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Synthesis of Polycaprolactone-Hydroxyapatite (PCL-HA) Biodegradable Nanofibres Via an Electrospinning Technique for Tissue Engineering Scaffolds

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ABSTRACT

The interest in biodegradable polymer nanofibres with tissue cell regeneration potential has increased in recent years. However, there are issues in the development of scaffolding to provide a favourable environment for cell proliferation and attachment. Such issues can be overcome by the addition of hydroxyapatite (HA), which is widely used in biomaterial applications. Biodegradable nanofibres of polycaprolactone (PCL) and hydroxyapatite (HA) have been produced by electrospinning. In this study, PCL was mixed with HA to synthesise nanofibres by single nozzle electrospinning. Furthermore, PCL-HA nanofibres were mixed with fibronectin to investigate the effect of adhesion of fibronectin to the surface of the PCL-HA nanofibres. The structure and morphology of nanofibres were determined by scanning electron microscopy (SEM), the chemical properties of nanofibres were analysed by Fourier transform infrared (FTIR), and the diameter and adhesive force of nanofibers and fibronectin were determined by an atomic force microscope (AFM). The SEM examination revealed the formation of cylindrical and smooth nanofibres with dense fibre networks when 10% HA was used, as HA can generate fibre. FTIR analysis indicated the presence of PCL and HA inside the nanofibres produced by electrospinning. The AFM examination showed that the PCL-HA nanofibres with 100 µg/ml of fibronectin gives the highest adhesion force which is important for the scaffold to resist the force from the external environment. This outcome resulted indicates that the PCL-HA nanofibers with fibronectin are promising for tissue engineering scaffold application. Hence, further investigations are needed to ensure the compatibility of living cells to survive and grow on the PCL-HA nanofibrous mats.

Keywords: Electrospinning; nanofibres; polycaprolactone; hydroxyapatite; tissue engineering

INTRODUCTION

The rapidly growing research area of tissue engineering provides new and promising approaches for replacing lost tissue, organ failure and damaged organs (Rezaei et al. 2013). This can be achieved by providing scaffolds for cell regeneration. The scaffold serves as a framework for cells by providing an appropriate environment on which to live and grow, promoting the development of an extracellular matrix and other biological molecules and by facilitating the formation of tissue and organ functions (Hassan et al. 2014). Furthermore, it provides a favourable environment for cell proliferation and attachment.

Several techniques have been used to synthesise tissue engineering scaffolds, such as solvent casting, gas foaming, freeze drying, rapid prototyping, phase separation and electrospinning. Recently, electrospinning has been used to produce polymeric fibres with controlled diameter, uniform structure and large surface area. This technique produces nanofibres by applying an electrostatic field to a polymer solution driven by a high voltage supply to create a strong repulsive electrical force to overcome the surface tension of the charged polymer solution. The benefits of electrospinning are the synthesis of nanofibres with high surface to volume ratio, uniform structure, tunable porosity, and malleability to conform to a wide variety of sizes and shapes (Bhardwaj & Kundu 2010).

Nanofibres are potential scaffolds for tissue engineering (Wang et al. 2018), and have been synthesised from PCL and chlorophyllin sodium copper salt (CSC) by electrospinning. The presence of CSC inside PCL nanofibres provided adequate space for living cells in the scaffold (Zulkifli et al. 2019). The potential of PCL/HA nanofibres for tissue engineering scaffold applications has also been suggested. The addition of HA resulted in bead-less fibers without agglomerates, with the fibers thicker than those of PCL nanofibers. SEM images further showed that the scaffolds were biocompatible, which suggested that the PCL/HA will be a suitable candidate for scaffold in tissue engineering applications (Hassan et al. 2014).

