



CONTACT ANGLE, CONDUCTIVITY AND MECHANICAL PROPERTIES OF POLYCAPROLACTONE/HYDROXYAPATITE/POLYPYRROLE SCAFFOLDS USING FREEZE-DRYING TECHNIQUE

Sharon Kalu Joseph Ufere¹ and Naznin Sultana^{1,2}

¹Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, Johor, Malaysia

²Advanced Membrane Technology Research Center (AMTEC), Universiti Teknologi Malaysia, Johor, Malaysia

E-Mail: naznin@biomedical.utm.my

ABSTRACT

For bone tissue engineering application, a porous scaffold with interconnected network is highly sorted for cell guidance, cell attachment and growth in the three dimensional structure. Using freeze drying technique, we produced three types of scaffolds. PCL scaffold, PCL/HA nanocomposite scaffold and a conductive scaffold PCL/HA/PPY. Conductive scaffold was produced to render the scaffold conductive. The scaffolds were characterized and evaluated using a number of techniques. Composite scaffolds with suitable pore size distribution were obtained by freezing the polymer solution mixture at -18°C, which was controlled by the polymer and solvent phase crystallization. From the result, the wettability and porosity of the sample were observed to increase more with the incorporation of Polypyrrole in the scaffold than in Polycaprolactone scaffold alone. PCL/HA/PPY scaffold had a lower porosity than PCL and PCL/HA scaffolds. The compressive modulus increased from 1.15Mpa (PCL) up to 6.10Mpa in PCL/HA/PPY scaffold. Incorporation of PPY polymer rendered the scaffold conductive. Hence, the characteristics of PCL/HA/PPY scaffold studied in this present work shows good prospect and may probably be employed for bone tissue engineering applications.

Keywords: bone tissue engineering, polypyrrole, freeze-drying, conductive scaffolds.

1. INTRODUCTION

Development of biological substitute for bone regeneration has emerged as the core objective in bone tissue engineering (TE). This is because of the several drawbacks associated in the use of the conventional methods; autograft, allograft and xenograft. Autograft is associated with high cost, second surgery requirement, and donor site morbidity. Allograft on the hand also has some limitations though can be used for larger defects. There could be disease transmission or even immune rejection. Meanwhile, xenograft share similar limitations as allograft and it really used because their structure and function do not accurately match that of human tissue [1]. To tackle the problems associated with conventional methods, and to develop bone construct that can copy the structure of natural bone, TE methods have been applied as a hopeful alternative.

Tissue engineering depends widely on the utilization of porous scaffolds for tissue and organ regeneration to provide the proper environment. When fabricating a scaffold, several factors must be put into consideration because performance of the scaffold is basically what determines the success of the bone tissue engineering. Hence, a good scaffold must be biocompatible, biodegradable, high porosity, correct pore size to enhance cell adhesion and growth, and mass transportation of nutrient. The mechanical properties of the scaffold ought to be constant with the anatomical site it is to be implanted.

Blending of synthetic polymer and ceramics is one of the most effective techniques for providing new, appropriate biocomposite for a specific application[2]. Ceramic and polymer composites demonstrate the best features of each constituent that is the toughness of

polymer and stiffness of ceramic [3] over scaffolds consisting of either only polymer or only ceramics [4]. Studies have shown that combining polymer (PCL) and ceramics (HA) will enhance mechanical properties, biodegradability as well as improve osteoconductivity[4, 5]. There are several methods that can be utilized to produce biodegradable scaffolds. These comprise freeze-drying [6], phase separation[7], self - assembly [8], and electrospinning [9]. Emulsion freezing/ freeze-drying technique owing to its importance in making extremely porous scaffolds and regulating of the pore size has been used for fabricating TE scaffolds in recent years [10].

