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Conductive Bio-Copolymers based on Pectin-Polycaprolactone/ Polyaniline and Tissue Engineering Application Thereof

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ABSTRACT

Development of biopolymers possessing both biodegradable and electrically conducting properties has attracted a huge interest in the biomedical field. These systems have some benefitials in wound healing and reducing the long-term health risks. In this study, the pectin-polycaprolactone (Pec-PCL) copolymers were synthesized by ring-opening polymerization. Subsequently, the solutions of the synthesized Pec-PCL and homopolyaniline (H-PANI) were blended in various ratios and their conductivity properties were measured by cyclic voltammetry and the composition of 80:20 was selected for electrospinning process because of the suitable electroactive behavior and biodegradability. The morphology, biocompatibility, hydrophilicity, and mechanical properties of the nanofibers were thoroughly investigated. Resulted scaffolds represented a porous structure with large surface area (110–130 nm) and Young's modulus of 1615 ± 32 MPa, which imitated the natural microenvironment of extra cellular matrix (ECM) to regulate the cell attachment, proliferation and differentiation. The results demonstrated that these electrospun nanofibers could be potentially applied in biomedical such as tissue engineering.

Keywords: *Conductive polymer; pectin; PCL; polyaniline; biodegradability.*

1. Introduction

Tissue engineering includes a deep knowledge about the cells, growth factors, and material engineering to provide the potential alternatives for tissue transplantation [1-3]. The design of three-dimensional (3D) scaffolds to construct the cell based matrices is a key factor [4–7]. The porous scaffolds with interconnecting pores act as analogues of an extracellular matrix (ECM), which is constituent of tissue for facilitating the cellular

functions like cell growth and differentiation [8–11]. Distinct synthetic and natural biomaterials have been investigated for their applications as scaffolds. Although the synthetic scaffolds are capable of controlling their mechanical features, they have limitations such as poor degradability and toxicity [12-16]. On the other side, the natural materials are expected to be better candidates for the efficient cell adhesion and are also fairly stable [17–20].

The controlled biodegradation and its rate are the vital characteristics in scaffolding [21]. There are a lot of biodegradable polymers can be used for scaffold construction, among which the biopolymers are of the most promising categories. They have been amply used in the scaffolds and regenerative medicine because of their biological and chemical similarities with natural tissues [22–26]. Pectins (Pec) are polysaccharides, composed of (1,4)-linked α-D-galacturonic acid residues, subsuming some 2-OsubstitutedL-rhamnopyranose residues. Pectins have also a wide range of physiological activity and exhibit hypocholesterolemic, enterosorptive, anti-inflammatory, antibacterial, antitumor, antiulcer, etc. [27–30]. They improve the cell adhesion, proliferation, and differentiation [31]. The pectin-based biomaterials are extensively employed in tissue engineering, wound dressing, drug delivery, and other biomedical applications [26,32,33].

The electrical signals can regulate the cell attachment, proliferation and differentiation [34]. An electric field also elevated the endochondral ossification [35], stimulates the healing of chronic wound and nerve regeneration [36,37], and assists in retrieving the function in the damaged rodent spinalcord [38]. The preparation of scaffolds for the central nervous system tissue engineering was extensively reported [39,40]. Several researchers have focused on incorporation of the conducting polymers into the biomaterials for their electrical stimuli [41,42]. In the current work, we prepared the Pec-PCL/H-PANI copolymers with various contents and, consequently, selected the proper composites to afford the electrospun nanofibers. The features of electrospun nanofibers were thoroughly investigated for the tissue engineering purposes. A research work has been reported in the field of pectin-based scaffolds [43], in which another scaffolding method was utilized. Furthermore, in the mentioned investigation, the Young's modulus was low because of the applied natural polymers. On the other hand, in our research, in addition to the PANI precursor for retrieving the conductivity in tissue engineering, the PCL was used to optimize the mechanical properties and also to increase the Young's modulus.

2. Experimental

2.1. Materials and Methods

Pectin (number average molecular weight (M_w) = 45000–50000 g mol^{-1}) were purchased from Sigma. Caprolactone (CL, 99%) was purchased from Merck and distilled under reduced pressure over calcium hydride (CaH_2) prior to use. The tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) was prepared from Sigma-Aldrich. Aniline monomer was purchased from Merck (Darmstadt, Germany) and was distilled under the reduced pressure prior to application. Ammonium peroxydisulfate (APS; Merck) was recrystallized at room temperature from ethanol. The poly(3-caprolactone) (PCL) (number average molecular weight (M_n) = 70000–90000 g mol^{-1}) was purchased from Merck. All other reagents were prepared from Merck and purified according to the standard methods.