PCL is a non-toxic and biodegradable polymer (Dash & Konkimalla, 2012), with a slow degradation rate, hence, is a suitable candidate for fabricating fibrous scaffolds. However, PCL is hydrophobic which may affect cell adhesion and the degradation rate, therefore, bioceramics, such as HA, are mixed with PCL to produce the scaffold to provide a favourable environment for cell attachment and proliferation. HA is osteoconductive and hydrophilic compared to PCL polymers and is a type of calcium phosphate with similar chemical morphology to human bones (Serra et al. 2016), thus, improves the biocompatibility and strength of electrospun PCL nanofibres (Jaiswal et al. 2013). Fibronectin is an extracellular matrix protein that promotes cell growth, adhesion, migration, differentiation and wound healing. In tissue engineering, cell adhesion is very important and generally, the cell will adhere to the synthetic surface through protein adsorption (Sun et al. 2019). Hence, the adhesion of fibronectin to the surface of the nanofibres is essential to develop a suitable scaffold for tissue engineering.

Very few literatures are reported for combined fibronectin, HA and PCL electrospun scaffolds for tissue engineering to the best of our knowledge. Therefore, this study aimed to investigate the synthesis of PCL/HA nanofibres via electrospinning for the production of scaffolding for tissue engineering applications, also adding fibronectin to improve the biocompatibility of the scaffold.

METHODOLOGY

MATERIALS

Polycaprolactone (MW 80,000 g/mol), phosphoric acid, calcium hydroxide and fibronectin were purchased from Sigma-Aldrich. The solvent 99% acetic acid, 30% ammonia solution and sodium hydroxide were purchased from System Chemicals (Malaysia). All reagents and materials were used as received without any further purification.

PREPARATION OF HYDROXYAPATITE

HA was synthesized through wet chemical precipitation in the ratio Ca/P=1.67. The mixture was stirred for 1.5 hr at 300 rpm and 80°C at pH 10, then cooled to room temperature for 24 hr and filtered. The suspension was dried in the oven at 95°C for 2 hr and calcined in a furnace at 700°C for 4 he (Syed & Qasim, 2014; Mohamed Rafie & Nordin, 2017).

PREPARATION OF COMPOSITE HYDROXYAPATITE/ POLYCAPROLACTONE (PCL-HA)

Different HA concentrations were dissolved in acetone and labelled as shown in Table 1. Stirring was performed using a magnetic stirrer at 40°C. Then, 0.5, 1.0 and 1.5 g of HA was added into the polymer solution samples and mixed using a magnetic stirrer for 24 hr at room temperature. Each polymeric solution was transferred into a 5 mL syringe (Moeini et al. 2017).

TABLE 1. Sample solutions prepared before electrospinning.

Sample	Solution
1	50 mg/mL of PCL
2	50 mg/mL of PCL with HA (5%)
3	50 mg/mL of PCL with HA (10%)
4	50 mg/mL of PCL with HA (15%)

ELECTROSPINNING OF HA-PCL NANOFIBRES

The equipment used for electrospinning (ES-106) was purchased from the Nfiber Company. During electrospinning, the flow rate of the solution was maintained at 1.5 mL/h, with an applied voltage of 25 kV, and a 12-cm distance was maintained between the tip nozzles and the collector (Rezk et al. 2019). This process was conducted for an hour at 22 ± 5°C and 35 ± 4% humidity. Then, samples 1-4 were taken from the aluminium foil and vacuum dried for 12 hr.

FIBRONECTIN ADHESION TO HA-PCL NANOFIBRES

The PCL-HA (10%) nanofibres from sample 3 were dissolved in fibronectin solutions for 40 minutes to prepare samples for fibronectin fixation as shown in Table 2. All samples were air-dried before use (Nordin et al. 2011).

TABLE 2. Concentration of fibronectin

Sample	Solution
5	PCL-HA without fibronectin
6	PCL-HA with 50 µl/mg of fibronectin
7	PCL-HA with 100 µl/mg of fibronectin
8	PCL-HA with 150 µl/mg fibronectin

CHARACTERISATION OF HYDROXYAPATITE

The crystal phase and degree of crystallinity were characterised using X-ray diffraction (XRD, D*-Advance, Bruker Company, Germany) with Cu Kα radiation (λ=0.15408). The HA was synthesised at pH 10 according to Mohamed Rafie and Nordin (2017). The XRD data were collected at room temperature.

