Abstrak

Eksperimen ini mengkaji sifat-sifat scaffold komposit yang diperbuat daripada campuran polimer dan hydroxyapatite (HA) yang dihasilkan melalui kaedah electrospinning. Degradasi secara in vitro telah dijalankan dengan merendam scaffold tersebut di dalam phosphate buffered saline (PBS) (pH 7.4 \pm 0.1) pada suhu $37 \pm 1^{\circ}$ C selama 8 minggu dimana cecair tersebut ditukar setiap 4 minggu. Tahap degradasi diukur melalui kehilangan jisim, perubahan pada permukaan scaffold dan juga perubahan pada spectrum FTIR. Keputusan eksperimen menunjukkan bahawa scaffold ini diperbuat daripada kombinasi gentian berdiameter 0.5 - 2.5 µm. Keadaan permukaan scaffold selepas degradasi selama 8 minggu tidak menunjukkan sebarang perubahan apabila dibandingkan dengan keadaan permukaan scaffold sebelum proses degradasi dan fenomena yang sama dapat diperhatikan pada scaffold lain. Selain daripada keadaan permukaan scaffold, kesan degradasi juga diukur melalui peratusan kehilangan jisim. Keputusan eksperimen menunjukkan peratusan kehilangan jisim adalah negatif (dalam kata lainnya, jisim scaffold bertambah selepas proses degradasi selama 8 minggu). Analisis menggunakan FTIR menunjukkan pertambahan jisim scaffold adalah disebabkan oleh penyerapan air oleh HA (yang terdapat di dalam scaffold) yang mempunyai sifat hidrofilik. Sementara itu, analisis liang (pore analysis) menggunakan perisian ImageJ menunjukkan bahawa peratus keliangan (percentage of porosity) adalah di antara 27.5 - 35.5%. Keputusan modulus keanjalan dan kekuatan tegangan muktamad pula menunjukkan keputusan yang tidak selari dengan pertambahan peratusan HA di dalam scaffold. Tiada perubahan juga dapat dikesan pada spektra FTIR selepas proses degradasi. Kesimpulannya, scaffold komposit ini masih dapat mengekalkan struktur yang agak stabil walaupun

selepas direndam selama 8 minggu di dalam cecair yang mempunyai komponen yang sama seperti cecair di dalam badan manusia.

iii

University of Malays

Abstract

The characteristic and hydrolytic degradation of polycaprolactone-hydroxyapatite composite scaffolds prepared by electrospinning method were studied. In vitro degradation was carried out in phosphate buffered saline (PBS) (pH 7.4 \pm 0.1) at $37 \pm 1^{\circ}$ C for 8 weeks. The extent of degradation was measured by physical mass loss, FESEM observations and FTIR. The results show that the scaffolds were made from a combination of fibers with diameter ranging from $0.5 - 2.5 \mu m$. The surface morphology of scaffolds after 8 weeks of degradation in PBS showed no change (when compared to the ones before the degradation) and this phenomenon was observed in all scaffold compositions. The effect of degradation was also observed by measuring the percentage of mass loss. Almost all the scaffolds showed no mass loss due to water uptake by HA particles (proven from FTIR analyses). Porosity analysis using ImageJ (NIH, USA) showed that the percentage of porosity of the scaffold is between 27.5%-35.5%. Young's modulus and ultimate tensile stress (UTS) of the scaffolds showed inconsistent results as the percentage of HA increase. FTIR spectra after degradation showed no difference with the ones before the degradation. Overall, it can be concluded that PCL/HA composite scaffolds still retain a relatively stable architecture after 8 weeks of degradation in PBS.

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Table of Contents

1.	Introduction	1
	1.1. Objectives	5
	1.2. Scope of study	5
2.	Literature review	6
3.	Materials and methods	
	3.1. Preparation of electrospun PCL and PCL/HA composite scaffolds	22
	3.2. Degradation set up	22
	3.3. Characterization methods	
	3.3.1. FESEM	23
	3.3.2. FTIR	24
	3.3.3. XRD	25
	3.3.4. Tensile testing	25
	3.4. Statistical analysis	26
4.	Results and discussion	27
5.	Conclusion	41
6.	References	43
7.	Appendix	48

Page

List of Figures

Figure 2.1	SEM micrographs of Composite–MC mats containing 30% HA– TCP: (a) at low magnification; (b) at high magnification; (c) displaying sub-micron web network between large micron-sized fibers; (d) 50% HA–TCP; (e and g) 30% HA–TCP cross section with corresponding (f and h) SEM-EDXA-mapping for calcium.	7
Figure 2.2	Field emission scanning electron microscopy (FESEM) images for the PCL nanofibers containing different amounts of hydroxyapatite, (A) 0 %, (B) 3 %, (C) 5 %, and (D) 7 % hydroxyapatite with respect to polymer solution.	8
Figure 2.3	XRD results for the standard (the vertical base lines) and prepared hydroxyapatite (calcined). Additionally, the spectra of pristine PCL and the prepared hydroxyapatite/PCL nanofiber mat with different concentrations of hydroxyapatite.	9
Figure 2.4	XRD spectra of HA powder, β-TCP powder, 30% Composite– MC + DMF, 30% Composite–MC and PCL electrospun mat.	10
Figure 2.5	The FTIR spectra of HA powder, β-TCP powder, 30% Composite–MC and 30% Composite–MC + DMF and unfilled PCL scaffolds.	11
Figure 2.6	FT-IR spectra of calcined bones (A); prestine PCL nanofiber (B); PCL nanofiber containing 3% HAp; (C) PCL nanofiber containing 5 % HAp; and (D) PCL nanofiber containing7 % HAp; (E).	12
Figure 2.7	Mechanical properties of pure polymeric and composite nanofibrous scaffolds.	13
Figure 2.8	Stress-strain curve of electrospun composites.	15
Figure 2.9	Mechanical characterization of the micro- and nanofibrous electrospun PCL scaffolds. (A) Representative stress-strain curves, (B) Ultimate stress measurements, (C) Young's Modulus, (D) Ultimate strain measurements.	16
Figure 2.10	Mean and root mean square errors for the: (A) fiber diameter [x] measurements, (B) porosity [e], (C, D) pore size [2] estimates for micro- (C) and nanofibers (D). Statistically significant differences at $p < 0.05$, *M-HD vs. N-HD, **M-HD vs. M-LD, pM -LD vs. N-LD, ppN -HD vs. N-LD (Student's t test).	19
Figure 2.11	FE-SEM images for the PCL nanofibers containing different amounts of hydroxyapatite; (A) 0 %, (B) 3 %, (C) 5 %, and (D) 7 % with respect to polymer solution after incubation in SBF at 37°C for 10 days.	21
Figure 3.1	The front view of scaffold mounted on Instron mechanical tester.	26
Figure 4.1	Photograph of the scaffold	27
Figure 4.2	Graph of fiber diameter versus types of scaffolds. $*$ (p < 0.05) indicates composite scaffold's fiber diameter is significantly	28

different from PCL scaffold.