2.2. Synthesis of pectin-PCL (Pec-PCL)

The Pec-PCL copolymers were synthesized in a reactor with the appropriate feeds of the CL monomer and pectin. The reaction mixture was agitated at 100 °C within 1 h for completely dissolving the pectin in the CL monomer. $Sn(Oct)_2$ (0.03 mol %) was subsequently added to the reaction mixture. The reaction was carried out at 140 °C for 18 h under nitrogen and the resultant brown product was dissolve in the dimethyl sulfoxide (DMSO) and precipitated in water and filtered, washed several times, and eventually dried in vacuum at room temperature (Fig. 1).

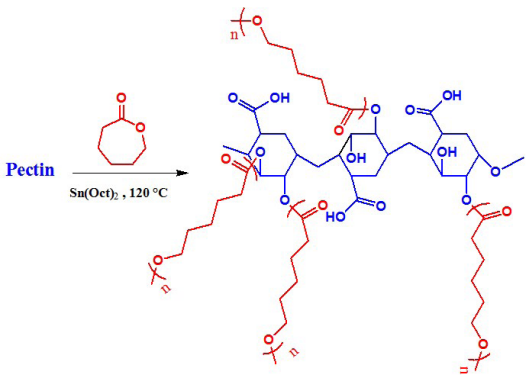

Fig. 1- Synthesis of Pec-PCL copolymer.

2.3. Synthesis of H-PANI

A reactor was filled with 30 mL distilled water, 12 mmol aniline monomer, and 24 mmol p-toluenesulfonic acid (p-TSA). The reaction mixture was stirred to reach a homogeneous solution and temperature was the decreased to 0 °C. Parallely, 12 mmol APS was dissolved in 50 mL deionized water and was gently added to the above-mentioned reactor. The reaction mixture was stirred for 1 day at 0 °C and the reaction was then terminated by pouring the reactor content into a large amount of methanol. The resultant product was filtered, washed several times with methanol, and dried in vacuum at room temperature.

2.4. Electrospinning of Pec-PCL/H-PANI

The Pec-PCL copolymers and homo-polyaniline (H-PANI) were dissolved in DMSO at 10% w/v at the ratios 90:10, 80:20, 70:30, 60:40 and 50:50. The PCL was also dissolved in CCl_3 at 5% w/v under stirring until the mixture was homogeneous. The solution blends were prepared with a ratio of 70:30 (v/v) from Pec-PCL/H-PANI and PCL, respectively. The prepared solutions were subsequently added to a syringe with a hypodermic needle used as the nozzle at room temperature. The solutions were injected at a rate of 0.3 mL/h and voltage of 23 kV. After the nanofiber deposition, the mats were dried at room temperature to remove the solvents residues.

2.5. Biocompatibility tests

2.5.1. Cell culture

The osteoblast MG63 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 10%(v/v) fetal bovine serum (FBS) with 100 mL^{-1} penicillin and streptomycin and incubated at 37 °C in 5% CO_2.

2.5.2. Morphology

So as to probe the biocompatibility of systems, the osteoblast MG63 cells grown onto the electrospun nanofibers were examined using the field emission scanning electron microscopy (FESEM). The cells were seeded onto the six-well plates pre-coated with the nanofibers at 10^5 cells/cm and grown within 1 day. The cells were washed with distilled water and fixed with 2% glutaraldehyde (Sigma-Aldrich) for 1 h at room temperature. The samples were subsequently dried, coated with gold/palladium mixture, and examined by FESEM

2.6. Characterization

Fourier transform infrared (FT-IR) spectra of the synthesized materials were recorded on a Shimadzu 8101M FT-IR (Shimadzu, Kyoto, Japan). The poroton nuclear magnetic resonance (^{1}HNMR) spectra were recorded by a FT-NMR (400 MHz) Bruker spectrometer (Bruker, Ettlingen, Germany) in the deuterated dimethylsulfoxide (DMSO-d_6). FESEM type 1430 VP (LEO Electron Microscopy Ltd, Cambridge, UK) was applied to determine the surface morphologies of the nanofibers and the electrochemical experiments were conducted on Auto-Lab PGSTA T302N.