Conductive polymers (CPs) have been investigated and used in numerous biomedical applications because of its acceptable biocompatibility since 1980s [11]. CPs mediates electrical stimulation and has the potential to be the stimulating factor that increase bone regeneration. Recent studies showed that the addition of CP can advance both the mechanical strength, the biodegradability of scaffolds including their in-vitro biocompatibility [12, 13]. Amongst the most investigated CPs is polypyrrole (PPY) as shown by the number of publication surrounding its properties and uses? We report the fabrication of a new conductive nanocomposite scaffold PCL/HA/PPY utilizing freeze-drying technique and comparing it's characteristics with both PCL and PCL/HA scaffolds. Firstly, PCL and PCL/HA composite scaffolds were fabricated with the incorporation of different amount of HA. PCL/HA scaffold with 10% (w/w) HA was found to be suitable. Then PPY was added to render it conductive. The wettability, mechanical properties and conductive characteristics of the scaffolds were investigated.



2. MATERIALS AND METHODS

2.1 Materials

Polycaprolactone (PCL) with molecular weight Mn 70,000-90,000, was purchased from Sigma-Aldrich (UK). Nanosized Hydroxyapatite (HA) was synthesized in-house by a nano-emulsion method and Polypyrrole was also purchased from Sigma-Aldrich (UK). The solvent 1,4 Dioxane and other chemicals used were all of analytical grade.

2.2 METHODS

2.2.1 Preparation of composite solutions

The following procedures was followed to fabricate PCL scaffold, composites of PCL/HA and PCL/HA/PPY. Firstly, PCL solution was prepared by dissolving 1g of PCL in 10ml of 1,4 Dioxane into a centrifuge tube. In order to obtain homogeneous solution, the mixture was stirred using magnetic stirrer for 3 hours at 50°C until the polymer was completely dissolved thus producing homogenize solution and was transferred into a glass vial. Secondly 0.1 g HA nanoparticles were incorporated into 1g of PCL, dissolved into 1,4 Dioxane to produce 10% HA in PCL/HA (10% w/v) composite scaffold and dispersed onto the polymer matrix using a hand held homogenizer. The same procedure was repeated and different amount of PPY (5%, 10% and 15% w/w) were incorporated into the mixture of 10% PCL/HA. It was mixed properly with a homogenizer and thereby producing a homogeneous solution.

2.2.2. Freeze –Drying

The prepared PCL, PCL/HA and different percentage ratios of PCL/HA/PPY were placed into separate glass vials. The glass vials containing the solutions were then transferred into a freezer at preset temperature of -18°C for overnight to solidify the solution. The frozen solution was then transferred into a freeze-drying vessel (Labconco-Freezone, USA) and freeze-dried for 48 hours to eliminate the solvent.

2.2.3. Contact angle measurement

The wettability of the scaffolds were determined using a water contact angle measuring system (VCA Optima, AST Product, Inc). By dropping 1µl size of deionized water onto the composite surface and measurement was done after 5 sec for each sample. Five measurements were performed for each sample at different positions of the scaffold surface and was analyzed using VCA Optima software.

2.2.4. Attenuated Total Reflectance (ATR-FTIR)

Chemical bonding examination of PCL, PCL/HA, PCL/HA/PPY scaffolds were executed using an ATR-FTIR spectroscopy in the range of 4000- 650 cm⁻¹. To examine the spectra obtained, Infra-red solution software was used.

2.3. Conductivity measurement

The electrical conductivity of PCL/HA and PCL/HA/PPY freeze-dried scaffolds were determined using a Digital Multimeter (Victor VC830L). Scaffolds circular discs with a thickness of 4mm and diameter of 13mm were hydrated with distilled water. By placing the positive and negative probes at the sides of the circular discs with 13mm distance from each other, the resistance of the scaffolds was determined three times for each sample and the average was estimated. Average resistance (R) was subsequently introduced into Pouillet's law equation to attain the conductivity (σ) [14].

$$\sigma = L / (R \times A) \quad (i)$$

where L is the thickness, D is the diameter of the circular disc scaffold, and A is surface area of the circular disc scaffold, $A = \pi \times (D/2)^2$.