- Figure 4.3 HA particles tend to agglomerate as its concentration increase. HA is not evenly distributed throughout the scaffold due to large particle size used (20-45 micrometer). Scale bar: 100μm. Magnification: x800
- Figure 4.4 Graph of percentage of porosity vs. types of scaffolds. Data is represented as mean $(\pm \text{ std dev})$ (n = 5). Error bar represents standard deviation.
- Figure 4.5Graph of frequency distribution of fiber diameter for (A) PCL (B)33PCL/HA 10% (C) PCL/HA 20% (D) PCL/HA 30% (E) PCL/HA40% (F) PCL/HA 50% scaffold. 74% of PCL fibers are in the
micrometer range while only 30-50 % of composite fibers are in
the micrometer range.(Note: Fibers are considered to be in
micrometer range when the fiber diameter is > 1000nm)33
- Figure 4.6 Graphical illustration of the mass and molecular weight loss over time for a resorbable polymer such as PCL. Initial hydration (0–6 months), through degradation and mass loss (6-12 months), resorption (post 12 months) and metabolisation (post 18 months).
- Figure 4.7 FTIR spectra of PCL and PCL/HA composite scaffolds.
- Figure 4.8 XRD spectra of PCL and PCL/HA composite scaffolds. The diffraction peaks for PCL occur at $2\theta = 20^{\circ} 25^{\circ}$ while diffraction peaks for HA occur at $2\theta = 30^{\circ} 40^{\circ}$. Graph of fiber diameter (μ m) vs. types of scaffolds. The diffraction peaks of PCL/ HA composite scaffolds contains a combination of both.
- 36

26

30

37

List of Tables

from PCL scaffold.

Table 2.1	Pore size, porosity and tensile properties of electrospun mats.	14
Table 4.1	Pore size/diameter of PCL and composite scaffolds	29
Table 4.2	Young's modulus (YM) and ultimate tensile strength (UTS) of the scaffolds. Data is represented as mean (\pm standard deviation). *(p < 0.05) indicates YM of the composite scaffold is significantly different	31

ix

List of Symbols and Abbreviations

DCM	Dichloromethane
FESEM	Field Emission Scanning Electron Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
НА	Hydroxyapatite/ Hydroxylapatite
НСА	Hydroxycarbonate apatite
In vitro	Experimentation using components of an organism
In vivo	Experimentation using a whole living organism
NIH	National Institutes of Health
PBS	Phosphate Buffered Saline
SBF	Simulated Body Fluid
ТСР	Tricalcium phosphate
UTS	Ultimate tensile stress
XRD	X-ray Diffractometer

List of Appendices

FESEM micrographs

FTIR spectra

university

Chapter 1. Introduction

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to develop biological substitutes that restore, maintain or improve tissue function. This concept involves 3 main strategies: the use of isolated cells or cell substitutes to replace limited functions of the tissue; utilization of tissue inducing substances such as growth factors, and scaffolds to direct tissue development.

Tissue engineering scaffold is an artificial structure that is capable of supporting three dimensional (3D) tissue formation. In bone tissue engineering, the scaffold is usually made from ceramic or polymers. Ceramic is chosen due to its ability to stimulate biochemical response from living tissues (Guarino *et al.*, 2009) while polymer is chosen because of its degradation property.

Polycaprolactone (PCL) is an example of a synthetic polymer. It is biocompatible (Jerome *et al.*, 2008, Vert *et al.*, 1992, & Sun *et al.*, 2006) and can be hydrolytically degraded into non-toxic products, making it a suitable candidate for bone tissue regeneration (Albertsson *et al.*, 2002, Smith *et al.*, 2005 & Rabetafika *et al.*, 2006). PCL degradation kinetics is slower compared to the other polyesters due to its strong hydrophobic nature (Ang *et al.*, 2007). Its hydrophobicity enables it to provide mechanical support during the period required by the bone to heal.

Hydroxyapatite (HA) with chemical formula $Ca_{10}(PO_4)_6(OH)_2$ is a calcium phosphate ceramic that is chemically similar to the mineral components and hard tissues in mammals. The calcium to phosphate ratio (Ca/P) of HA is similar to the (Ca/P) of bone which is 1.67. Due to this factor, this bioactive material is able to support bone ingrowth and osseointegration when used in orthopedic, dental and maxillofacial application. Apart from that, it is also known for being able to form strong bond between the scaffold and the adjacent tissue (Guarino *et al.*, 2009).

As mentioned earlier, bone tissue engineering scaffold can be made from either ceramic or polymer. However, ceramic or polymer alone as a scaffold is not enough since each of these materials has its own limitations. To overcome the limitations, these materials are usually combined together through various processes. PCL/HA is one of the examples of a composite scaffold. HA is added to improve osteoconductivity of the composite while PCL is added to overcome the brittleness of HA. Combination of HA and PCL can increase the mechanical toughness and resistance to brittle failure of HA while guiding the reparative growth of the natural bone.

Conventional scaffold fabrication techniques such as solvent-casting particulateleaching, gas foaming, fiber meshes/ fiber bonding, phase separation, melt moulding and etc. present inherent limitations that compromise the activity of cells on the scaffold. Due to problems with conventional method, many researchers have explored new method of preparing scaffold such as solid freefrom fabrication, hierarchical scaffold fabrication, electrospinning and many more.

Electrospinning is a method used to produce nanofibers. The way it works is that the material (polymer) is melted and electrically charged by a high voltage source. This electrically charged polymer solution is then ejected out of a needle upon which it evaporates/solidifies before hitting a collection screen (Huang, 2003). Scaffolds produced using this method usually have high surface to volume ratio and interconnecting pores which facilitate cell adhesion and formation of cell-cell junctions (Phipps *et al.*, 2011). Not only that, the cost of fabricating scaffold with nano-scale resolution fibers by electrospinning is relatively cheap and fast compared to other methods. Another advantage of this method is the porosity of the scaffold can be controlled by modulating the fiber diameter and packing density of the fibers (Soliman *et al.*, 2010).

A good electrospun scaffold should have porosity between 70-90% to allow 3D cell infiltration besides effective diffusion of nutrients and oxygen. The pore diameter must be bigger than the size of the cells that will be deposited onto it and the pores must be interconnected to promote migration and infiltration of cells into scaffold. Apart from that, the scaffold also should have surface morphology that allows cell adhesion and must be able to degrade and resorb at a controlled rate at the same time as specific tissue cells seeded into the 3D construct attach, spread and increase in quantity (no of cells/void volume) and quality. The rate of scaffold degradation must be equal to the rate of tissue formation because if the scaffolds were to last for a long time compared to the time required for tissue growth, it may be detrimental or a hindrance to the tissue engineering endeavor.

According to (Lyman, 2002), bulk and surface properties of a biomaterial must be equally characterized since this provides fundamental information on how the performance of the material will be. Initial tests such as determination of chemical composition, tensile strength, elastic modulus, surface roughness, hardness and IR transmission spectrum must be carried out to determine the characteristic of the material before it can be used as implant material.

Another characterization which is just as important the initial tests mentioned previously is the in vitro biodegradation. This characterization is equally important especially for 3D scaffold material as the material has to degrade slowly and release non-toxic by-products upon formation of a tissue. In vitro biodegradation can reduce many problems associated with tissue replacement such as rejection and chronic inflammation and although conclusion derived from in vitro assays are not extrapolable to in vivo conditions, they do provide valuable information on the potential in vivo behavior of the material.

