Hindawi
International Journal of Polymer Science
Volume 2021, Article ID 6689789, 18 pages
https://doi.org/10.1155/2021/6689789

Research Article

Poly(ε-caprolactone)/Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Blend from Fused Deposition Modeling as Potential Cartilage Scaffolds

Wasana Kosorn and Patcharaporn Wutticharoenmongkol

Department of Chemical Engineering, Faculty of Engineering, Thammasat University, Pathum Thani, Thailand 12120

Correspondence should be addressed to Patcharaporn Wutticharoenmongkol; tpatchar@engr.tu.ac.th

Received 25 November 2020; Revised 26 February 2021; Accepted 8 March 2021; Published 23 March 2021

Academic Editor: Marta Fern ndez Garc a

The scaffolds of poly(ε-caprolactone)/poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PCL/PHBV) blends were fabricated from fused deposition modeling. From indirect cytotoxicity testing based on mouse fibroblasts, all scaffolds with various blend ratios were nontoxic to cells. The surface-treated scaffold with a blend ratio of 25/75 PCL/PHBV exhibited the highest proliferation of porcine chondrocytes and total glycosaminoglycans (GAGs) after 21 days of culture. The scaffolds with a blend ratio of 25/75 with local pores (LP) were prepared from FDM along with a salt leaching technique using NaCl as porogens. The effect of NaOH in surface treatment on the biological property of scaffolds was investigated. The scaffolds with LP and with 1 M NaOH surface treatment exhibited the highest proliferation of cells and total GAGs after 28 days of culture. The degradation behaviors of the scaffolds were studied. The nonsurface treated, surface treated without LP, and surface treated with LP scaffolds were degraded in phosphate buffer (pH 7.4) for 30 days at 37°C and 50°C for nonenzymatic condition and at 37°C for enzymatic condition. The surface treated with LP scaffold showed the highest amount of weight loss, followed by the surface treated without LP, and the nonsurface-treated scaffolds without LP, respectively. The results from Fourier-transform infrared spectroscopy indicated degradation of PCL and PHBV through hydrolysis of the ester functional group. The compressive strengths of all scaffolds were sufficiently high. The results suggested that the scaffolds with the existence of LP and with surface treatment showed the highest potential for use as cartilage scaffolds.

1. Introduction

The cartilage is one of the tissues existing in the human and animal bodies that are subjected to large mechanical loads. Therefore, cartilage tissues are easily injured. In cartilage tissue engineering, the challenge goals are to support and accelerate the healing and repairing process of cartilage tissues until the restoration of tissues to normal structure and function is achieved [1, 2]. Scaffolds can mimic the three-dimensional structure of cartilage tissues which support cell adhesion and proliferation. The ideal scaffolds should have acceptable mechanical properties that can bare loads at the injured tissues. Moreover, in terms of biochemical property, the scaffolds should be nontoxic to cells, be able to support adhesion of cells, proliferation, can mediate cell-to-cell signaling, and maintain phenotype of cells [3, 4].

Various biodegradable polymers including poly(lactide) (PLA) [5], poly (glycolic acid) (PGA) [6], poly-L-lactide (PLLA) [7], poly(ε-caprolactone) (PCL) [5, 8, 9], poly (lactic-*co*-glycolic acid) (PLGA) [10], poly(ethylene glycol) (PEG) [11, 12], poly(hydroxybutyrate) (PHB) [6, 13], and its copolymer poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) [9, 14] are frequently used to fabricate into scaffolds for tissue engineering. The thermogel of stereocomplex of PLA and PEG modified with cholesterol were fabricated as cartilage scaffolds which exhibited high mechanical strength and preserved gene expression of chondrocytes [11]. The hydrogel fabricated from the copolymer of PEG and

poly(L-alanine-*co*-L-phenylalanine) exhibited the great potential for use in cartilage repair as observed from high mechanical properties and *in vivo* regeneration of hyaline-like cartilage [12]. The nanofiber membrane of PCL and lignin copolymer was reported as a good candidate for use in osteoarthritis treatment because of its biocompatibility, biodegradability, and antioxidant activity [15]. In the present work, the blends of PCL and PHBV were chosen to fabricate into the cartilage scaffolds. PCL and PHBV are aliphatic semicrystalline polymers. They are biodegradable, biocompatible, and environmental friendly polymer. Their degradation was due to the hydrolysis of the aliphatic ester linkage under physiological conditions [16, 17]. PCL exhibited slow degradation rate because of its crystallinity and the long hydrophobic $-CH_2$ moieties in repeating units. PHBV is more fragile and has lower rate of hydrolytic degradation due to its higher degree of crystallinity. The blends of PCL and PHBV could provide the compromise properties that suitable for use as scaffolds [9, 18].

Many techniques are utilized to fabricate the scaffolds in tissue engineering. The solvent-free techniques, for example, injection [19], extrusion [20], thermoforming [21], selective laser sintering [22–24], and fused deposition modeling (FDM) [7, 9, 25, 26], have drawn attention in the field of scaffolding. FDM can produce three-dimensional objects directly from the filaments of melted polymer or resin assisted by the computer-aided design program. FDM has no limit on the use of thermoplastic polymers as raw materials and can produce the scaffolds with interconnected pores that mimic the structure of the natural extracellular matrix. Various kinds of polymer scaffolds were successfully fabricated by using FDM. The porous scaffolds of PCL with alkaline treatment and supplemented with chondroitin sulfate fabricated from FDM were found to enhance chondrogenic differentiation [26]. Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBH), PHB, and PHBV were fabricated into scaffolds using FDM [25]. However, PHB and PHBV showed the great decrease in their viscosities and molecular weight after the FDM process. The scaffolds of PCL/PHBV blends with plasma-treated surface were successfully fabricated by FDM and were investigated for use as cartilage scaffolds [9]. The plasma-treated scaffolds with higher PHBV content exhibited higher hydrophilicity and higher proliferation of porcine chondrocytes.

In the present work, FDM was utilized to fabricate the three-dimensional scaffolds of PCL/PHBV blends. Effects of processing parameters of FDM on filament size and pore size were investigated. The scaffolds with local pores were prepared by using salt leaching technique. The alkaline treatment was performed to improve wettability of scaffold surfaces. The proliferation of porcine chondrocytes and amounts of glycosaminoglycans (GAGs) after cell cultures were evaluated. Lastly, the degradation behaviors of the scaffolds were studied.

2. Experimental Part

2.1. Materials. PCL (M_n 80,000 g/mol, Sigma-Aldrich, USA) and PHBV (M_W 180,000 g/mol, TianAn Biopolymer, China) were used to prepare polymer scaffolds. Sodium chloride (NaCl, particle size = 106-250 μm) and sodium hydroxide (NaOH) were purchased from Ajax Finechem (Australia). Candida antarctica lipase B (Novozyme 435) was purchased from Sigma-Aldrich (USA). Anhydrous disodium hydrogen orthophosphate (Na_2HPO_4), sodium dihydrogen orthophosphate ($NaH_2PO_4.H_2O$), and sodium azide (NaN_3) were purchased from Carlo Erba (Italy).