2.4 Mechanical property

The mechanical property of the scaffolds; 10% PCL, 10%HA/PCL and 10%PPY/HA/PPY composite scaffolds were determined at room temperature using mechanical testing machine (Instron mechanical tester) at a crosshead speed of 0.5mm/min.

3. RESULTS AND DISCUSSIONS

3.1. Water Contact Angle (WCA) measurement

The wettability of freeze-dried scaffolds were measured. The results from the contact angle measurements of the five scaffolds as represented in Table 1, showed the angles within the range of 115° to 67.8°. As shown in the table, the surface of the freeze- dried 10% PCL scaffold was very hydrophobic, having average water contact value of $115.1 \pm 2.6^\circ$. However 10% PCL/HA scaffold exhibited the lowest contact angle value of $67.8 \pm 13.5^\circ$. With regards to these results it can be seen that the contact angle for the samples PCL/HA and PCL/HA/PPY scaffolds were generally lower than that of PCL alone indicating that those samples were more hydrophilic than PCL scaffold. This results correlates with the fact that PCL polymer is a known hydrophobic material and HA being a bioactive and hydrophilic material. When HA was added to PCL, the composite material was observed to have hydrophilic properties. On the other hand, for the PCL/HA/PPY composite scaffolds, it was also observed that addition of 15% PPY polymer to PCL/HA mixture further increased the hydrophobicity of the scaffolds. And as the ratio of PPY increased, the wettability of the scaffolds reduced. Combination of the polymers helped to reduce the hydrophobicity of the scaffolds up to $108 \pm 4.2^\circ$ and maintained their individual properties. The enhancement in the wettability can improve the cell attachment, cell proliferation and cell- scaffold interactions [15].



3.2 Attenuated Total Reflectance (ATR)- fourier Transform Infrared Spectroscopy (FTIR) analysis

Figure-1 compares ATR-FTIR spectra of 10% PCL, 10% PCL/HA and 10% PCL/HA/PPY scaffolds. The ATR-FTIR was performed in order to determine the chemical bonding present in PCL, PCL/HA as well as in PCL/HA/PPY scaffolds. The FTIR spectrum of 10% PCL and 10% PCL/HA scaffolds, the transmittance bands were observed at 2865, 2944 cm^{-1} (related to CH and CH_3 group); 1721 cm^{-1} (C=O stretching). The IR peaks were designated as 3436, 2944 cm^{-1} (CH, and CH_3 group); 1471, 1365 cm^{-1} (C-O-C stretching vibrations). In this figure, PCL and HA corresponding bands materials were detected in the spectrum, this confirmed the formation of both compounds in the nanocomposite. C=O, C-O, and C-H bands were related to PCL and the P-O and O-H bands were ascribed to HA. Comparing PCL/HA composite scaffold with HA alone and pure PCL peaks, small displacements of PCL/HA nanocomposite peaks were insignificant owing to the chemical bonding that existed between HA and PCL during the chemical process. In this study owing to the small weight percentage of HA, its characteristic bands had lower transmittance percentage. Kim *et al.* [16] has also reported this phenomenon. HA formation was first indicated by the formation of broad FTIR band highlighted at about 1005-1168 cm^{-1} .

In PCL/HA scaffold, the transmittance bands were observed as 2865, 2895 cm^{-1} (related to CH and CH_3 group); 1720 cm^{-1} (C=O stretching). The bands at 960-965 cm^{-1} and 560-605 cm^{-1} correspond to symmetric P-O stretching vibration of the PO_3^{4-} ion [17, 18]. The ATR-FTIR spectrum of PCL/HA/PPY composite scaffold. Here, the absorption peak was observed as 2865, 2943 cm^{-1} (CH, CH_3 groups) and C=O stretching was observed at 1721 cm^{-1} . It was observed that all the peaks in PCL/HA/PPY scaffold spectra were almost similar as that of PCL and PCL/HA. This could be owing to the presence of small amount of PPY polymer on the PCL/HA sample.