In vitro degradation studies usually use buffer solution such as simulated body fluid (SBF) or phosphate buffered saline (PBS) to maintain stable pH. The scaffolds remain in the solution for certain number of days or weeks at 37°C and various parameters such as weight loss, polymer molecular weight and mechanical properties are monitored. There are a lot of factors that influence the degradation profile of the scaffold namely properties of the polymer, presence of filler, polymer crystallinity, polymer molecular weight, morphology and porosity of the scaffold, pH of the degradation medium and etc. The degradation process can be controlled by optimizing scaffold material, design and dimensions. Although in vitro conditions are meant to imitate the in vivo environment, there are still many differences between the two. Nevertheless, degradation study is just as important as it gives preliminary insight on the characteristics of scaffold when immersed in media that mimic the human blood plasma in vitro.

The characteristics of PCL and PCL/HA composite scaffolds can be described by conducting various tests using different techniques and equipments. Field emission scanning electron microscopy (FESEM) is conducted to identify the surface properties or surface morphology of the composite scaffold. FESEM images are used to determine the pore size, pore shape, fiber diameter and the porosity of the scaffold. Fourier Transform Infrared Spectroscopy (FTIR), microtensile test as well as hydrolytic degradation tests are carried out to determine the intrinsic properties of PCL and PCL/HA scaffolds.

1.1. Objectives

- a) To characterize of electrospun Polycaprolactone/Hydroxyapatite (PCL/HA) composite scaffolds
- Electrospun polycaprolactone/ hydroxyapatite (PCL/HA) composite scaffolds of various composition will be characterized to study the physical, chemical and mechanical properties. XRD, FTIR, SEM and tensile test will be used in this work.
- b) To identify the effect of in-vitro degradation on the structure and properties of electrospun Polycaprolactone/ Hydroxyapatite (PCL/HA) composite scaffolds.
- The in vitro degradation behavior of electrospun polycaprolactone/ hydroxyapatite (PCL/HA) composite scaffolds of various composition will be studied using a hydrolytic degradation method. The structural properties of these scaffolds before and after degradation will be characterized to observe the effect of the degradation mechanism on these properties.

1.2. Scope of study

This study is mainly about determining the characteristics of PCL and PCL/HA scaffolds. XRD, FTIR, SEM, tensile test and degradation test were carried out to identify the physical (mechanical) and chemical properties of the scaffold. The results were used to justify the feasibility of the scaffold to be used in human to repair bone defects caused by various factors. No cells were introduced to the scaffold to expedite tissue ingrowth or regeneration.

Chapter 2. Literature review

Electrospun scaffold consists of a self-entangled polymer fiber randomly overlapping segments of itself (Chakradeo *et al.*, 2011). Since the scaffold thickness is built up slowly by sequential deposition, the fiber orientation is typically viewed as a quasiplanar phenomenon. Figure 2.1 shows the SEM micrographs of PCL/HA-TCP composite scaffold produced by electrospinning method. It is observed that the fibers of these mats were irregular in geometry and had a rough surface which indicates presence of ceramic granules at the surface. The surface morphology of the scaffold became rougher as the percentage of HA-TCP increased and this can be seen in Figure 2.1 (d). According to Patlolla *et al.*, higher concentrations of HA/TCP were not attempted because the scaffold will become too weak and harder to handle (Patlolla *et al.*, 2009).



Figure 2.1: SEM micrographs of Composite–MC mats containing 30% HA– TCP: (a) at low magnification; (b) at high magnification; (c) displaying submicron web network between large micron-sized fibers; (d) 50% HA–TCP; (e and g) 30% HA–TCP cross section with corresponding (f and h) SEM-EDXAmapping for calcium (Reproduced from Patlolla *et al.*, 2009)

A study by Kanjwal *et al.* showed that pure PCL nanofibers produced by electrospinning had smooth morphology and uniform diameter as well as bead-free appearance (Figure 2.2 A) (Kanjwal *et al.*, 2010). Other scaffolds which were

produced from the same technique but with different percentage of HA also had not been affected much by the addition of HA. Figure 2.2 D shows that HA particles that had smaller diameter than the fibers were encapsuled inside the fibers while HA particles with larger diameter attached to the nanofiber mats.



Figure 2.2 Field emission scanning electron microscopy (FESEM) images for the PCL <u>nanofibers</u> containing different amounts of hydroxyapatite, (A) 0 %, (B) 3 %, (C) 5 %, and (D) 7 % hydroxyapatite with respect to polymer solution (Reproduced from Kanjwal *et al.*, 2010)

Phase analysis by X-ray diffraction is conducted to determine phase composition and crystallinity of the composite scaffold. The XRD pattern of pure HA, pure PCL and PCL/HA composites are shown in Figure 2.3. Strong diffraction peaks for HA can be seen at $2\theta = 25.5^{\circ}$, 21.02° , 31.77° , 32.90° , 34.08° , 40.45° , 46.71° , 48.62° , 49.46° , 51.28° , 52.10° and 53.14° . According to Kanjwal and co-workers, both PCL and HA spectra did not show any additional impurities peaks which indicate that the component of the scaffold has high crystallinity and high purity respectively (Kanjwal *et al.*, 2010). The PCL/HA composite spectra shows combination of spectrum from HA and pure PCL and the HA intensity increased as the concentration of HA in the scaffold increase.



Figure 2.3: XRD results for the standard (the vertical base lines) and prepared hydroxyapatite (calcined). Additionally, the spectra of pristine PCL and the prepared hydroxyapatite/PCL nanofiber mat with different concentrations of hydroxyapatite (Reproduced form Kanjwal *et al.*, 2010).

The same phenomenon was also found in the study conducted by (Patlolla *et al.*, 2009). The XRD data also confirmed the presence of HA at $2\theta = 25.9^{\circ}$, 31.8° , 32.9° and 34.1° . The peaks corresponding to PCL and β -TCP were clearly seen in the spectra while peaks corresponding to HA were diminished due to small percentage of the particles in the composites (Figure 2.4).



Figure 2.4: XRD spectra of HA powder, β-TCP powder, 30% Composite – MC + DMF, 30% Composite–MC and PCL electrospun mat (Reproduced form Patlolla *et al.*, 2009).

FTIR analysis is mainly conducted to identify functional groups of HA and to determine the bonds between the HA phase and PCL phase. Patlolla and coworkers found out that there were two bands corresponding to HA which are OH⁻ and PO₄³⁻(Patlolla *et al.*, 2009). Broad peak corresponding to absorbed water were found in HA and β -TCP at 3300cm⁻¹ (Figure 2.5). Missing peak at 1023 cm⁻¹ corresponding to β -TCP and shifted PCL peak (from 1107 cm⁻¹ to 1112cm⁻¹) was deduced to be caused by reaction between PCL and HA.