2.2. Fabrication of Polymer Filaments before FDM. PCL and PHBV pellets were ground to obtain a particle size of 500 μm by using Ultra Centrifugal Mill ZM 200 (Retsch). The obtained PCL and PHBV powders were mixed at various weight ratio including 100/0, 75/25, 50/50, and 25/75 and were milled with alumina balls at 180 rpm for 16 h at room temperature (25°C). The mixed polymers with the specified ratios were then extruded through a twin screw extruder (HAAKE MiniLab) with a diameter of nozzle of 1.64 mm at a rotational speed of 80 rpm at 80, 140, 150, and 150°C, respectively. It should be noted that the pristine PHBV was too fragile and unable to prepare into a continuous filament for further use in the fabrication process; therefore, it was not used in this research work.

The fabrication of dual-pore scaffolds was carried out using FDM accompanied with salt leaching method. The global pore (GP) was obtained from the process of FDM. The local pore (LP) which is much smaller than GP was obtained from the salt leaching method by using NaCl as leaching salt. The definition of GP and LP is presented in Figure 1(a). The blend of 50/50 PCL/PHBV powders was mixed with NaCl at 50/50% *w*/*w* of polymer/salt. The particle size of NaCl was in a range of 106-250 μm. Similar to the polymer blends without salt, the mixture of polymer and NaCl was ground using a ball mill under the same condition and extruded through twin screw extruder with the same rotational speed at 150°C.

2.3. Fabrication of Polymer Scaffolds through FDM. The schematic diagram of FDM is illustrated in Figure 1(b). The obtained filament of the polymer blend with a diameter of 1.64 mm was fed into in-house FDM machine and extruded at 160-170°C using the angles of nozzle of 0, 45, 90, and 135° (see Figure 1(c)) which were designed using a computer-aided design (CAD) software (SolidWorks, Dassault Systémes S.A.). The fiber diameter (FD) and spacing distance (SP) were set at 200 and 400 μm, respectively (see Figure 1(d)). The filament feeding speed was controlled at 0.5 mm/sec. The hatch speed was varied at 8, 10, 12, and 14 mm/sec. The diameter and the thickness of scaffold were 8 and 2.5 mm, respectively. The obtained scaffolds without LP were as follows and designated as 100/0 PCL/PHBV, 75/25 PCL/PHBV, 50/50 PCL/PHBV, and 25/75 PCL/PHBV. The scaffold with LP was designated as 50NaCl_50/50 PCL/PHBV. The extrusion temperature for all samples was 160°C, except for 25/75 PCL/PHBV which was 170°C.

2.4. Surface Modification of Polymer Scaffolds. The PCL/PHBV scaffolds without LP were immersed in 1.0 M NaOH solution at 50°C for 60 min in order to improve the

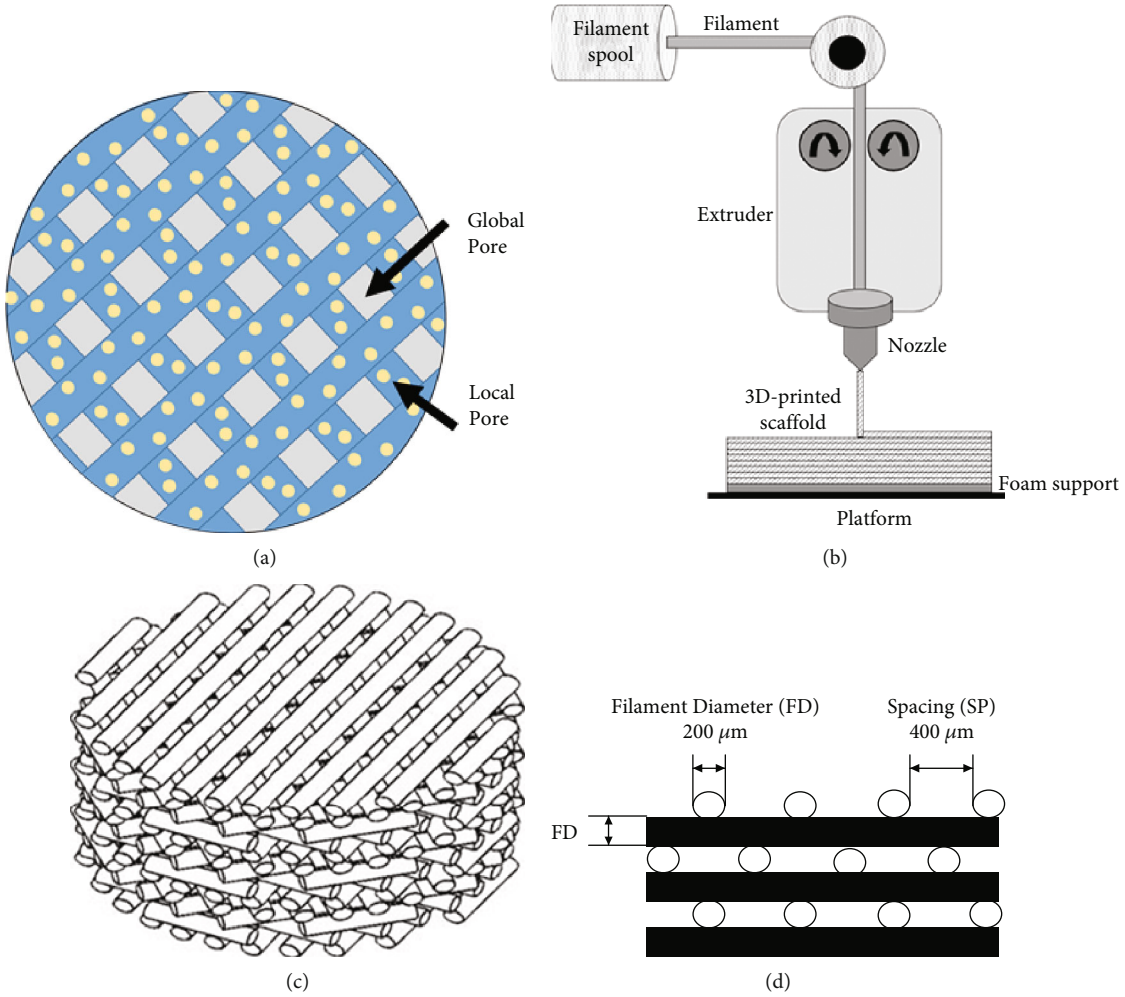


Figure 1: Schematic pictures of (a) global pores and local pores of scaffold, (b) FDM machine, (c) polymer scaffold fabricated from FDM, and (d) fiber diameter and spacing distance of the obtained scaffold.

hydrophilicity of their surfaces. After that, they were washed 3 times with deionized water for 30 min at room temperature (25°C) under shaking at 100 rpm using orbital shaker. The scaffolds were lastly washed with deionized water overnight (15 h) and freeze-dried. The obtained scaffolds were as follows and designated as 1H100/0 PCL/PHBV, 1H75/25 PCL/PHBV, 1H50/50 PCL/PHBV, and 1H25/75 PCL/PHBV. The effect of the concentration of NaOH solution on the hydrophilicity and degradation behaviors of scaffolds was also investigated. The PCL/PHBV scaffolds with 50% NaCl were immersed in 0.5, 1.0, or 3.0 M NaOH solution at 50°C for 60 min in order to remove salt particles and also to improve hydrophilicity of scaffolds. The scaffolds were than washed with deionized water in a same manner. The obtained scaffolds were as follows and designated as 50NaCl_0.5H50/50 PCL/PHBV, 50NaCl_1H50/50 PCL/PHBV, and 50NaCl_3H50/50 PCL/PHBV.