3. Results and discussion

The electrical stimulation could modify the cellular activities like cell migration [44], cell adhesion [45], DNA synthesis [46,47] and protein secretion [48] and also regulate the cellular activities in an artificial scaffold in order to control the regeneration of damaged tissues [49].

3.1. Characterization of Pec-PCL copolymers

3.1.1. FT-IR and ^{1}HNMR spectroscopies

FT-IR spectra of the pristine pectin and Pec-PCL copolymers are displayed in Fig. 2. The stretching vibrations of aliphatic C–H at 2950–2800 cm^{-1}, C–H bending vibrations at 1261 and 1436 cm^{-1} and C–O stretching vibrations at 1033 and 1153 cm^{-1} were detected for the pure pectin. In addition, the hydroxyl end groups were observed as a broad strong band centered at 3423 cm^{-1}. The conspicuous

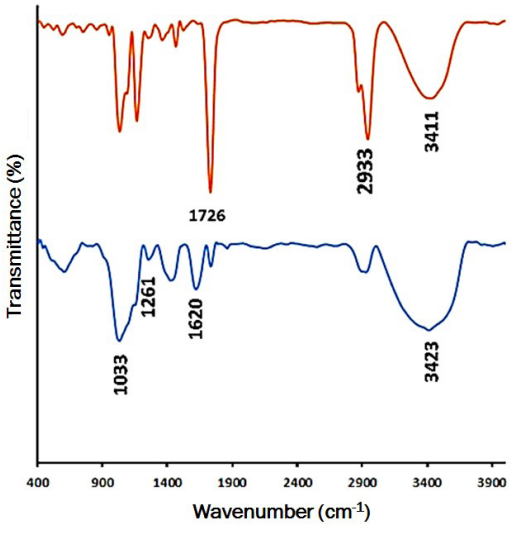

Fig. 2- FT-IR spectra of pectin (blue) and Pec-PCL (red) copolymers.

alterations were detected in FT-IR spectra of the Pec-PCL copolymers with respect to those obtained for the pristine pectin, i.e., stretching vibration of carbonyl group at 1726 cm^{-1} and stretching vibrations of aliphatic C–H at 2950–2800 cm^{-1}. In addition, the intensity of the hydroxyl stretching vibration remarkably decreased to further verify the synthesis of Pec-PCL.

The synthesized Pec-PCL copolymers were further characterized via ^{1}HNMR spectroscopy. ^{1}H-NMR spectrum of the Pec-PCL is depicted in Fig. 3. The prominent resonance peaks (1–4) and (a–d) were attributed to the pectin and PCL, respectively. In addition, a chemical shift associated with the –COOH groups of pectin is detected at 12 ppm (Fig. 3).

3.1.2. GPC analysis

The gel permeation chromatography (GPC) traces of Pec-PCL copolymers are reported in Fig. 4. The polydispersity index (PDI) of Pec-PCL copolymers synthesized through the ring opening polymerization was to some extent low (M_n = 87981 g/mol and PDI = 1.23).

3.2. Characterization of H-PANI

3.2.1. FT-IR spectroscopy

FT-IR spectra of the H-PANI are exhibited in Fig. 5 possessing the stretching vibrations of aromatic C–H at 3050–3200 cm^{-1}, γ(C–H) in the aromatic ring at 674, and 769 cm^{-1}, the N–H stretching vibration at 3517 cm^{-1}, stretching vibration of the C=C in the benzenoid units at 1551

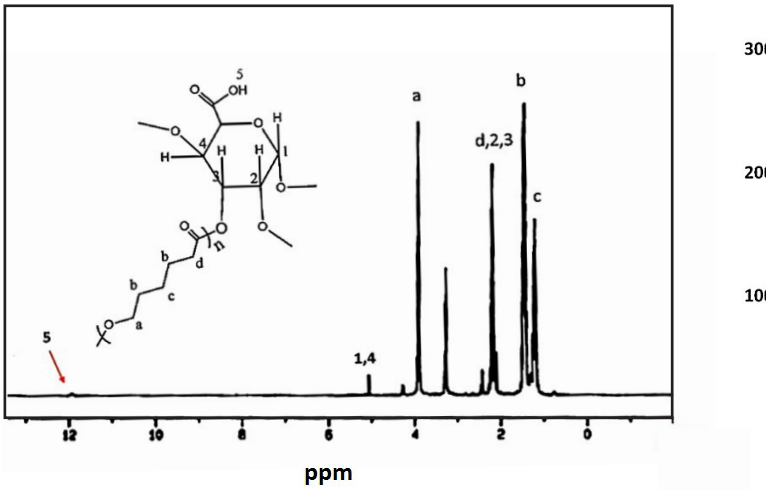

Fig. 3- ^{1}H-NMR spectra of the Pec-PCL.