Figure 2.5: The FTIR spectra of HA powder, β - TCP powder, 30% Composite -MC and 30% Composite – MC + DMF and unfilled PCL scaffolds (Reproduced from Patlolla *et al.*, 2009)



Figure 2.6: FT-IR spectra of calcined bones (A); prestine PCL nanofiber (B); PCL nanofiber containing 3% HAp; (C) PCL nanofiber containing 5 % HAp; and (D) PCL nanofiber containing7 % HAp; (E) (Reproduced from Kanjwal *et al.*, 2010).

A study by Kanjwal *et al.* also shows presence of phosphate and hydroxyl groups in the spectra. PO_4^{3-} is characterized by strong and complex band in the 1732-1037 cm⁻¹ (Figure 2.6) range and a medium intensity band at about 961 cm⁻¹ (Kanjwal *et al.*, 2010). Crystalline HA particles also generate characteristic OH⁻ bands at about 3446 cm⁻¹ and this phenomenon is noted in all the nanofiber combinations containing HA particles. There were also small peaks at 1700-1450 cm⁻¹ which indicates the existence of a Ca-O phase in the structure. A medium sharp peak at 628-635 cm⁻¹, assigned to the O-H bending deformation mode was found to increase in intensity as the amount of HA particles used in the scaffold increase.

A strong peak representing C=O carbonyl ester stretching of PCL occur at 1720 cm^{-1} while CH₂ bending of caprolactone chains occur at 720 cm⁻¹. There was also a

new absorption band at 1639 cm⁻¹ observed in all combination of nanofibers which accounts for asymmetric stretching vibrations of carbonyl group of caprolactone in the PCL side chains.

The data obtained from tensile test such as ultimate tensile stress, elastic modulus as well as elongation at breaking point is usually used to determine the strength of the electrospun scaffold. Polini *et al.* found that presence of 2-3.5% of HA and 2-3.5% of tricalcium phosphate (TCP) nanoparticles embedded inside PCL matrix cause decrease in UTS, elastic modulus (also known as Young's modulus) and elongation at breaking point (Polini *et al.*, 2011). Figure 2.7 shows the mechanical properties of pure polymeric and composite nanofibrous scaffolds. A tensile test conducted by Venugopal *et al.* also showed that the ultimate tensile strength of composite scaffold was 1.07MPa – a value less than the tensile strength of PCL meshes which is 3.37 MPa, deduced to be caused by addition of HA which is known to be brittle in nature (Venugopal *et al.*, 2008).



Figure 2.7: Mechanical properties of pure polymeric and composite nanofibrous scaffolds (Reproduced from Polini *et al.*, 2011)

Contrary to the finding by Polini *et al*, the tensile modulus of PCL/HA-TCP 30% scaffold was almost the same as the modulus of PCL mesh (Table 2.1) in a study conducted by (Patlolla *et al.*, 2009). This shows that additional HA-TCP has no effect on the Young's modulus of the scaffold. In spite of that, the ultimate tensile stress results of the composite scaffold decrease when HA-TCP was added into the scaffold (Figure 2.8) and this result is in agreement with previous findings by (Polini *et al.*, 2011).

	Only PCL	30% HA–TCP (Composite – MC)	30% HA-TCP (Composite- MC + DMF)
Porosity (%)	65 ± 6	76 ± 4	68 ± 4
Average pore size (µm)	2.4 ± 1.7	79.6 ± 67	7.0 ± 4.2
Pore size range (µm)	0.6 to 5.3	7.6 to 210.3	1.6 to 14.2
Modulus (MPa)	21.4 ± 1.4	24.4 ± 1.8	21.5 ± 1.2
Maximum tensile stress (MPa)	3.2 ± 0.9	1.9 ± 0.4	3.7 ± 1.0
Tensile strain at maximum tensile stress (%)	190 ± 18	29 ± 8	85 ± 15
Yield stress (MPa)	1.7 ± 0.3	1.6 ± 0.2	1.9 ± 0.4

Table 2.1: Pore size, porosity and tensile properties of electrospun mats (Reproduced from Patlolla *et al.*, 2009).



Figure 2.8: Stress-strain curve of electrospun composites (Reproduced from Patlolla *et al.*, 2009).

Another factor that may affect the tensile strength of electrospun scaffold is fiber diameter and fiber packing density. A study by Soliman and co-workers on PCL electrospun scaffold showed that scaffolds made of densely packed fibers and larger fiber diameter were more mechanically robust compared to their low density and smaller diameter counterparts (Soliman *et al.*, 2011). Figure 2.9 shows the UTS of micrometer-scale-densely-packed fibers is higher than the nanometerscale-less-packed fibers. The stiffness, as measured by the Young's Modulus on the other hand, decreased in the following order M-HD, M-LD, N-HD, and N-LD. (Note: M = micrometer, N = nanometer, HD = high density, LD = low density)



Figure 2.9: Mechanical characterization of the micro- and nanofibrous electrospun PCL scaffolds. (A) Representative stress-strain curves, (B) Ultimate stress measurements, (C) Young's Modulus, (D) Ultimate strain measurements. (Reproduced from Soliman *et al.*, 2011)

Porosity is void volume relative to the total volume of the scaffold. It represents the fraction of scaffold available for cell infiltration and tissue formation. Typical porosity for electrospun scaffold is known to be between 70-90% (Lowery, 2009) and the porosity can be measured using techniques like gravimetric method, image analysis, mercury intrusion porosimetry, using pycnometer a.k.a specific gravity bottle or liquid intrusion method, to name a few.

Measurement by gravimetric method and mercury porosimetry normally will give accurate porosity value. A study by Croisier *et al.* whom uses mercury porosimetry found that the porosity of electrospun PCL scaffold obtained was $87.1 \pm 4.4\% \text{ v/v}$ which is in agreement with typical porosity of scaffold (i.e 70-90%) (Croisier *et al.*, 2012). However, the downside of this method is that, high mercury pressure

applied might cause erroneous results since the pressure applied might result in deformation of the structure of the scaffold. Porosity determination using gravimetric method is known to be simple and produces accurate results. However, careful sample preparation, precise determination of scaffold dimensions and sufficiently large samples for weighing are required in order to achieve good accuracy.

Image analysis is another method that is usually used to measure the porosity of the scaffolds. Basically, porosity measurement is done by using software that will calculate the voids in the cross-sectional images of the scaffold. A study by Patlolla *et al.* found that the porosity of electrospun PCL and PCL/HA-TCP 30% scaffold was 65 ± 6 and 76 ± 4 respectively (Patlolla *et al.*, 2009). However, this method possesses its own limitation as it only measures porosity in 2 dimensions. In addition, the degree of porosity measured will be less than the actual value due to loss of small fundamental details during the image manipulation. Nevertheless, 2D image manipulation represented valid instrument for description of additional morphological parameters such as size, distribution and orientation of pores not achieved by other methods.

Pore size in electrospun scaffolds refers to an average pore size with the underlying pore sizes distributed over a wide range of values. There are a lot of methods that can be used to measure pore size namely image analysis, capillary flow porometry, liquid intrusion/extrusion porosimetry, high resolution NMR, and mathematical model, each with its own pros and cons (Chakradeo *et al.*, 2011).