2.5. Morphology of Polymer Scaffold. Morphological appearance of the scaffolds before and after degradation was observed by a Hitachi S-3400 N scanning electron microscope (SEM). Prior to observation by SEM, the sample was doubly coated with gold using a JEOL JFC-1200 sputter-coater under 15 mA for 60 sec.

2.6. Hydrophilicity of Polymer Scaffolds. Surface hydrophilicity of the scaffolds was determined at 25°C by using an optical bench-type contact angle goniometer at each time points in a range of 0-60 sec.

2.7. Indirect Cytotoxicity of Polymer Scaffolds. The indirect cytotoxicity determination of the scaffolds was carried out in adaptation from the ISO 10993-5 standard test method. Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA), supplemented by 10% fetal bovine serum (FBS; Biochrom AG, Germany), 1% L-glutamine (Invitrogen Corp., USA), and 1% antibiotic and antimycotic formulation (Invitrogen Corp., USA), was used as a medium for cell culture. The scaffolds were sterilized by immersion in 70% ethanol for 30 min. They were later incubated in the culture media for 24 h at 37°C before the extraction medium was collected. The surface area-to-volume extraction ratio of 3 cm^2/mL was used. In the same time, the mouse fibroblast

cells (L929, ATCC CCL1, NCTC 929, of Strain L) were separately cultured in wells of tissue culture polystyrene plate (TCPS) for 16 h. The culture medium was then removed and replaced with an extraction medium. The cells were then further incubated for 24 h. Finally, the viability of cells was measured by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The indirect cytotoxicity was tested for both the fresh scaffold (Day 0) and the degraded scaffolds which were immersed in phosphate buffer (pH 7.4) at 37°C for 30 and 150 days (Day 30 and Day 150).

2.8. Proliferation of Cells. Porcine chondrocytes were harvested from knee joints of new born pigs and cultured until passage 3. The scaffolds were sterilized by immersion in 70% ethanol for 30 min before placing in 24-well tissue-cultured polystyrene plate. Porcine chondrocytes (passage 3) were seeded onto each scaffold at a density of 1×10^6 cells/well and cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, USA) in an incubator at 37°C under humidified atmosphere of 5%CO_2. The culture medium was changed every 3 days. The number of viable cells was quantified by Alamar blue assay based on the redox reaction between metabolite substances from active cells with resazurin (Sigma Aldrich, USA) redox indicator. The cell-scaffold samples were incubated with resazurin for 4 h in darkness. Color of resazurin was changed from blue to red which corresponded to colors of the oxidized, nonfluorescent form and the reduced, fluorescent form, respectively. Fluorescence intensity of each sample was determined at excitation/emission wavelength of 530/590 nm using a fluorescence microplate reader. The experiments were performed in triplicate.

2.9. Total Glycosaminoglycans (GAGs). Porcine chondrocytes (passage 3) were cultured on scaffolds for either 21 or 28 days to observe the total amounts of glycosaminoglycans (GAGs). At a specified cultured time, the cell-scaffold samples were freeze-dried. Subsequently, proteins synthesized from cultured cells were digested with 5 mg/mL papain (crude powder from papaya latex, Sigma Aldrich, USA) solution followed by incubation at 60°C for 16 h. Aliquot of 100 μL sample was mixed with 100 μL of 0.032 mg/mL 1,9-dimethylmethyleneblue (DMMB) (Sigma Aldrich, USA) solution in 96-well tissue-cultured polystyrene plate. The absorbances of the resulting solutions were measured using a microplate spectrophotometer at 525 nm. The experiments were carried out in triplicate. The total amount of GAGs of each sample was calculated from its absorbance. Bovine chondroitin sulfate (Sigma Aldrich, USA) was used as a standard substance.

2.10. Degradation of Polymer Scaffolds

2.10.1. Preparation of Phosphate Buffer. In the degradation study, phosphate buffer was used instead of phosphate buffer saline in order to avoid the precipitation of salt on scaffolds. For the preparation of the phosphate buffer, 28.66 g of Na_2HPO_4, 5.59 g of $NaH_2PO_4.H_2O$, and 0.2 g of NaN_3 as an antibacterial and antimicrobial agent were dissolved in distilled water. The final volume was adjusted to 1,000 mL. The pH of the as-prepared phosphate buffer was 7.4.

2.10.2. Degradation of Polymer Scaffolds without Enzyme. Each scaffold sample was put in a plastic tube containing phosphate buffer solution at a ratio of 10 mg/2 mL of scaffold/phosphate buffer. The tube was shaken at 100 rpm using an orbital shaker at 37 and 50°C. The scaffolds were collected at 30 days of degradation. The medium, phosphate buffer solution, was changed once at Day 15.

2.10.3. Degradation of Polymer Scaffolds with Enzyme. For the enzymatic degradation, each scaffold sample was placed in a plastic tube containing phosphate buffer solution at a ratio of 10 mg/2 mL of scaffold/phosphate buffer. Candida Antarctica lipase (Novozyme 435) was added into the medium at 10 wt.% based on the weight of scaffold. The tube was shaken at 100 rpm using an orbital shaker at 37°C for 30 days. The medium was changed once at Day 15.

2.10.4. Weight Loss of Polymer Scaffold. Weight loss behaviors of the scaffolds were examined for both nonenzymatic and enzymatic conditions. The scaffolds were immersed in phosphate buffer medium without enzyme at either 37°C or 50°C and at 37°C with enzyme for 30 days. The samples were then dried in an oven at 80°C for 5 h. The degree of weight loss of sample was calculated according to Equation (1):

$$\text{Weightloss}\,(\%) = \frac{M_i - M_t}{M_i} \times 100, \quad (1)$$

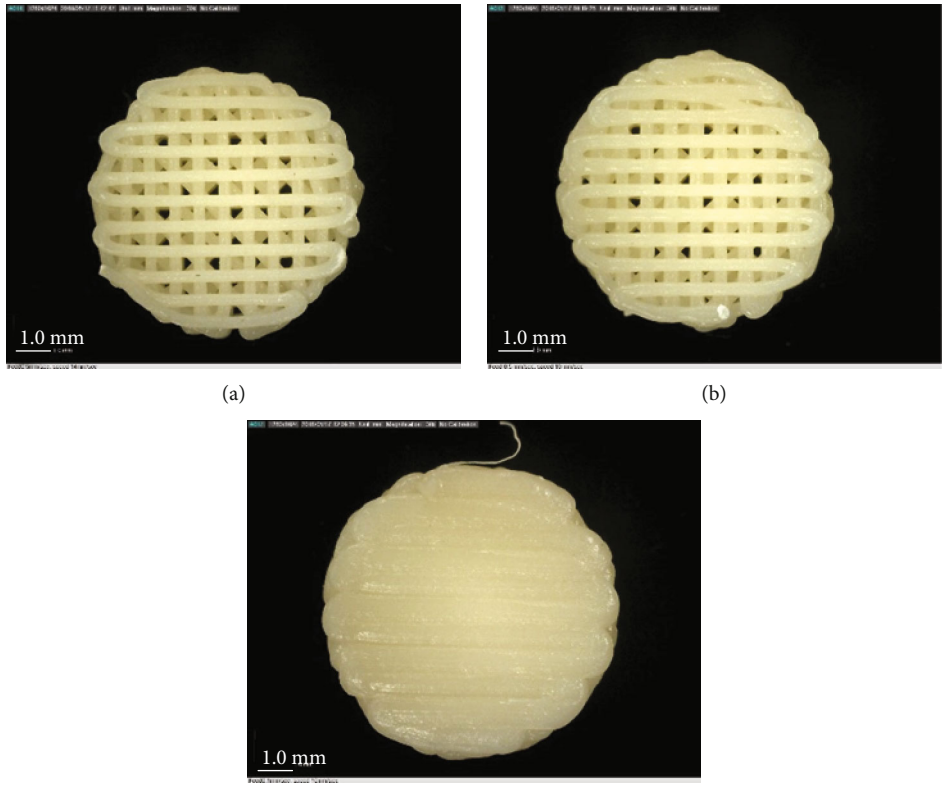
where M_i is the initial weight of the sample and M_t is the dry weight of the sample after degradation for 30 days.