3.3 Compressive mechanical properties

Table-2 summarized the corresponding mechanical parameters, compressive modulus and compressive strength. It was noticeable that by incorporating 10% w/v HA nanoparticle the mechanical features of PCL

scaffold was enhanced owing to the high strength property of hydroxyapatite. Consequently, compressive modulus, compressive strength and maximum load of HA/PCL and PCL/HA/PPY nanocomposite scaffolds increased 4.20 ± 0.05 and 6.10 ± 0.03 respectively in contrast to PCL scaffold alone with compressive modulus value of 1.25 ± 0.04 .

3.4. Electrical properties of PCL/HA and PCL/HA/PPY scaffolds

To determine the resistance of the scaffolds, a digital Multimeter was utilized. Conductivity was estimated using Pouillet's law equation. Table-3 shows the resistance and the corresponding conductivity of the scaffolds. The result shows that the sample in hold electrical properties after the addition of Polypyrrole into PCL/HA. No electrical resistance or conductivity was observed in PCL/HA scaffold as PCL and HA are regarded as being non-conductive scaffolds. The conductivity of 15% PPY in PCL/HA was significantly higher than 5% and 10 % respectively. It was observed that the higher the ratio of PPY to PCL and HA, the higher the conductivity.

Nevertheless, 10% PPY with conductivity $95.81 \mu\text{S/m}$ was considered as the most appropriate as too high conductivity could cause the scaffold to become cytotoxic to the cells. However, this conductive property of the scaffold could ameliorate cell-scaffold interaction in vivo, this possibly could be owing to enhanced intracellular signaling amid the cells [14].

CONCLUSIONS

Highly porous composite scaffolds and conductive scaffolds were fabricated and characterized using freeze-drying technique. As HA was incorporated into PCL, the water contact angle reduced, but addition of conductive polymer rendered it hydrophobic. The compressive modulus increased from 1.15 Mpa (PCL) up to 6.10 Mpa in PCL/HA/PPY scaffold. Incorporation of PPY polymer rendered the scaffold conductive. Therefore, the features of PCL/HA/PPY scaffold studied in this present work showed good prospect for bone tissue engineering applications.



Table-1. Contact angle measurement of the scaffolds representing its calculated average and standard deviation.


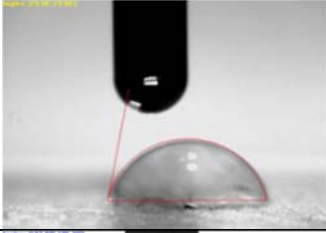
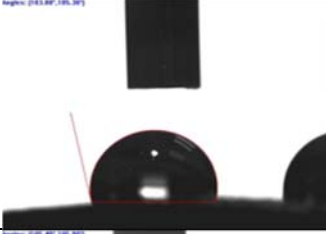
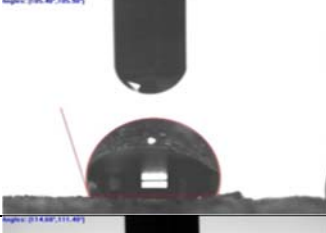
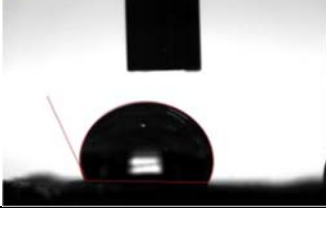
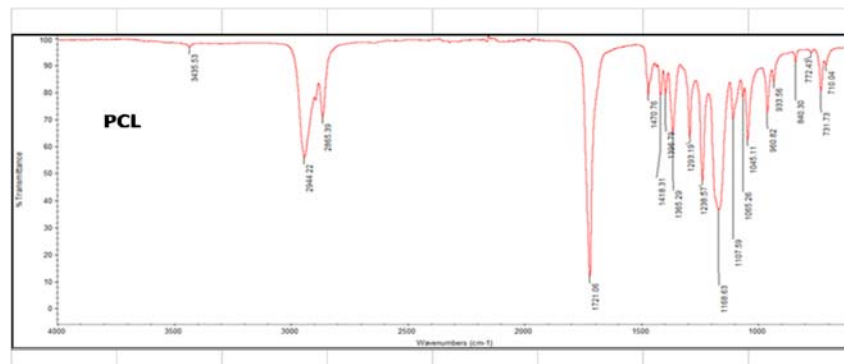
Sample		Water contact angle
PCL10%		$115.1^{\circ} \pm 2.6$
10% PCL/HA		$67.8^{\circ} \pm 13.5$
5% PPY in (PCL/HA)		$108^{\circ} \pm 4.2$
10% PPY in PCL/HA		$109.9^{\circ} \pm 3.6$
15% PPY in PCL/HA		$113.6^{\circ} \pm 9.6$

