The third journal paper entitled “Development of Tissue Scaffolds using Selective Laser Sintering of Polyvinyl Alcohol/hydroxyapatite Biocomposite for Craniofacial and Joint Defects” based on the laser sintering of PVA has been accepted for publication in **Journal of Materials Science: Materials in Medicine**.

5.3 Future Work

The research work conducted in the past two years had ascertained the possibility of employing the dual material systems comprising of polymer blend of PEEK and HA, and PVA for processing in **SLS** to produce TE scaffolds. However, there are still certain areas in this research that can be improved upon. The following studies are suggested:

1. Based on the parametric scaffolds library developed by the author's research group, scaffolds with complex **3D** structures using PEEK-HA blend can be fabricated in which various characterisation such as mechanical strength and porosity can be investigated. Similarly, the same set of designs can be used on PVA to assess its tensile strength and porosity. As PVA has the potential of being used as the replacement of cartilages, the tensile strength of the scaffolds need to be examined. Furthermore, the dissolution behaviour of PVA has to be studied as well because being a soluble polymer, the dissolution rate would affect the structural and mechanical strength of the scaffolds.
2. Positive result in cell culture has shown the potential of PEEK for use in TE applications. However, as only fibroblast cells were used in the analysis, it is proposed that osteoblast cells should be used for further cell culture. Since HA is a major component of the in vivo surface in which osteoblast cells interact, the usage of fibroblast cells would only verify biocompatibility. As PEEK-HA scaffolds are meant for usage as bone scaffolds, the effect of variance in the weight percentage of HA in the scaffolds would give a better understanding on

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the in vitro behaviour of the intended scaffolds when seeded with osteoblast cells.

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APPENDIX A

10W @ 200 times

10W @ 500 times

Appendix A-1 : Micrographs of Pure PEEK sintered at 110°C

12W @ 200 times

12W @ 500 times

Appendix A-1: Micrographs of Pure PEEK sintered at 110°C

14W @ 200 times

14W @ 500 times

Appendix A- 1: Micrographs of Pure PEEK sintered at 110°C

16W @ 200 times

16W @ 500 times

Appendix A-1: Micrographs of Pure PEEK sintered at 110°C

9W @ 200 times

9W @ 500 times

Appendix A-3: Micrographs of Pure PEEK sintered at 140°C

9W @ 200 times

10W @ 500 times

Appendix A-3: Micrographs of Pure PEEK sintered at 140°C

12W @ 200 times

12W @ 500 times

Appendix A-3: Micrographs of Pure PEEK sintered at 140°C

14W @ 200 times

14W @ 500 times

Appendix A-3: Micrographs of Pure PEEK sintered at 140°C

16W @ 200 times

16W @ 500 times

Appendix A-3: Micrographs of Pure PEEK sintered at 140°C

APPENDIX B

Appendix B-1: Micrographs of 90 % **wt** PEEK and 10 % **wt**

Appendix B-1 : Micrographs of 90 % **wt** PEEK and 10 % **wt**

Appendix B-2: Micrographs of 20 % **wt** HA and 80 % **wt** PEEK

Appendix B-2: Micrographs of 20 % wt HA and 80 % wt PEEK

Appendix B-3: Micrographs of 70 % wt PEEK and 30 % wt

Appendix B-3: Micrographs of 70 % wt PEEK and 30 % wt