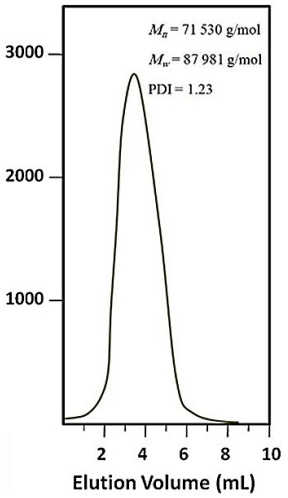

Fig. 4- GPC traces of Pec-PCL in the dimethylformamide (DMF) as eluent.

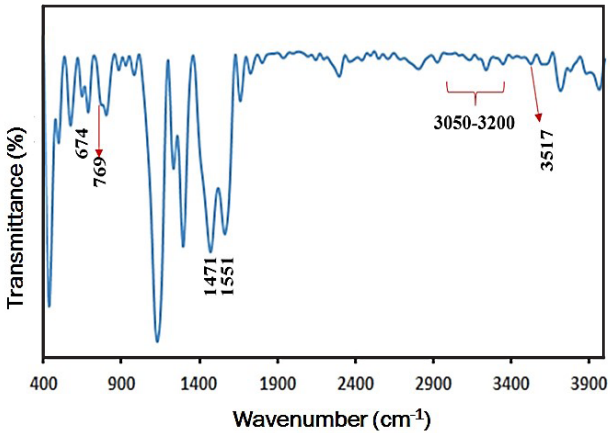

Fig. 5- FT-IR spectra of the H-PANI.

cm^{-1}, $C_{aromatic}$–N stretching vibration at 1294 cm^{-1} and the combination bands at 1650–1900 cm^{-1} [35].

3.3. Electroactivity features

Electrically conductive polymers have attracted remarkable attention in recent decades because they simultaneously own the physical and chemical properties of organic polymers and the electrical characteristics of metals. The PANI is one of the most charming electrically conductive polymers thanks to its unique properties, e.g., controllable electrical conductivity, environmental stability, and rich redox chemistry, and also for its potential applications such as anticorrosion coatings [50], batteries [51,52], sensors [53,54], separation membranes [55,56], antistatic coatings [57] and electromagnetic interference shielding [58,59]. Recently, PANI has been introduced as novel intelligent scaffolds for the cardiac and/or neuronal tissue engineering [60–64]. The peaks in the cyclic voltammogram (CV) are attributed to the electrochemical responses of PANI. The mentioned peaks also shed light on the charge injection during the interconversion of two oxidation states of PANI. The CVs of Pec-PCL/H-PANI with varied ratios for 5 cycles in constant rate of 25 mV s^{-1} in the sulfuric acid (1 mol L^{-1}) between –0.20 and +1.20 V versus the reference electrode are illustrated in Fig. 6. The CVs of the Pec-PCL/H-PANI films exhibited different anodic and cathodic peaks by elevating the PANI content. Moreover, the amount of current increased parallel with the PANI enhancement. The chemical reversibility was promoted in the ratio of 80:20 for the Pec-PCL/H-PANI films. The effect of the potential scanning rate (V) on the peak currents for the Pec-PCL/H-PANI was investigated in the range of 10 to 60 mV s^{-1} scan rate in the sulfuric acid between –0.20 and +1.20 V against the reference electrode. According to Fig. 7, the anodic peaks of the Pec-PCL/H-PANI films were shifted towards the higher potentials with raising the scan rates and PANI contents.