Table-2. Compressive Mechanical strength of PCL, PCL/HA and PCL/HA/PPY scaffolds.

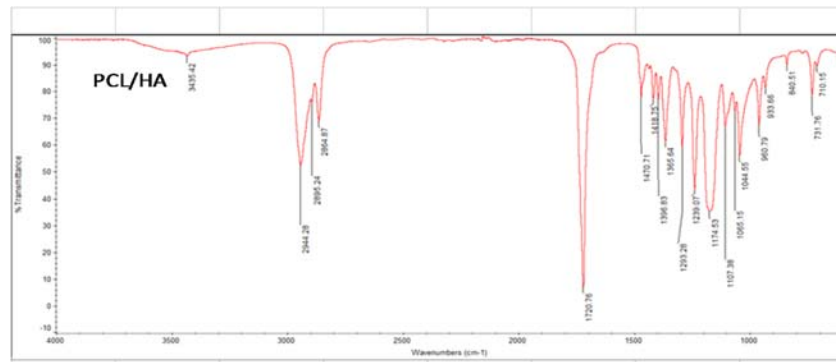
Scaffolds	Compressive strength (Mpa)	Compressive modulus (Mpa)
10% PCL	0.2 ± 0.05	1.15 ± 0.04
10% HA/PCL	0.5 ± 0.04	4.20 ± 0.05
10%PPY/10%HA/PCL	0.7 ± 0.02	6.10 ± 0.03

**Table-3.** The measurement electrical properties of PCL/HA and PCL/HA/PPY composite scaffolds.

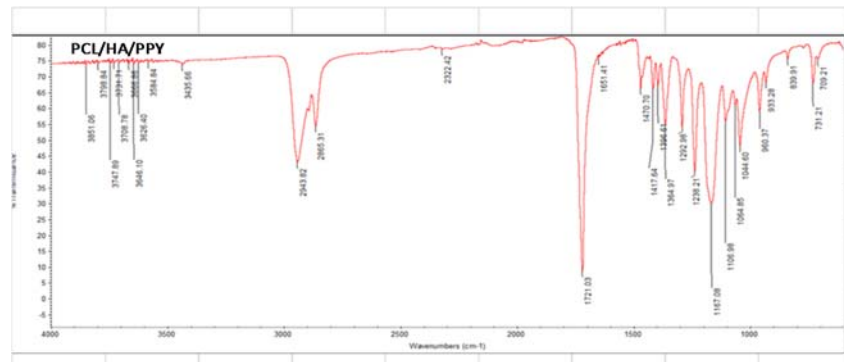
Sample	Thickness (mm)	Diameter(mm)	Resistance (MΩ)	Conductivity(μ/Sm)
10% HA/PCL	4.12	13.60	-	-
5% PPY/PCL/HA	3.11	13.22	10.53	2.15
10% PPY/PCL/HA	4.90	13.64	0.35	95.81
15% PPY/PCL/HA	3.48	13.28	0.027	921.65



(a)



(b)



(c)

Figure-1. FTIR Spectra of (a) PCL, (b) PCL/HA and (c) PCL/HA/PPY composites scaffolds prepared by freeze-drying method.



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