2.10.5. Chemical Composition of Polymer Scaffolds. A PerkinElmer Spectrum Spotlight 300 Fourier-transformed infrared spectroscope (FT-IR) was used to characterize the important functional groups of the polymer scaffolds. Transmittance was recorded in a range of 600–4000 cm^{-1} wave number.

2.10.6. Mechanical Property of Polymer Scaffolds. The compressive strength of the polymer scaffolds was analyzed by the NRI Narin Instrument (Thailand) Universal Testing Machine. The sample size was $6.0 \times 6.0 \times 6.0\,mm^3$. The strain rate of 0.5 mm/min with a 1 kN load cell was applied to the sample at 25°C. For each type of sample, at least five measurements were carried out.

3. Results and Discussion

3.1. Fabrication of PCL/PHBV Blend Scaffolds through FDM. The polymer blend scaffolds were prepared with various ratios of PCL and PHBV through FDM. The diameter and the thickness of scaffolds were 8 and 2.5 mm, respectively. The FD and SP which were designed by using CAD software were set at 200 and 400 μm, respectively. However, there are many parameters affected to the FD and SP of the obtained scaffolds. The FD of sample is normally larger than the size of nozzle (a preset FD) due to the die swell effect and subsequently resulted in the smaller SP value than that of the preset one. The value of SP can be simply called as the pore size of scaffold.



(a) (b) (c)

Figure 2: The 50/50 PCL/PHBV scaffolds fabricated using the filament feeding speeds of (a) 0.25, (b) 0.5, and (c) 1.0 mm/s under a constant hatch speed of 10 mm/s.

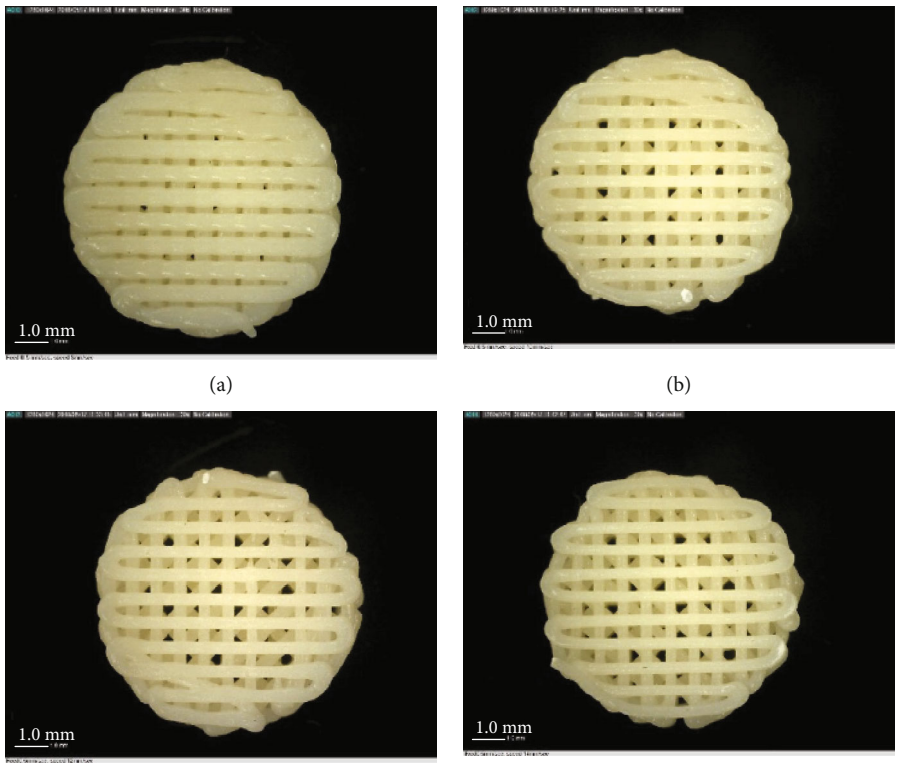
One of the processing parameters in FDM is an extrusion temperature. The filaments of all types of polymer blends were fed into an extrusion part and extruded at 160°C to prepare the scaffolds. The 25/75 PCL/PHBV was the only sample that was extruded at 170°C. According to the higher T_m of PHBV (165-175°C) than that of PCL (60°C), the 25/75 PCL/PHBV blend composed of a high fraction of PHBV could not be completely melted at 160°C. It should be noted that the use of temperature lower than 160°C provided incompletely melting of polymer blends and the use of temperature up to 200°C resulted in degradation of polymers.

3.1.1. Filament Feeding Speed. The first step of FDM started with feeding the filaments of polymer blends into the heated extrusion chamber. The filament feeding speed is an important parameter affected to the obtained FD and pore size of the scaffolds. The filament of 50/50 PCL/PHBV was fed into the extrusion part with various feeding speed including 0.25, 0.5, and 1.0 mm/s and subsequently extruded with the hatch speed of 10 mm/s. The printed 3D polymer scaffolds are shown in Figure 2. It was observed that the obtained FD was increased, and the pore sizes were decreased with increasing feeding speed (see Table 1). There was no data for the FD and pore size for the scaffold obtained from extrusion with the feeding speed of 1.0 mm/sec because all filaments fused together and there was no any pore. It is obvious that the feeding speed of 1.0 mm/s could not be used to fabricate the scaffolds. From these results, the filament feeding speed of 0.5 mm/s was chosen for further study because the obtained scaffold had the pore size in a range of 200-400 μm which suitable for use as either cartilage or bone scaffolds [27].

Table 1: Filament diameters and pore sizes of 50/50 PCL/PHBV from FDM with different filament feeding speed and hatch speed.