Capillary flow porometry characterizes pore size distribution with respect to gas flow through a scaffold (Chakradeo *et al.*, 2011). Generally, this method measures the smallest diameter of the pore. This method is normally used in filtration applications and can be used to determine the cell retention characteristics of a scaffold. A study by Patlolla and co-workers which used the same technique found that the pore diameter of electrospun PCL was between 0.6-5.3 μ m while the pore size of PCL/HA-TCP 30% was between 7.6-210.3 μ m (Patlolla *et al.*, 2009). However, a study by Jena *et al.* found that the pore diameter obtained using this technique differs considerably from the diameter determined by other techniques (Jena *et al.*, 2005).

Another famous method used to measure pore size is SEM image analysis (Ziabari *et al.*, 2008 and Tomba *et al.*, 2010). This technique can be used to characterize individual pores and for description of cell retention characteristics. It measures void area between fibers by using a representative slice of finite thickness. Since this is one of the simplest and cheapest methods to find the pore size, the representative slice thickness (fiber mat thickness or depth of focus) must be chosen carefully to avoid erroneous measurement.

Liquid intrusion/ extrusion porosimetry is known to provide the complete picture of pore structure by measuring pore diameter and volume (Jena *et al.*, 2003 & Rutledge *et al.*, 2009). It measures pore structure by forcing mercury into or forcing wetting liquid from pores through the application of pressure. Mercury intrusion requires much higher pressures than liquid extrusion and such high pressures could cause elastic deformation and buckling in PCL fibers with diameter less than 10µm. Furthermore, high pressure applied to fill mercury into small pores might cause underestimation of the small diameter pores.



Figure 2.10: Mean and root mean square errors for the: (A) fiber diameter, (B) porosity, (C, D) pore size estimates for micro - (C) and nanofibers (D). Statistically significant differences at p < 0.05, *M-HD vs. N-HD, **M-HD vs. M-LD, +M-LD vs. N-LD, ++N-HD vs. N-LD (Student's t test) (Reproduced from Soliman *et al.*, 2011)

Soliman *et al.* computed pore sizes using statistical model (Model 1 and Model 2) (Soliman *et al.*, 2011). The results showed that pore size measured using Model 1 consistently showing lower values compared to pore size measured using Model 2 (Figure 2.10 (C) & (D)). It was found that the pore size of the tested scaffolds was influenced by the fiber diameter and their packing density. Larger fiber diameter and lower packing density produced bigger pore size compared scaffold with smaller fiber diameter and higher packing density. As a result, the M-LD was found to have a significantly larger pore size (44–64 μ m) than the other considered scaffolds.

Phosphate buffered saline (PBS) and simulated body fluid (SBF) are 2 types of solutions that are normally used in in vitro biodegradation experiment. Simulated body fluid, as the name implies, has almost similar ion concentration as the human blood plasma (Kokubo *et al.*, 1990). However, SBF is particularly used in determination of bioactivity of artificial material and coating of apatite on various materials under biomimetic conditions but rarely in biodegradation experiment. Formation of apatite on the surface of artifical material when immersed in SBF is used as an indicator to whether the material will bond to the bone when implanted inside animal's body (in vivo).

In the study by Kanjwal and co-workers, the SBF was exploited to precipitate biological apatite (Kanjwal et *al.*, 2010). After 10 days of incubation at 37°C, it was found that pure PCL did not induce apatite formation on its surface. The composite scaffolds on the other hand showed formation on its surface and it was deduced that embedded HA stimulated the precipitation. Figure 2.11 shows the FESEM images of scaffolds of different HA percentages after immersed in SBF for 10 days.

The downside of SBF is that it is a supersaturated solution which means it can easily form apatite on the surface of the specimen if not controlled (Kokubo *et al.*, 1990). Easy formation of apatite will cause increase in weight of the specimen and this does not favour the biodegradation experiment which focuses on measuring the mass loss of the scaffold after a certain period of time. PBS on the other hand has lesser ionic components but nonetheless represents the isotonic property and pH of the human blood plasma.



Figure 2.11: FE-SEM images for the PCL nanofibers containing different amounts of hydroxyapatite; (A) 0 %, (B) 3 %, (C) 5 %, and (D) 7 % with respect to polymer solution after incubation in SBF at 37° C for 10 days (Kanjwal *et al.*, 2010).

Chapter 3. Materials and methods

3.1. Preparation of electrospun PCL and PCL/HA composite scaffolds

In this experiment, the scaffold was prepared by a graduate researcher from Chemistry Department, University of Malaya. The scaffold was fabricated using electrospinning method. Polycaprolactone (PCL) was mixed with co-solvent (chloroform and dichloromethane, (DCM)) until the solute is dissolved. Hydroxyapatite (HA) obtained from grinded cow bones with the size of 20-45 micrometer was then added to the mix and the mixture was sonicated using ultrasonic mixture to avoid agglomeration. The solution was poured into the syringe and then pumped out onto an aluminum sheet laid beneath using syringe pump at DC current of 12V. There were 6 types of composite scaffolds produced: PCL, PCL/HA 10%, PCL/HA 20%, PCL/HA 30%, PCL/HA 40% and PCL/HA 50%. The proportion of HA added was based on mass. For instance, PCL/HA 50% is consists of equal amount (in mass) of HA and PCL.

3.2. Degradation set-up

The in vitro degradation of PCL/HA scaffold can be determined by measuring percentage of mass loss of the scaffold. The in vitro hydrolytic degradation was carried out by immersing pristine PCL and composite PCL with different HA percentages into phosphate buffered saline (PBS) at 37°C.

PBS is a water-based salt solution that contains sodium chloride, sodium phosphate and in some formulations, potassium chloride and potassium phosphate. This solution helps maintaining constant pH and the osmolarity and ion concentration usually match those of human body (isotonic). In this experiment,

PBS solution made from tablet PBS (Sigma-Aldrich, USA) which yields 0.01M phosphate buffer, 0.0027M potassium chloride and 0.137M sodium chloride at pH 7.4 \pm 0.1 was mixed with 3ml of penicillin streptomycin (to prevent bacterial contamination) was used.

Degradation test was performed under sterile condition. To keep the scaffold sterile, the specimens were kept in 70% ethanol overnight and dried for another 24 hours before immersed in the degradation media.

Scaffolds were cut into pieces of 10mm x 30mm. The specimens were precisely weighed (W_{init}) using analytical balance (Shimadzu, Kyoto, Japan) and then immersed in 8 ml PBS (pH 7.4±0.1) and were placed inside an incubator at 37°C for 8 weeks. The degradation medium was renewed every 4 weeks (Liu et al., 2012). The biodegradation was conducted under static condition.

After 8 weeks, the specimens were recovered and prepared for weight measurement and tensile strength test. For the weight measurement, the specimens were washed with distilled water and dried at in an oven at 40° C for 2 days plus 1 day inside a vacuum desiccator before weighed. The weight loss percentage = 100 (initial weight – weight after degradation) / initial weight.

SEM and FTIR were carried out after 8 weeks of degradation to determine the effect of degradation on the scaffolds.