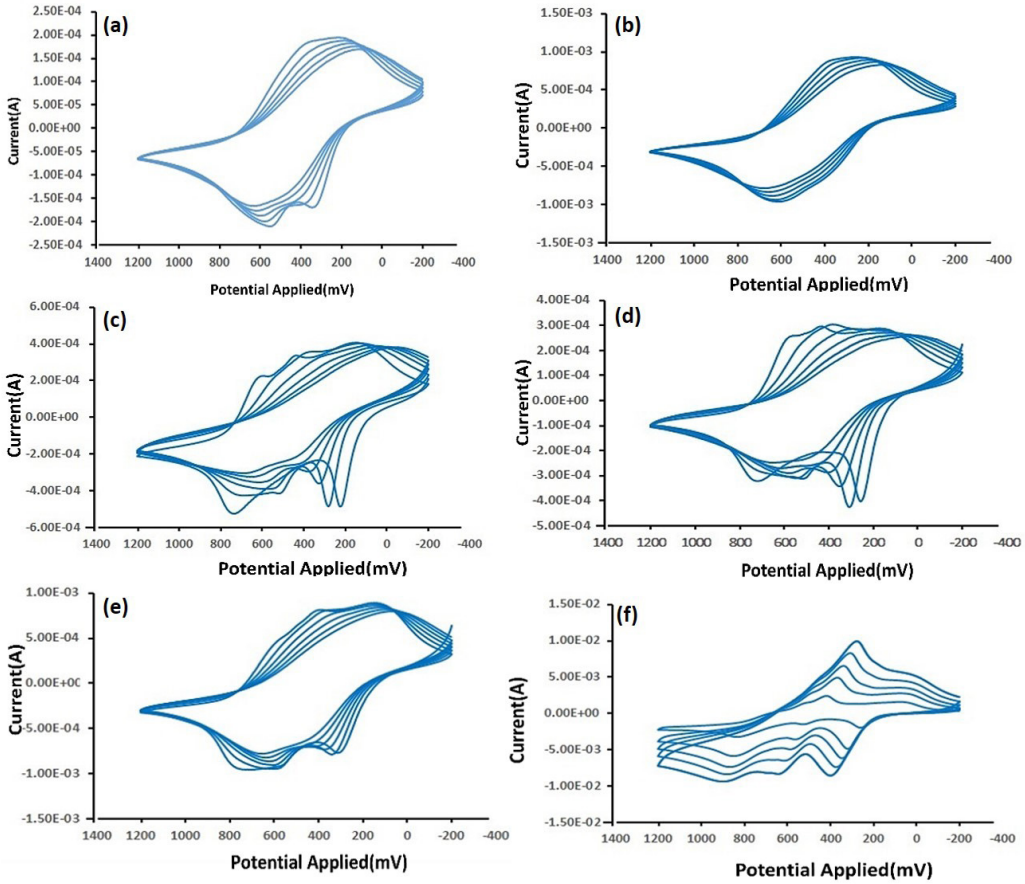

Fig. 6- Cyclic voltammograms of Pec-PCL:H-PANI comosites in the scan rate of 25 mV s^{-1} in concentrations of 90:10, 80:20, 70:30, 60:40, 50:50 and 0:100 (a–f), respectively, in the aqueous solution of sulfuric acid (1 mol L^{-1}) between –0.20 to +1.20 V.