Filament feeding speed (mm/s)	Hatch speed (mm/s)	Filament diameter (μm)	Pore size (μm)
0.25	10	268.8 ± 24.5	446.2 ± 21.2
0.5		410.6 ± 27.4	235.9 ± 27.9
1.0		n/a	n/a
0.5	8	604.6 ± 23.2	109.3 ± 22.6
	10	410.6 ± 27.4	235.9 ± 27.9
	12	343.4 ± 28.5	355.8 ± 32.4
	14	323.0 ± 14.8	365.9 ± 27.3

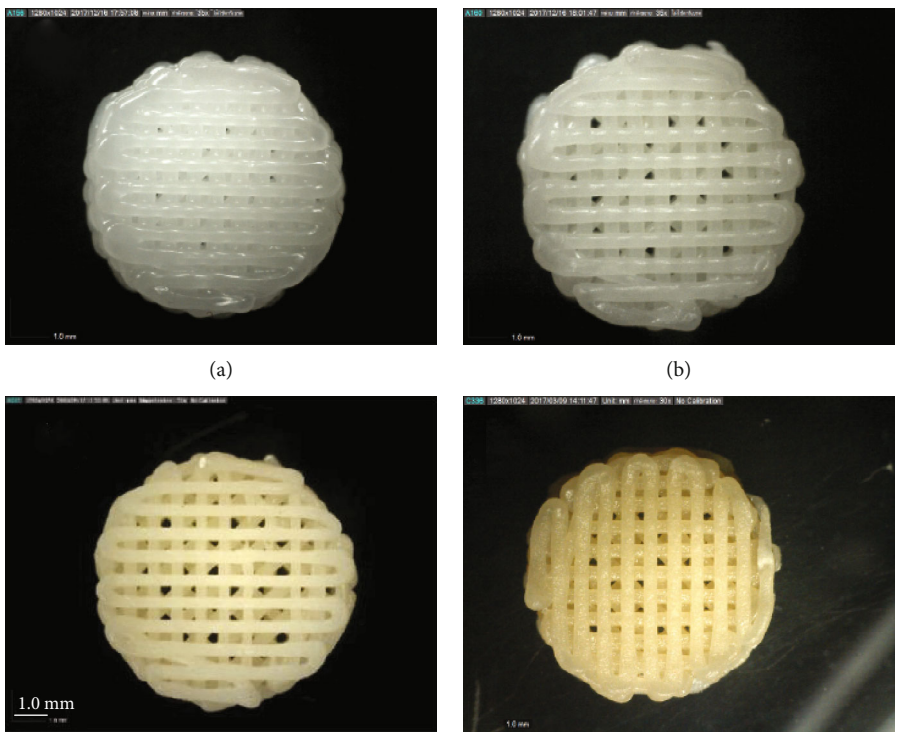
3.1.2. Hatch Speed. The effect of hatch speed on the FD and pore size of the polymer scaffolds was also investigated. The filament of 50/50 PCL/PHBV was fed into the FDM machine at 0.5 mm/s and printed with various hatch speeds including 8, 10, 12, and 14 mm/s. Figure 3 shows the polymer scaffolds



(a) (b)

(c) (d)

Figure 3: The 50/50 PCL/PHBV scaffolds fabricated using the hatch speeds of (a) 8, (b) 10, (c) 12, and (d) 14 mm/s under a constant filament feeding speed of 0.5 mm/s.

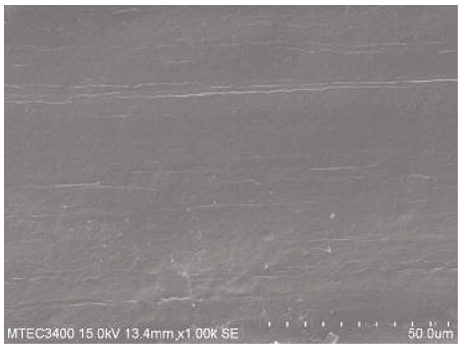
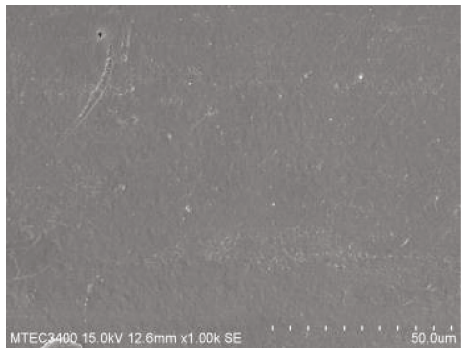
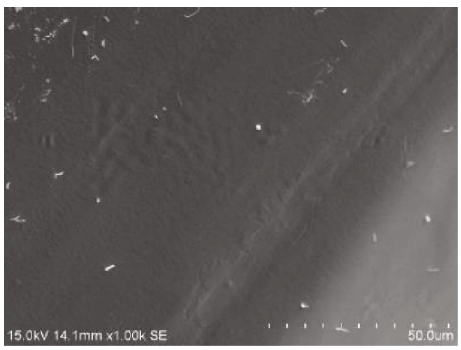
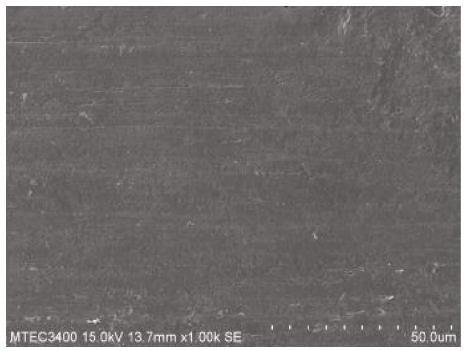
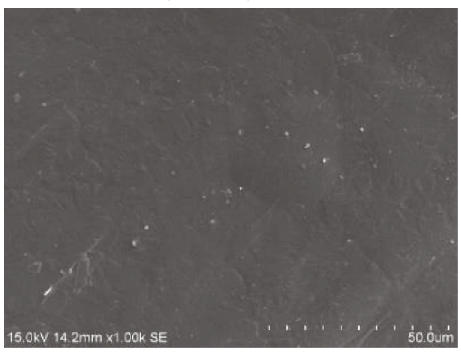
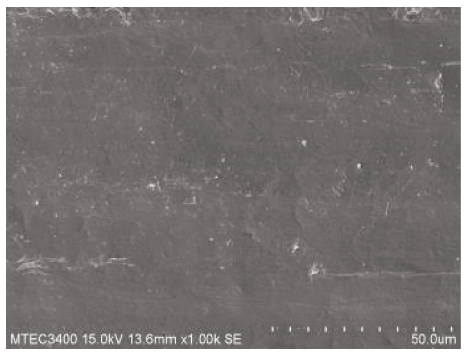
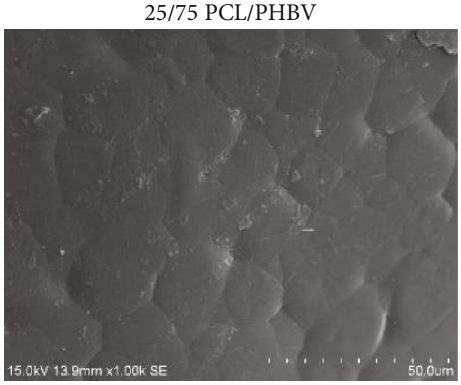
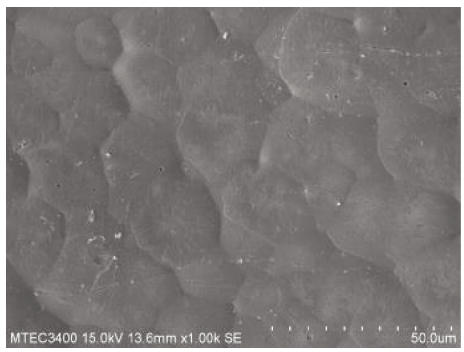


(a) (b)

(c) (d)

Figure 4: Scaffolds fabricated from the ratios of blending at (a) 100/0, (b) 75/25, (c) 50/50, and (d) 25/75 PCL/PHBV using a filament feeding speed of 0.5 mm/s and a hatch speed of 12 mm/s.