3.3. Characterization methods

3.3.1. FESEM

The surface morphology of the electrospun scaffold was studied using Field Emission Scanning Electron Microscopy (FESEM) (Nova Nanosem 230, Hillsboro, Oregon, USA). No sample preparation (i.e gold coating) was done prior to analysis. An accelerating voltage of 15-22 kV and a working distance of 5 mm were used for viewing the samples. High magnification images (5000x) were taken at random fields of the scaffolds. The fiber size, pore diameter and percentage of porosity of electrospun scaffolds were determined from the FESEM images using ImageJ 1.4s software (National Institutes of Health, USA). Fiber diameters and pore diameters of 50 fibers and 50 areas for each scaffold were measured respectively. The degree of porosity was measured by measuring void fraction on black and white micrographs.

3.3.2. FTIR

FTIR is conducted to identify functional groups of HA and to determine the bonds between HA and PCL phase. FTIR works by passing IR radiation through the sample. Some of IR radiation will be absorbed by the sample while some will be passed through. The resulting spectrum will represent the molecular absorption and transmission, and each spectrum produced is unique. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis of the scaffolds was performed using the Perkin Elmer FTIR-ATR 400 series. The spectra were collected in the range 4000–400 cm⁻¹ with a resolution of 4 and scans of 32. The spectra were collected for PCL, PCL/HA 10%, PCL/HA 20%, PCL/HA 30%, PCL/HA 40% and PCL/ HA 50% before and after 8 weeks of degradation in PBS. X-ray diffraction analysis is conducted to determine phase composition and crystallinity of the PCL/HA scaffold. PCL and HA are both known to be partly crystalline and partly amorphous. Although crystallinity helps strengthen and improve the performance of the scaffold over wide range of temperature, scaffold that is too high in crystalline part tend to be brittle. In the XRD spectrum, the crystalline part will give sharp and narrow diffraction peak while the amorphous component will give a broad peak (halo).

X-ray diffraction (XRD) patterns of samples were recorded at ambient temperature on an PANanaytical Diffractometer (Philips, Netherlands). The samples were irradiated with a monochromatized Cu K_{α} (1.54056 Å) X-ray source with a step size (2-theta) of 0.026 and scan step time (s) of 8.67. The operating voltage and current used were 45 kV and 40 mA, respectively. The samples were scanned in the 2-theta range 5–60° (Patlolla *et al.*, 2009).

3.3.4. Tensile testing

Tensile test was carried out to determine the strength of the scaffolds. The bulk tensile property of each scaffold was measured in dry condition because mechanical properties under dry condition will give information regarding durability during surgical handling. The specimens were cut into rectangular strips of 30mm x 10mm. To improve the grip of the specimen during tensile testing, the ends of the specimen were individually fixed to pieces of cardboard using slow-setting adhesive (adopted from Bosworth & Downes, 2010). The sample width was measured using an electronic vernier caliper before gripped within the tensile tester clamps of the Instron 5848 mechanical tester (Instron Corporation, Norwood, MA, USA). Sample mounting was performed gently by aligning each strip axially and

lightly gripping it at the ends to avoid pulling and pre-stressing the material. Figure 3.1 shows how the specimen is fixed to the mechanical tester. Each sample was loaded into the Instron mechanical tester and subjected to load cell of 2kN and 0.2mm/s speed. The modulus of elasticity was determined from the initial 10% strain at the linear regime for each specimen. The mechanical properties reported are averages of 8-10 test samples per scaffold type.



Figure 3.1: The front view of scaffold mounted on Instron mechanical tester.

3.3.5. Statistical analysis

Acquired data was statistically analyzed using IBM SPSS. Data was analyzed with one way ANOVA and Tukey Post-Hoc test with 95% confidence level.
Chapter 4. Results and discussion

This chapter explains the results of the research. In general, it describes the properties of the scaffolds obtained by using various techniques and equipments. The results include the surface morphology, fiber diameter, porosity, pore size, tensile properties and the effect of in vitro degradation on the scaffolds. This is followed by discussion to support the findings.



Figure 4.1: Photograph of the scaffold.

Figure 4.1 shows how the scaffold looks like. Morphologically, scaffold with higher percentage of HA will show higher agglomeration compared to its lower concentration counterparts (Figure 4.3). In terms of fiber diameter, addition of HA > 30% does affect the size of fiber diameter. The diameter of PCL/HA 30%, 40% and 50% fiber is smaller compared to PCL, PCL/HA 10% and PCL/HA 20% (Figure 4.2). However, there is no significant difference in fiber diameter between PCL/HA 30%, 40% and 50%. This means addition of HA = 10% - 20% has no effect on fiber diameter of PCL/HA 40 and 50%. Therefore it can be concluded that addition of HA of size between 20-45 microns does not increase the size of fiber diameter but instead makes the diameter much smaller. Scaffolds with higher percentage of HA are preferred because they mimics the ECM of bone which has fiber diameter between 50-500 nm (Dvir *et al.*, 2011) and help stimulates bone growth better compared to the lower HA percentages counterparts.



Figure 4.2: Graph of fiber diameter versus types of scaffolds. * (p < 0.05) indicates composite scaffold's fiber diameter is significantly different from PCL scaffold.



Figure 4.3: HA particles tend to agglomerate as its concentration increase. HA is not evenly distributed throughout the scaffold due to large particle size used (20-45 micrometer). Scale bar: 100µm. Magnification: x800.

Statistical analysis on the size of pore diameter shows addition of HA has no effect on the size of the pore diameter (Table 4.1). The pore diameter measured is between 2.38- 7.22 μ m. The result from this study contradicts the findings by Yoshimoto *et al.* and Grainger *et al.* whom stated that pore size between 100-350 μ m is recommended for bone regeneration. However, a cell study conducted using bone marrow stromal cells (BMSC) showed the bone cells were alive despite the small pore size and the same phenomenon was observed in all scaffold compositions. According to Heydarkhan-Hagvall *et al.*, this is possible because the scaffold is made from overlapping fibers which were loosely bonded to each other. This unique structure allows the bone cells to push the fibers to enlarge the pores for efficient oxygen, nutrient and waste transfer as they grow.

Types of scaffolds	Pore size (µm)	
PCL	4.55 (±2.17)	
PCL/HA 10%	5.05 (±2.17)	
PCL/HA 20%	4.10 (±1.55)	
PCL/HA 30%	4.35 (±1.62)	
PCL/HA 40%	4.73 (±1.46)	
PCL/HA 50%	4.70 (±1.92)	

Table 4.1: Pore size/diameter of PCL and composite scaffolds

Fiber diameter and fiber packing density are factors that affect porosity of the scaffold (Soliman *et al.*, 2011). Scaffold with large fiber diameter and high fiber packing density is less porous compared to its small fiber diameter and low fiber packing density counterparts. Scaffold with porosity between 70-90% (Lowery, 2009) is desired because it allows efficient oxygen and nutrient supply to as well as waste removal from the cells which will ensure undisturbed growth of new bone. There are many methods to measure the degree of porosity and one of them is by measuring void fraction using ImageJ software (NIH). This method works by measuring the average projected porosity deduced from the percent void fraction as seen in top view of SEM micrographs of the scaffolds (Soliman *et al.*, 2011). The percentage of void fraction is determined by counting and summing the voids in 2D SEM images. ImageJ was used to convert grey scale images into a binary format (black and white format) – with fibers associated with black pixels

while voids with white pixels, to compute the void fraction (the fraction of white pixels). Statistical analysis on the effect of HA percentage on the porosity shows HA has no effect on the porosity of the scaffolds (Figure 4.4). In general, the porosity of the scaffolds is between 27.5-35.5% - a range that is very small compared to the porosity of an ideal scaffold. The results obtained by measuring void fraction using ImageJ is known to give smaller reading compared to other methods because the software only measures voids on 2D micrographs. In addition, image manipulation that involves conversion of the micrographs into black and white (binary) images before void measurement cause loss of details which resulted in erroneous reading.