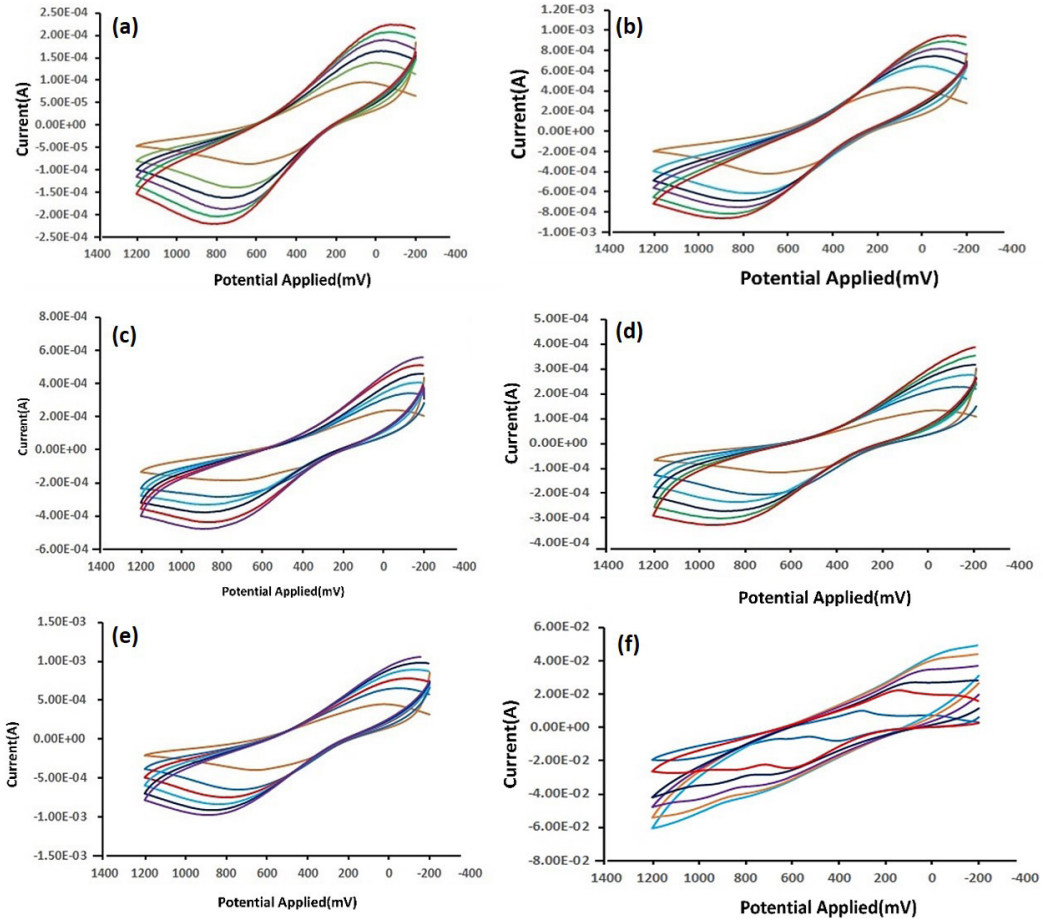

Fig. 7- Cyclic voltammograms of Pec-PCL/H-PANI in the scanning range of 10–70 mV s^{-1} and the ratios of 90:10, 80:20, 70:30, 60:40, 50:50 and 0:100 (a–f), respectively, in aqueous solution of sulfuric acid (1 mol L^{-1}) between –0.20 to +1.20 V.

Therefore, the electrochemical oxidation/reduction of the films were chemically reversible and the resulting polymers owned the proper stabilities. Notably, the composition of 80:20 was selected for electrospinning process because of the suitable electroactive behavior and biodegradability.

3.4. Characterization of electrospun nanofibers

3.4.1. Morphology

The surface morphologies of Pec-PCL/H-PANI/PCL electrospun nanofibers were studied by FESEM. As illustrated in FESEM image of Fig. 8, the samples presented the uniform nanostructures. Herein, the 3D interconnected pore structures with the average diameters ranged in 110–130 nm were formed.

3.4.2. Contact angle

The surface hydrophilicity of scaffolds affects their capability in the cell attachment. The water contact angles of the electrospun nanofibers were measured at room temperature to investigate their surface properties. The contact angle of electrospun nanofibers with the water was 72 ° ± 5. Furthermore, a typical photograph of water drop on the electrospun nanofiber is shown in Fig. 9.

3.4.3. Mechanical properties

Selection of type and material of biological scaffolds is very important for designing the properties of target tissue. The scaffolds not only induce the cell connections but also cause the cell migration, transfer of biochemical factors, and the release of nutrients. In this regard, a scaffold must possesses the appropriate structural features like prominent mechanical properties [35]. The prepared samples exhibited a linear elastic behavior before failure. At a deforming speed of 5 mm/min, Young's modulus, tensile strength, and elongation

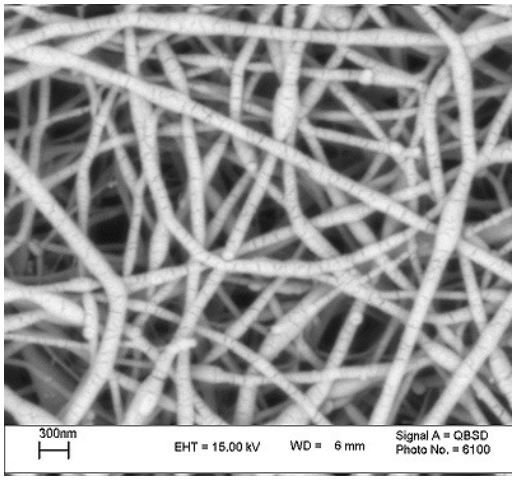

Fig. 8- FESEM image of the Pec-PCL/H-PANI /PCL electrospun nanofibers.