MORPHOLOGICAL ANALYSIS

The strip was coated with Pd for 45 seconds before SEM under vacuum. The distance between the edge of the electron gun and the sample surface was set at 7.2 mm with an accelerating voltage of 15 kV. The magnification rates of 300 ×, 1000 × and 5000 × were used for testing the structural analysis and morphology for samples 1–4.

FTIR ANALYSIS

Samples 1–4 were placed on the plate of the IR-microscope and the chemical content in the nanofibres determined using the FTIR analysis software.

FIBRE DIAMETER AND ADHESIVE FORCE DETERMINATION BY AFM

The strip was attached to the metal plate using adhesive tape and 10 µm x 10 µm images were analysed to determine the average diameter of the samples 5–8. Measurements were taken at 50 different locations using the XEI software. The adhesive force of the nanofibres was determined by attaching the AFM probe.

RESULTS AND DISCUSSION

XRD STUDY OF HA

The structure and phase composition morphology of crystalline hydroxyapatite were analysed by XRD with radiation Cu Kα (λ=0.15408) as shown in Figure 1. From the XRD pattern, the highest peak is at 30° to 35°, indicating pure HA as in a previous report (Mohamed Rafie & Nordin, 2017). Furthermore, the HA had high crystallinity, as evidenced by the narrow diffraction peak (Wijesinghe et al. 2014).

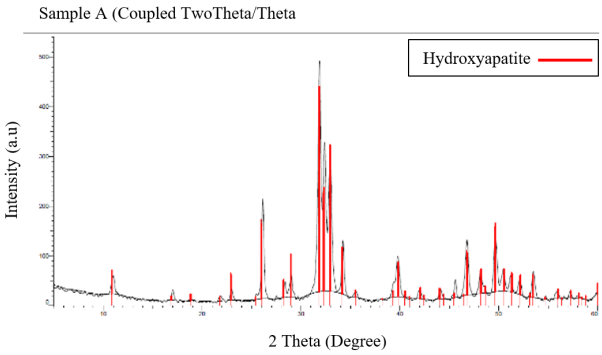
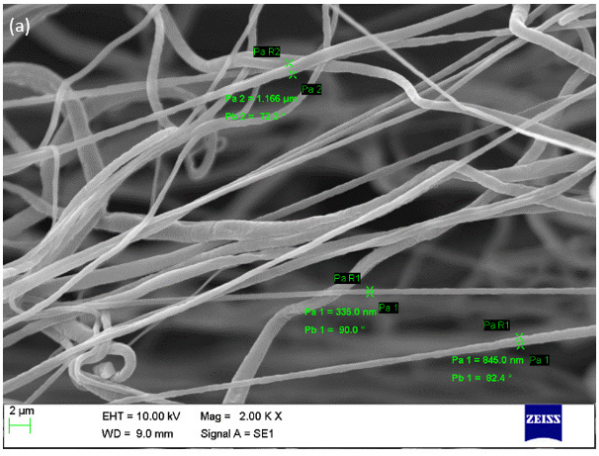


FIGURE 1. XRD pattern of HA

MORPHOLOGY OF THE NANOFIBRES

The SEM analysis for samples 1–4 is presented in Figure 2, showing that the nanofibres were on a nanometre scale with porous surfaces. The PCL sample only possessed smooth, beadless and cylindrical fibres, whereas the PCL-HA nanofibres were thicker and denser because HA can generate fibres, thereby enhancing the formation of PCL nanofibres. This confirmed that HA was well mixed with the PCL polymer solution and embedded into the PCL fibres, and was in agreement with a previous study which showed that incorporation of HA made the PCL-HA nanofibres smoother with almost unnoticeable bead defects compared to PCL nanofibres (Hassan et al. 2014). Sample 3 was selected for fibronectin fixation as it forms a denser, smooth and uniform fibre network suitable for scaffold preparation.