Figure 4.4: Graph of percentage of porosity vs. types of scaffolds. Data is represented as mean $(\pm \text{ std dev})$ (n = 5). Error bar represents standard deviation.

Young's modulus, also known as tensile modulus, is a ratio of tensile stress over tensile strain while ultimate tensile stress (UTS) is the maximum stress a material can withstand while being stretched before necking (Degarmo, 2003). These tensile properties are influenced by fiber diameter, fiber packing density and HA concentration. ANOVA and Tukey-HSD post hoc analysis on the effect of HA concentration on tensile modulus shows that Young's modulus of all composite scaffolds is significantly different from pure PCL scaffold. PCL scaffold shows the highest value for Young's modulus and this result is in agreement with finding by Soliman et. al (Table 4.2). There was no pattern observed in Young's modulus and UTS of composite scaffolds. Referring to bar charts of frequency distribution of composite scaffolds (Figure 4.5), we can see that 30-50% of the fibers are in the micrometer scale region while the rest are in nanometer scale region. The YM and UTS results obtained from this experiment varies between scaffolds and the values fall between YM and UTS of micrometer scale fibers and nanometer scale fibers in a study by Soliman *et al.* Factors that cause variation in readings include mixed-size fibers and defects in fibers (caused by addition of micrometer scale HA particles into the PCL and twisting of the fibers). This is in accordance with a statement by Fakirov *et al.*, & Bhattacharyya *et al.* whom stated that the defects in fibers are responsible for large scatter observed in properties of the fibers.

Table 4.2: Young's modulus (YM) and ultimate tensile strength (UTS) of the scaffolds. Data is represented as mean (\pm standard deviation). * (p < 0.05) indicates YM of the composite scaffold is significantly different from PCL scaffold.

	Types of scaffolds	YM (MPa)	UTS (MPa)
	PCL	11.22 (±2.56)	1.13 (±0.16)
	PCL/HA 10%	6.53 (±2.23) *	1.21 (±0.43)
	PCL/ HA 20%	7.89 (±3.00) *	1.54 (±0.44)
	PCL/HA 30%	1.55 (±0.60) *	0.33 (±0.17)
	PCL/HA 40%	3.59 (±1.15) *	0.94 (±0.30)
	PCL/HA 50%	1.59 (±1.59) *	0.24 (±0.08)





Figure 4.5: Graph of frequency distribution of fiber diameter for (A) PCL (B) PCL/HA 10% (C) PCL/HA 20% (D) PCL/HA 30% (E) PCL/HA 40% (F) PCL/HA 50% scaffold. 74% of PCL fibers are in the micrometer range while only 30-50 % of composite fibers are in the micrometer range. (Note: Fibers

are considered to be in micrometer range when the fiber diameter is > 1000nm)

Hydrolytic degradation means breakdown of material in presence of water, which in this case is the degradation of PCL in PBS – a solution that mimics human blood plasma. According to Pitt *et al.*, PCL degrades by random chain scission of the ester group in the material. Chain scission occurs in PCL's amorphous region due to loose structural packing within this area which makes ester bonds more exposed to attack from water molecules (Yuan *et al.*, 2003 & Cook *et al.*, 1981). Ester bonds cleaved from polymer backbone cause formation of carboxylic acid known as capronic acid. Presence of capronic acid decreases pH of the degradation medium and this phenomenon was observed when measurement of pH was done after 8 weeks of immersion in PBS.

In this experiment, the degradation was measured in terms of percentage of mass loss on day 28 (4th week) and day 56 (8th week). Mean weight of 5 scaffolds per composition was measured and it was observed that PCL and PCL/ HA composite scaffolds increase in mass (negative percentage of mass loss) after 8 weeks of degradation. FTIR spectra of scaffolds 8 weeks after degradation show presence of broad band at 3400⁻¹ which signifies water intake by hydrophilic HA.

In spite of this, increase in mass does not mean absence of degradation. SEM micrographs on Week 8 were compared with the ones on Day 0 to identify the effect of degradation on the scaffolds. Presence of snapped fibers and decrease in fiber diameter size were used to justify degradation. Micrographs after 8 weeks demonstrated lack of surface change to the samples when compared with the images on Day 0.

Water intake by PCL and HA is the first step of hydrolytic degradation process. Figure 4.6 shows graphical illustration of mass loss of PCL. The graph shows the first 6 months is the period when the polymer is hydrated. Small mass loss (up to 10%) is expected to occur in the first 2 months. This finding is also supported by (Gopferich, 1996) whom stated that polymers that are capable of undergoing hydrolytic degradation will initially absorb water in to the material bulk and hydrating the molecular chains.

PCL takes 1-2 years to fully degrade inside the body (Dunkelman *et al.*, 1995) and the rate of degradation of PCL depends on its structural and morphological formations and surface area to volume ratio (Kim *et al.*, 2003 & Chin *et al.*, 2006). Samples with higher surface to volume ratio degrade faster due to greater water penetration into the sample which increases the rate of chain scission (Bolgen *et al.*, 2010). In spite of this, this statement contradicts the finding by Athanasiou *et al.* who proposed that scaffolds with higher porosity tend to degrade at a slower rate since larger surface area allowed easier removal of acidic breakdown products which consequently reducing the effect of autocatalysis. It can be concluded that the rate of PCL degradation in PBS is influenced by a combination of all the factors stated above. Apart from that, the fabrication method applied (i.e electrospinning) also contributed to making the scaffolds less wettable thus slowing the rate of degradation of the polymer (Bosworth *et al.*, 2010)





A complete chemical characterization is important in order to get information regarding chemical functions involved in the adhesion bonding of fibers and polymers in composite processing and IR spectroscopy is one of the techniques used in chemical characterization of fibers.



Figure 4.7: FTIR spectra of PCL and PCL/HA composite scaffolds.

The FTIR spectra of PCL and PCL/HA composites are shown in Figure 4.7. The chemical composition of fibrous scaffolds, investigated by FTIR spectroscopy confirmed the presence of HA particles within the scaffolds. This is proved by presence of IR bands resulting from vibrational modes from phosphates (PO_4^{3-}) and hydroxyl groups (OH) in the spectra. The PO_4^{3-} asymmetric stretching mode of vibration is characterized by a strong and complex band in the 1732-1037 cm⁻¹ region and a medium intensity band at about 961 cm⁻¹ that resulted from symmetric stretching vibrations. The OH⁻ bending deformation mode of vibration on the other hand, is characterized by a medium sharp peak at 628-635 cm⁻¹ (Kanjwal *et al.*, 2011). The intensity of these peaks increases with increase in amount of HA. Apart from that, the crystalline HA also generates characteristic OH⁻ band at about 3400 cm⁻¹. This broad peak corresponds to water absorbed by HA and it occurs in all the scaffolds' spectra after 8 weeks of immersion inside

degradation media. This explains scaffolds' increase in weight after 8 weeks of degradation.