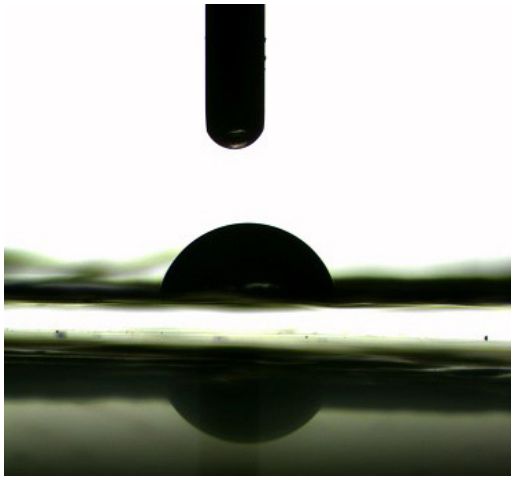

Fig. 9- Photograph of water drop on the Pec-PCL/H-PANI/PCL electrospun nanofiber.

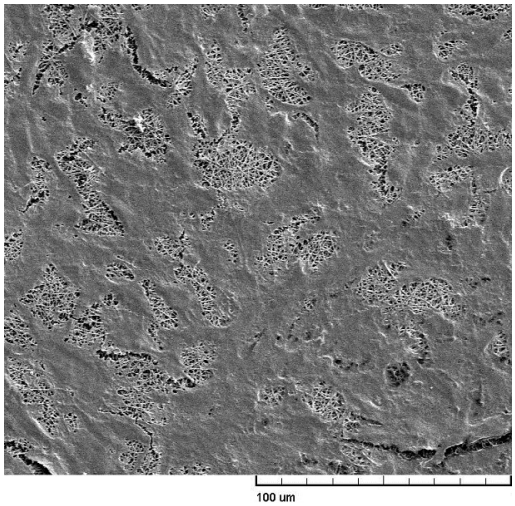

Fig. 10- FESEM image of the mouse osteoblast MG63 cells onto the Pec-PCL/H-PANI/PCL electrospun nanofibers after 7 days.

at break were 1615 ± 32 MPa, 159.21 ± 10 MPa and 28.10 ± 3.7%, respectively.

3.5. Biocompatibility of electrospun nanofibers

3.5.1. Cell morphology

Fig. 10 depicts the morphology of osteoblast cells cultured on the scaffold after 7 days in culture. These osteoblast cells tightly adhered to the Pec-PCL/H-PANI/PCL nanofibers and fabricated the integrated cell-fiber configurations. The cells in these constructs displayed a wide cell-cell contact which is helpful for the cell activity and function and thus promotes the cell proliferation [41]. The novel 3D Pec-PCL/H-PANI/PCL scaffolds were capable of providing an interconnected porous structure and large surface area for the osteoblast cell adhesion and proliferation. Fig. 10 shows FESEM image of the mouse osteoblast MG63 cells onto the Pec-PCL/H-PANI/PCL electrospun nanofibers after 7 days. FESEM results approved that the nanofibers provided more active sites for the live cells to spread and thus the cells could be further expanded on the surface.

4. Conclusions

The Pec-PCL copolymers were synthesized through the ring opening polymerization of PCL with the pectin as an initiator and the PANI precursors were obtained from the chemical oxidation polymerization of aniline. The electrospun

nanofibers were fabricated based on the Pec, PCL, and PANI constituents. The proper electroactivity could in turn provide the special function to improve the adhesion, growth, and proliferation of cells. Owing to the presence of the PCL and pectin segments in the copolymer configuration, it was biodegradable, solving the application problem of conducting or electroactive polymers in vivo for a long time. Moreover, the synthesized copolymers represented a good solubility, so it was easy to process the copolymer materials as scaffold. The Pec-PCL/H-PANI copolymers and their electrospun nanofibers could be prosperously considered as a novel biomaterial and a proper candidate for the electroactive polymers employed in the biomedical fields.

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