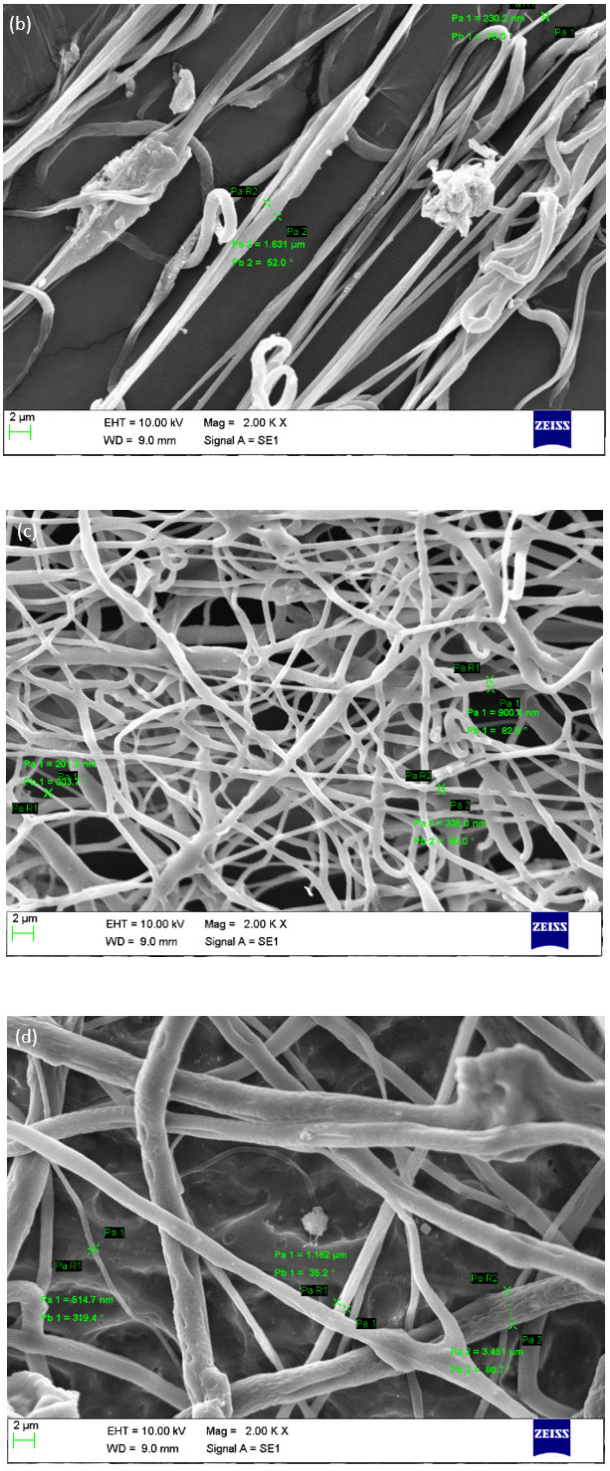


FIGURE 2. SEM images of nanofibres (a) Sample 1 (b), Sample 2 (c), Sample 3 and (d) Sample 4

FTIR OF THE ELECTROSPUN NANOFIBRES

Chemical characterisation of nanofibres was performed by FTIR as shown in Figure 3. All samples had a similar pattern, showing the presence of a carbonyl group of C = O at 1500–1800 cm-1 (Elzein et al. 2004). The band at 1294 cm-1 was assigned to the C-C backbone and C-O stretching modes in the crystalline PCL (Coleman & Zarian, 1979). For Figure 3(b), 3(c) and 3(d), the band at 3500 cm-1 was attributed to the bending of a hydroxyl group (OH-) and the band at 650 cm-1 is related to bending vibration of the hydroxyl group. HA characteristics were found at 1400–1500 cm-1, which represent the carbonate group, indicating that the presence of HA in the PCL nanofibres for Figure 3(b), 3(c) and 3(d).