Table 2: SEM images of surfaces of PCL/PHBV scaffolds with different ratios of blending before and after surface modification with NaOH.

Ratio of PCL/PHBV	Before surface modification	After surface modification
100/0	100/0 PCL/PHBV 	1H100/0 PCL/PHBV 
75/25	75/25 PCL/PHBV 	1H75/25 PCL/PHBV 
50/50	50/50 PCL/PHBV 	1H50/50 PCL/PHBV 
25/75	25/75 PCL/PHBV 	1H25/75 PCL/PHBV 

fabricated from FDM by using different hatch speeds. The FD and pore sizes are presented in Table 1. The FD was decreased, and the pore sizes were increased with increasing hatch speed. The obtained FD and pore sizes of polymer scaffolds were in a range of 323–605 μm and 109–366 μm, respectively. Evidently, the hatch speed of 8 mm/s was not

Table 3: Water contact angles as function of time of PCL/PHBV scaffolds before and after surface modifications with NaOH.

Sample	Contact angle (θ) (degree)			
	0 s	15 s	30 s	60 s
100/0 PCL/PHBV	91.35 ± 1.91	88.80 ± 0.71	80.45 ± 0.92	80.20 ± 0.57
75/25 PCL/PHBV	95.00 ± 6.93	95.15 ± 6.58	95.10 ± 6.65	94.60 ± 6.51
50/50 PCL/PHBV	86.30 ± 6.22	85.30 ± 5.94	85.00 ± 5.66	84.45 ± 5.44
25/75 PCL/PHBV	102.60 ± 3.96	100.65 ± 2.19	100.35 ± 2.47	100.20 ± 2.69
1H100/0 PCL/PHBV	0	—	—	—
1H75/25 PCL/PHBV	0	—	—	—
1H50/50 PCL/PHBV	0	—	—	—
1H25/75 PCL/PHBV	0	—	—	—

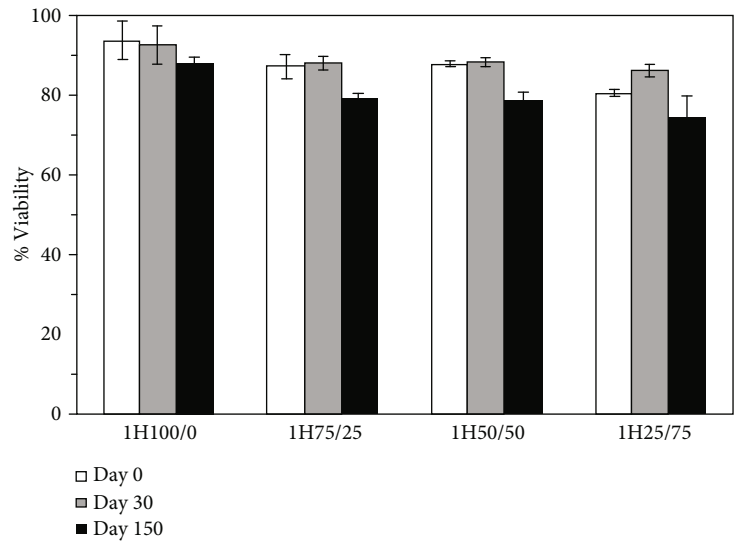


Figure 5: Indirect cytotoxicity of scaffolds with different blending ratios with surface modification with 1 M NaOH as evaluated by the viability of mouse fibroblast (L929) cells.

suitable to be used because the pore size of the polymer scaffold was too small. For further study, the hatch speed of 12 mm/s was used to fabricate the scaffolds because it gave the suitable pore size for cartilage or bone scaffolds [28]. Collagen-hyaluronic acid scaffolds with the mean pore size of 300 μm exhibited the highest cell proliferation, chondrogenic gene expression, and cartilage-like matrix deposition comparing with those with smaller pore sizes of 94 and 130 μm [28].

3.2. Polymer Blend Scaffolds without LP. The polymer scaffolds without LP were fabricated by using FDM with various ratios of polymer blends including 100/0, 75/25, 50/50, and 25/75 PCL/PHBV. Figure 4 shows photographs of the obtained scaffolds in which the 0/100 PCL/PHBV blend was not fabricated in this work because it was unable to be extruded into a continuous filament. The filament feeding speed and the hatch speed were 0.5 and 12 mm/s, respectively. Color of the 25/75 PCL/PHBV scaffold was yellow whereas others were white. Surface modification was carried out by immersing the scaffolds in 1.0 M NaOH solution at 50°C for 60 min. Table 2 shows photographs and SEM images of the scaffolds with different blend ratios before and after surface modification. From macroscopic observation, the 25/75 PCL/PHBV scaffold had the least elasticity and showed yellow color.

3.2.1. Contact Angles of Scaffolds. From the SEM images in Table 2, the surface morphology of each scaffold before and after surface modifications by using NaOH was not much different. However, the contact angles of the scaffolds after surface modification were significantly changed. Table 3 presents the contact angles of the polymer scaffolds before and after surface modifications. The experiments were done in triplicate. For the scaffold after treatment with NaOH solution, the water droplet was immediately absorbed on the surface of scaffolds ($\theta = 0^\circ$). Obviously, the scaffolds had higher hydrophilicity after surface modification as observed by the decreasing of the contact angles. The ester functional group of PCL and PHBV could be hydrolyzed by NaOH to be more hydrophilic (i.e., carboxylic and hydroxyl) groups [16]. The results suggested that the surface modification by using

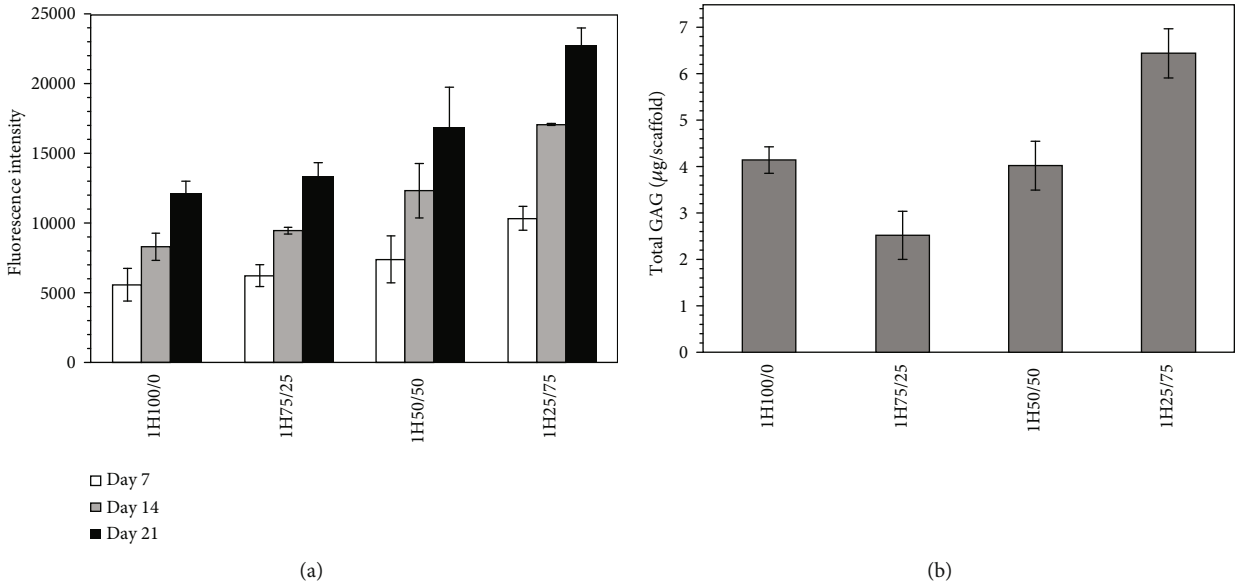


Figure 6: (a) Proliferation of porcine chondrocytes at 7, 14, and 21 days of culture and (b) amounts of extracted glycosaminoglycans after cultured for 21 days on the surface-modified scaffolds with NaOH at different blending ratios.