Typical infrared bands for PCL were also notable in pure PCL and PCL/HA scaffolds. At 2935.37-2942.52 cm⁻¹ (asymmetric CH₂ stretching), 2865.33-2866.61 cm⁻¹ (symmetric CH₂ stretching), 1721.96-1722.76 cm⁻¹ (carbonyl stretching), 1293.80-1293.95 cm⁻¹ (C–O and C–C stretching in the crystalline phase) and 1239.38-1239.81 cm⁻¹ (asymmetric COC stretching) were observed.

According to Nejati *et al.*, shifts in peak positions and missing peaks are normally associated with interaction between PCL and HA (Nejati *et al.*, 2009). The results from Patlolla *et.al*, showed that there was a loss of a peak of ceramic at 1023 cm⁻¹ and shifting of the peak of PCL from 1107 cm⁻¹ to 1112 cm⁻¹ which suggest interaction between PO₄³⁻ and C-O (Patlolla *et.al*, 2009). In this experiment, shifts in PO₄³⁻ peaks were observed in PCL/HA 20%, PCL/HA 30%, PCL/HA 40% and PCL/HA 50% spectra. However, no ceramic peak loss was observed. Therefore it can be concluded that PCL did not react with the HA.

Regular arrangement of atoms and molecules produce sharp diffraction XRD peaks whereas amorphous regions produce broad halos. The x-ray diffraction patterns of polymers contain a combination of both. Figure 4.8 shows the XRD spectra of PCL and PCL/HA composite scaffolds. The diffraction peaks of pure PCL occur at $2\theta = 20-25^{\circ}$ which is in agreement with finding by Raucci *et al.*, while the diffraction peaks of HA occur at $2\theta = 30 - 40^{\circ}$.

Ideally, the intensity corresponding to PCL peaks will decrease distinctly with the increase of the HA content in PCL/HA scaffolds. However, this is not the case in this experiment because the pattern of spectrum observed is pretty inconsistent. The absence of HA peaks may be caused by the limitation of the XRD equipment

itself. Since the HA particles are scattered throughout the scaffold there is a chance that the electron beam missed the particles thus causing absence of HA peaks.



Figure 4.8: XRD spectra of PCL and PCL/HA composite scaffolds. The diffraction peaks for PCL occur at $2\theta = 20^{\circ} - 25^{\circ}$ while diffraction peaks for HA occur at $2\theta = 30^{\circ} - 40^{\circ}$. The diffraction peaks of PCL/ HA composite scaffolds contains a combination of both.

Chapter 5. Conclusion

This study looked at a wide range of scaffold characteristics and how the addition of different percentage of HA and different method of fabrication (i.e electrospinning) would affect the characteristics of PCL and PCL/HA composite scaffold. FTIR, FESEM, tensile test and degradation test were used to identify the physical, chemical and mechanical properties of the scaffolds and to measure the extent of degradation on the scaffolds.

Image analysis on FESEM micrographs using ImageJ software (NIH, USA) showed that the scaffolds were made from nanometer-scale to micrometer-scale fibers of sizes between 0.5-2.5 μ m. The fiber morphology of scaffold after 8 weeks of immersion in PBS showed no difference from the fiber morphology of scaffold before degradation and this phenomenon was observed in all scaffold compositions.

The physical mass loss which is often associated with the effect of degradation was absent except in PCL/HA 10% scaffold. FTIR analyses on the scaffolds showed presence of broad peak at 3400cm⁻¹ which indicates water uptake by HA after 8 weeks of degradation. It can be concluded that mass loss could have occurred but it may not be detected due to limitation of the analytical balance machine which only measures up to 0.1mg or it might have been masked by water uptake. Another method for instance using gas permeation chromatograph (GPC) which measures molecular weight could be used to detect percentage of molecular weight loss after degradation in the future.

The Young's modulus and ultimate tensile stress (UTS) of the scaffolds are influenced by the three factors namely fiber diameter, fiber packing density and HA particles. The larger the fiber diameter and the higher the packing density the lower the Young's modulus and the higher the UTS will be. However, it is deduced that high percentage of HA particles will weaken the fibers and cause the Young's modulus to increase and the UTS to become low. No specific pattern can be observed in Young's modulus and UTS as the percentage of HA increase.

The activity of cells in/on the scaffolds and efficiency of nutrient transportation as well as oxygen diffusion is influenced by two factors namely porosity and pore size. These properties are often regarded as interdependent since the size of pores affects porosity and vice versa. In general, porosity of the scaffold is dependent on two factors: fiber diameter and packing density. Scaffolds with large fiber diameter and high packing density will have low porosity while scaffolds with smaller fiber diameter and lower packing density will have high porosity value. Porosity measurement using image analysis on FESEM micrographs showed that the porosity of the scaffold was between 27.5-35.5%

Overall, after in vitro degradation of 8 weeks, it can be concluded that PCL/HA composite scaffolds still retain a relatively stable architecture. However, the conclusion made from an 8 weeks of study cannot be solely used to represent the exact properties of the scaffolds. Further work should be carried out to confirm the findings in the preliminary study. Additional measurement techniques using more precise and reliable equipments should be adopted to overcome shortcomings in the apparatus used in this study.

Chapter 6. References

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Figure 7.1: Step-by-step measurement of percentage of porosity using ImageJ software (National Institutes of Health, USA) (A) Original image; (B) Inverted image; (C) Image after the contrast was increased to 70%; (D) Adjust threshold; (E) Image after the edges were smoothed; (F) Analyzed pores.



Figure 7.2: FESEM micrographs of (A) PCL; (B) PCL/HA 10%; (C) PCL/HA 20%; (D) PCL/HA 30%; (E) PCL/HA 40% and (F) PCL/HA 50% magnified to x800.



Figure 7.3: FESEM micrograph of PCL (A) before degradation; (B) after 8 weeks of degradation in PBS.



Figure 7.4: FESEM micrograph of PCL/HA 10% (A) before degradation; (B) after 8 weeks of degradation in PBS.



Figure 7.5: FESEM micrograph of PCL/HA 20% (A) before degradation; (B) after 8 weeks of degradation in PBS.



Figure 7.6: FESEM micrograph of PCL/HA 30% (A) before degradation; (B) after 8 weeks of degradation in PBS.



Figure 7.7: FESEM micrograph of PCL/HA 40% (A) before degradation; (B) after 8 weeks of degradation in PBS.



Figure 7.8: FESEM micrograph of PCL/HA 50% (A) before degradation; (B) after 8 weeks of degradation in PBS.







Figure 7.11: PCL/ HA10% spectrum before degradation.



Figure 7.12: PCL/ HA10% spectrum after 8 weeks of degradation in PBS.
















Figure 7.20: PCL/ HA50% spectrum after 8 weeks of degradation in PBS.



Figure 7.21: Graph of percentage mass loss on Day 0, 28 and 56.