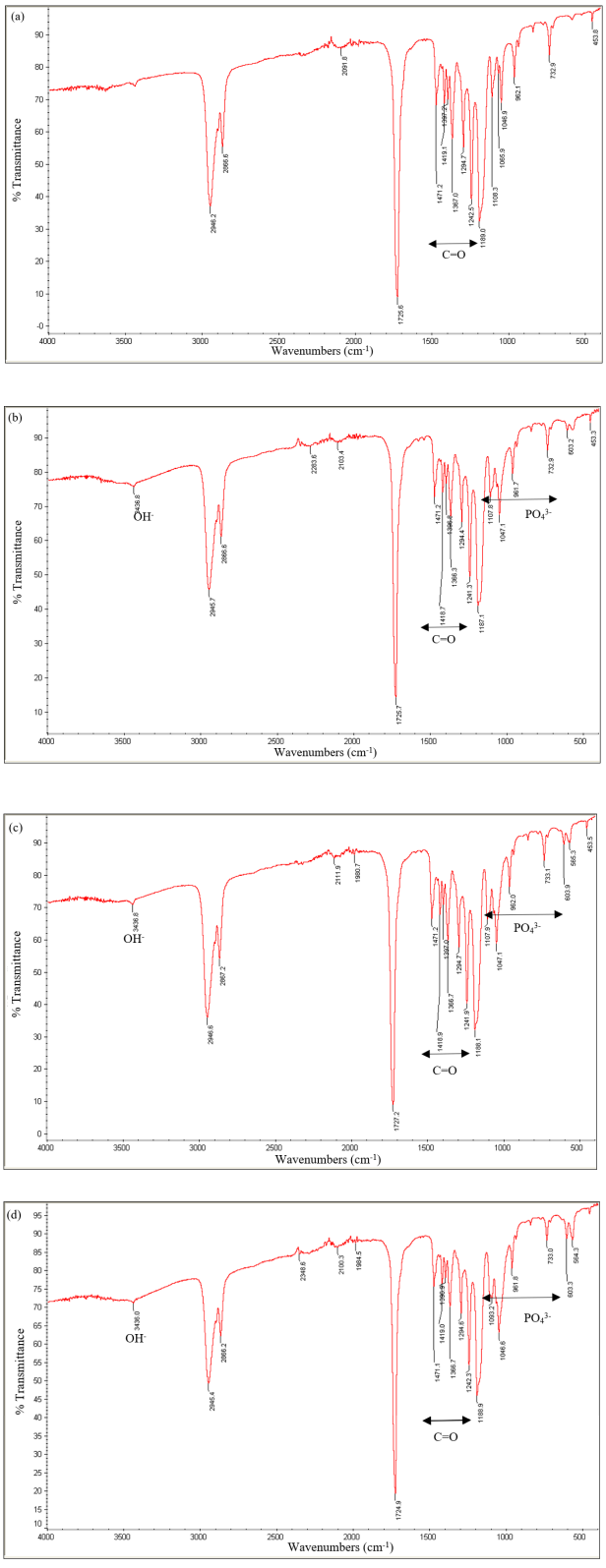


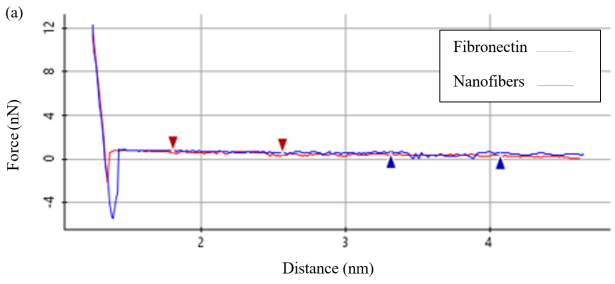
FIGURE 3. FTIR spectra of nanofibres (a) Sample 1 (b), Sample 2 (c), Sample 3 and (d) Sample 4

DIAMETER AND ADHESIVE FORCE OF NANOFIBRES WITH FIBRONECTIN

The diameter and adhesive force of the nanofibres represent important properties that must be considered for tissue engineering scaffolds (Xing et al. 2010). The average diameter of nanofibres in samples 5–8 are presented in Table 3, showing an increase in the diameter of PCL-HA nanofibres with increasing fibronectin concentration. This is because there is the adhesion of fibronectin to the PCL-HA nanofibre membrane which makes the structure of the PCL-HA nanofibres network thicker and denser compared to PCL-HA nanofibres without fibronectin.