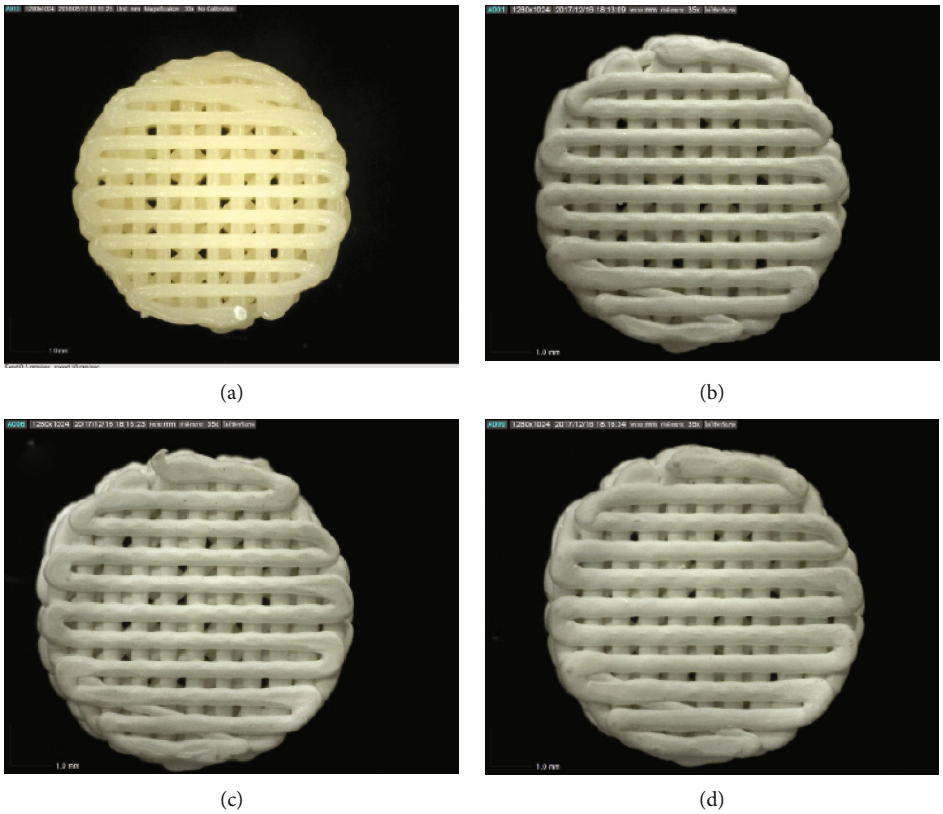


Figure 7: Scaffolds of (a) 50NaCl_25/75 before removing of salt, (b) 50NaCl_0.5H25/75, (c) 50NaCl_1H25/75, and (d) 50NaCl_3H25/75 PCL/PHBV.

NaOH solution could improve the hydrophilicity of the PCL/PHBV scaffolds and therefore enhance the potential for use in tissue engineering applications.

3.2.2. Indirect Cytotoxicity of Scaffolds. According to high hydrophilicity of the surface-modified PCL/PHBV samples including 1H100/0 PCL/PHBV, 1H75/250 PCL/PHBV, 1H50/50 PCL/PHBV, and 1H25/75 PCL/PHBV, their potential for use as scaffolds in tissue engineering were examined. The indirect cytotoxicity of the scaffolds based on the mouse fibroblast (L929) cells was investigated. Not only the cytotoxicity of the fresh scaffold (Day 0) was investigated, but also

Table 4: SEM images of 25/75 PCL/PHBV scaffolds without local pore and with local pores obtained from different concentrations of NaOH.

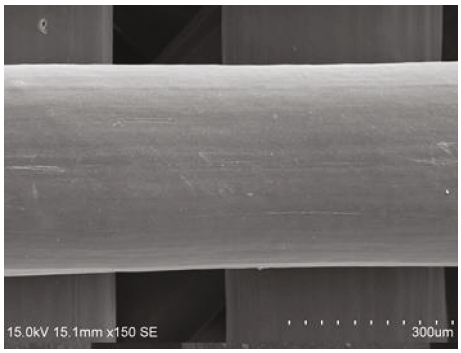
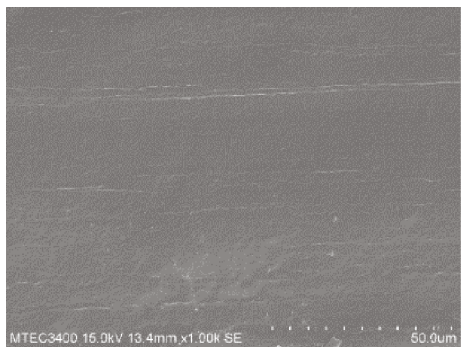
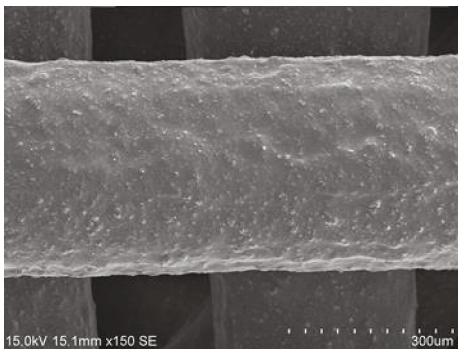
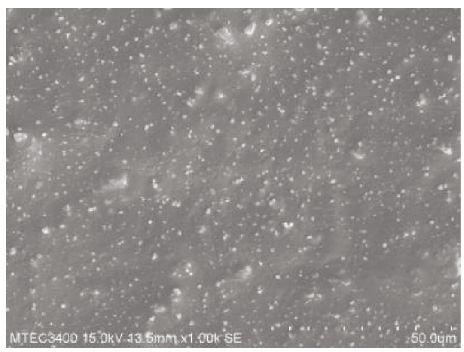
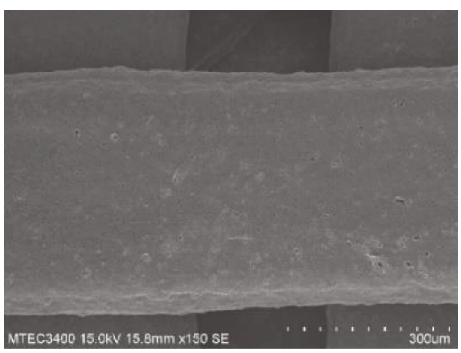
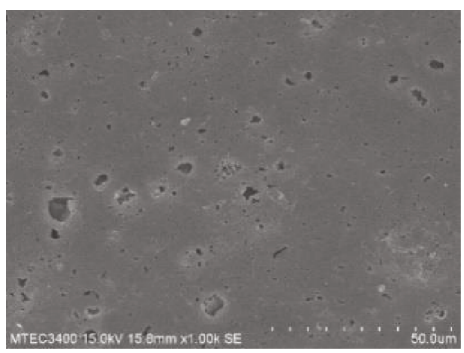
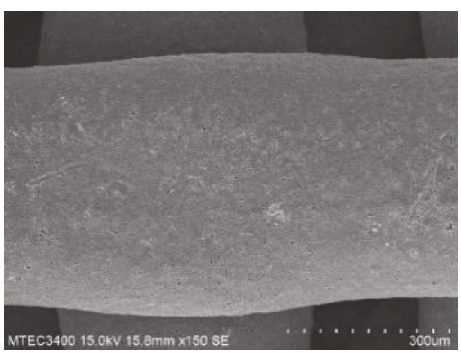
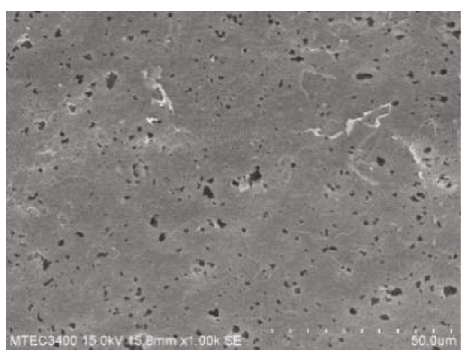
Sample	Magnification ×100	Magnification ×1000
25/75 PCL/PHBV		
50NaCl_25/75 PCL/PHBV before removing of salt		
50NaCl_0.5H25/75 PCL/PHBV		
50NaCl_1H25/75 PCL/PHBV		