Figure 4 shows the fibronectin molecules (red line in the graph) interacting with the fibres (blue line in the graph). The location of fibronectin within the complexes was characterised through the forced unfolding of the protein (Li & Xia, 2004). The interaction between fibronectin and surface of nanofibres occurs through the covalent attachment of the protein to the AFM tip and subsequent ripping of the molecule from the surface. Fibronectin adhered to the surface can be seen on the sawtooth pattern. The adhesion force and energy for samples 5–8 are shown in Table 4. For sample 5, the adhesion energy is zero because the adhesion force only occurs on the tip of AFM, hence, the adhesion force for sample 5 cannot be considered. The maximum adhesion force was obtained for sample 7 (PCL-HA with 100 µg/ml of fibronectin), hence, it was a better option for a tissue engineering scaffold as it is strong enough to resist the force from body movements or the external environment (Lee et al. 2017).

To further prove that fibronectin adhered to the surface of PCL-HA nanofibres, FESEM images are taken for samples 5–8 to observe the morphology and structure of the PCL-HA nanofibres as shown in Figure 5. Figure 5(a), (b) and (c) show that there is fibronectin on the nanofibres which makes the structure more compact and denser compared to nanofibres in Figure 5(a) with no fibronectin. Thus, nanofibres with adhered fibronection have the potential to be used for developing tissue engineering scaffolds.



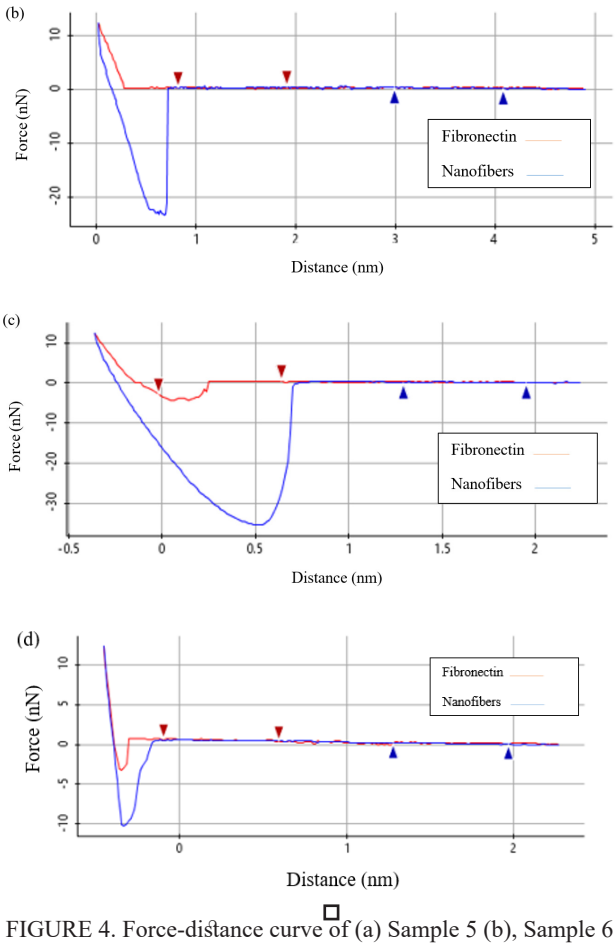
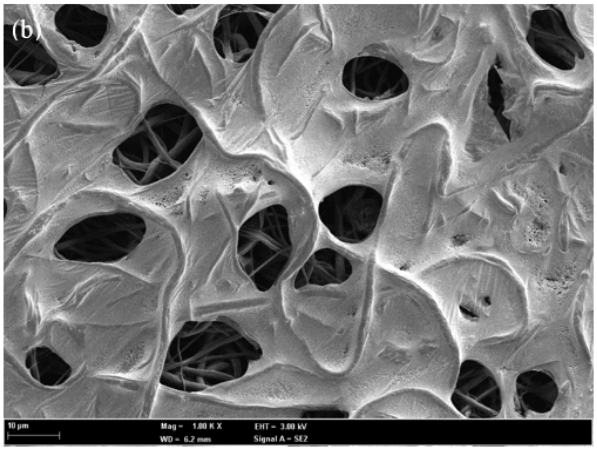


FIGURE 4. Force-distance curve of (a) Sample 5 (b), Sample 6 (c), Sample 7 and (d) Sample 8

TABLE 3. Adhesion force, energy and average diameter for samples 5–8

Sample	Average diameter (nm)	Adhesion force (nN)	Adhesion energy (J)
5	201.3	382.03 × 106	0
6	322.05	23.75	8.56
7	535.49	35.58	21.93
8	801.97	10.27	1.31



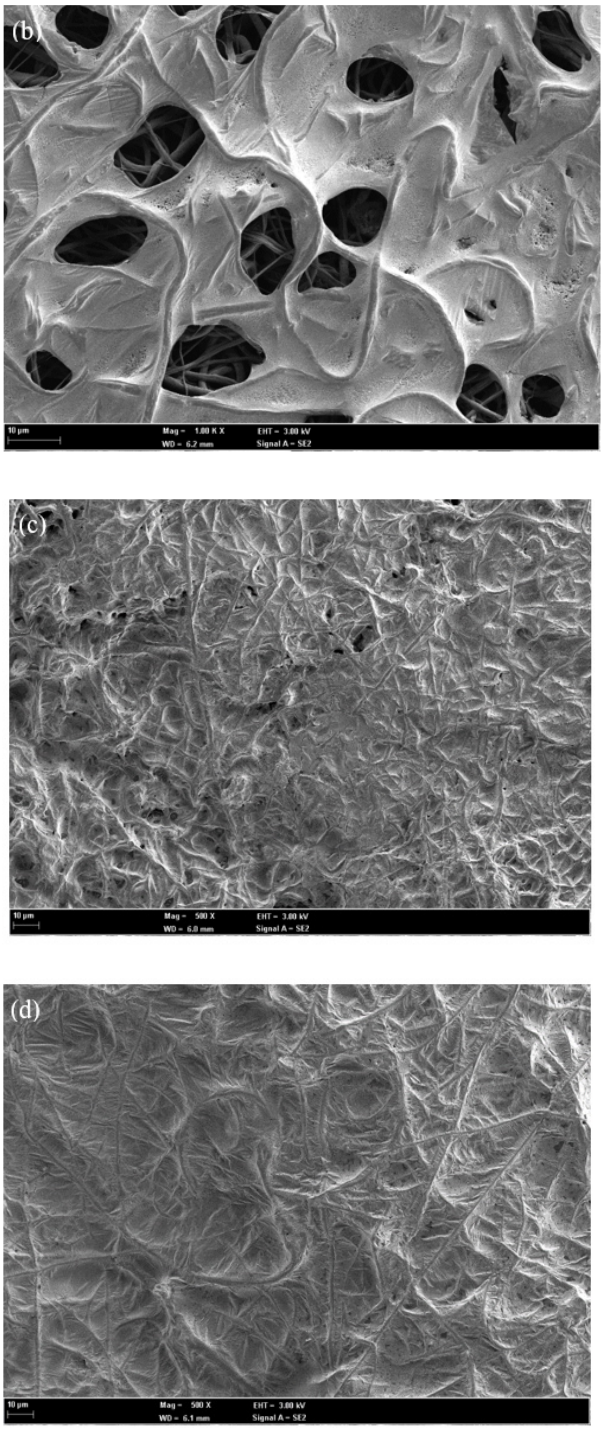


FIGURE 5. FESEM image for a) Sample 5 b), Sample 6 c), Sample 7 and (d) Sample 8

CONCLUSION

PCL-HA nanofibres were successfully prepared by electrospinning. Based on SEM result, the presence 10% of HA on PCL nanofibre forms a cylindrical and smooth nanofibre with dense fibre networks due to HA can generate fibre. The adhesion of 100 µg/ml fibronectin provided PCL-HA nanofibers (Sample 7) suitable for tissue engineering applications because it provided the highest adhesion force, which is important for the scaffold to resist the force from body movements or the external environment. Furthermore, the PCL-HA nanofibres are expected to be beneficial for cell growth as HA is osteoconductive, possessing bone-bonding ability useful for tissue engineering fibrous scaffolds. The biodegradable PCL-HA nanofibres produced are promising for tissue engineering scaffold applications but further investigations are required to determine the compatibility of living cells to survive and grow on the PCL-HA nanofibrous mats.

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DECLARATION OF COMPETING INTEREST

None

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