Table 4: Continued.

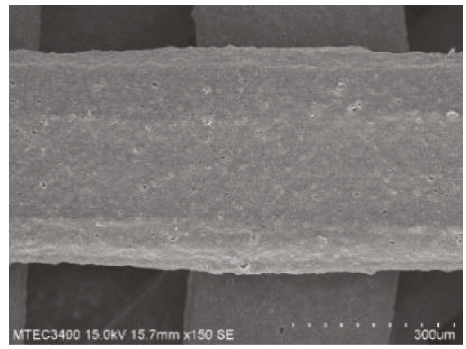
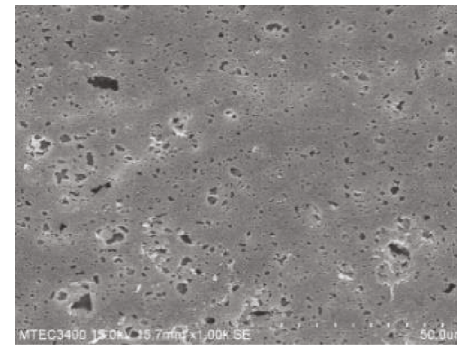
Sample	Magnification ×100	Magnification ×1000
50NaCl_3H25/75 PCL/PHBV		

Table 5: Water contact angles as function of time of 25/75 PCL/PHBV scaffolds without local pore and with local pores obtained from different concentrations of NaOH.

Sample	Contact angle (θ) (degree)					
	0 s	5 min	6 min	7 min	10 min	11 min
25/75 PCL/PHBV	100.69 ± 7.94	80.98 ± 4.74	N/A	66.65 ± 7.23	46.30 ± 1.89	0
50NaCl_0.5H25/75 PCL/PHBV	0	—	—	—	—	—
50NaCl_1H25/75 PCL/PHBV	0	—	—	—	—	—
50NaCl_3H25/75 PCL/PHBV	0	—	—	—	—	—
50NaCl_DI25/75 PCL/PHBV	92.38 ± 1.88	23.36 ± 5.88	0	—	—	—

those for scaffolds after immersion in phosphate buffer at 37°C for 30 and 150 days were also studied. Figure 5 reveals the viability of cells for each scaffold tested on Day 0, Day 30, and Day 150 of degradation. For each scaffold, the viability of cell at Day 30 was not much different from that of Day 0. However, the viability of cells at Day 150 was significantly lower than that of Day 0. Comparing among different types of scaffold, the higher ratio of PCL in scaffold exhibited the higher viability of cell. The 1H100/0 PCL/PHBV showed the highest viability of cell (88-94%), whereas those of 1H75/25 PCL/PHBV (79-88%) and 1H50/50 PCL/PHBV (79-89%) were comparable. Lastly, the viability of cell of 1H25/75 PCL/PHBV was the lowest (74-86%). However, all of these values were greater than 70% which indicated that the scaffolds were nontoxic to cells. Even the scaffolds have been in degradation for 150 days, there was no toxicity to cell.

3.2.3. Proliferation of Cells and Total GAGs. The surface-modified PCL/PHBV scaffolds were further studied for the proliferation of chondrocytes and the amounts of total GAGs produced from the culture in order to reveal the potential to be used as cartilage scaffolds. Figure 6(a) showed the proliferation of chondrocytes after culture on the 1H100/0, 1H75/25, 1H50/50, and 1H25/75 PCL/PHBV scaffolds for 7, 14, and 21 days. Alamar blue assay was conducted to determine the fluorescence intensity which is directly proportional to the number of viable cells. Interestingly, the higher ratio of PHBV in the scaffolds resulted in the higher proliferation of chondrocytes. Furthermore, the total GAGs extracted from the culture at Day 21 was also observed (see Figure 6(b)). Glycosaminoglycans (GAGs) are one of the key roles to indicate cell signaling, cell adhesion, cell proliferation, anticoagulation, and wound repair [29, 30]. Similar to the result of cell proliferation, total GAGs from the cell culture on 1H25/75 was the greatest (6.4 ± 0.5 μg/scaffold), whereas those for 1H100/0, 1H75/25, and 1H50/50 were 4.1 ± 0.3, 2.5 ± 0.5, and 4.0 ± 0.5 μg/scaffold, respectively. The results of cell proliferation and total GAGs suggested that the 1H25/75 PCL/PHBV has the greater potential than others for use as cartilage scaffolds. Even if the indirect cytotoxicity of the 1H25/75 PCL/PHBV was not as good as others, it will still be considered a nontoxic material.

3.3. Polymer Blend Scaffolds with LP. The polymer scaffolds with LP were fabricated by using FDM accompanied with salt leaching technique. NaCl particles were used as porogen to produce tiny pores in the scaffolds. According to the good biological properties in terms of cytotoxicity, cell proliferation, and total GAGs, the 1H25/75 was chosen for the further study. NaCl was mixed with the polymer bland at 50% *w*/*w* and was leached out by using NaOH solution at different concentrations (i.e., 0.5, 1, and 3 M) in order to produce LP and to improve the surface hydrophilicity in the same time. Photographs of the 50NaCl_25/75 PCL/PHBV before salt leaching, 50NaCl_0.5H25/75, 50NaCl_1H25/75, and 50NaCl_3H25/75 PCL/PHBV are shown in Figure 7. After salt leaching and surface modification, the scaffolds became whiter. Table 4 shows SEM images of 25/75 PCL/PHBV scaffolds without LP and with LP obtained from different concentrations of NaOH at ×100 and ×1,000 magnifications. The sizes of LP were in a range of 1-5 μm. The higher concentration of NaOH gave higher numbers of LP. However, 3 M

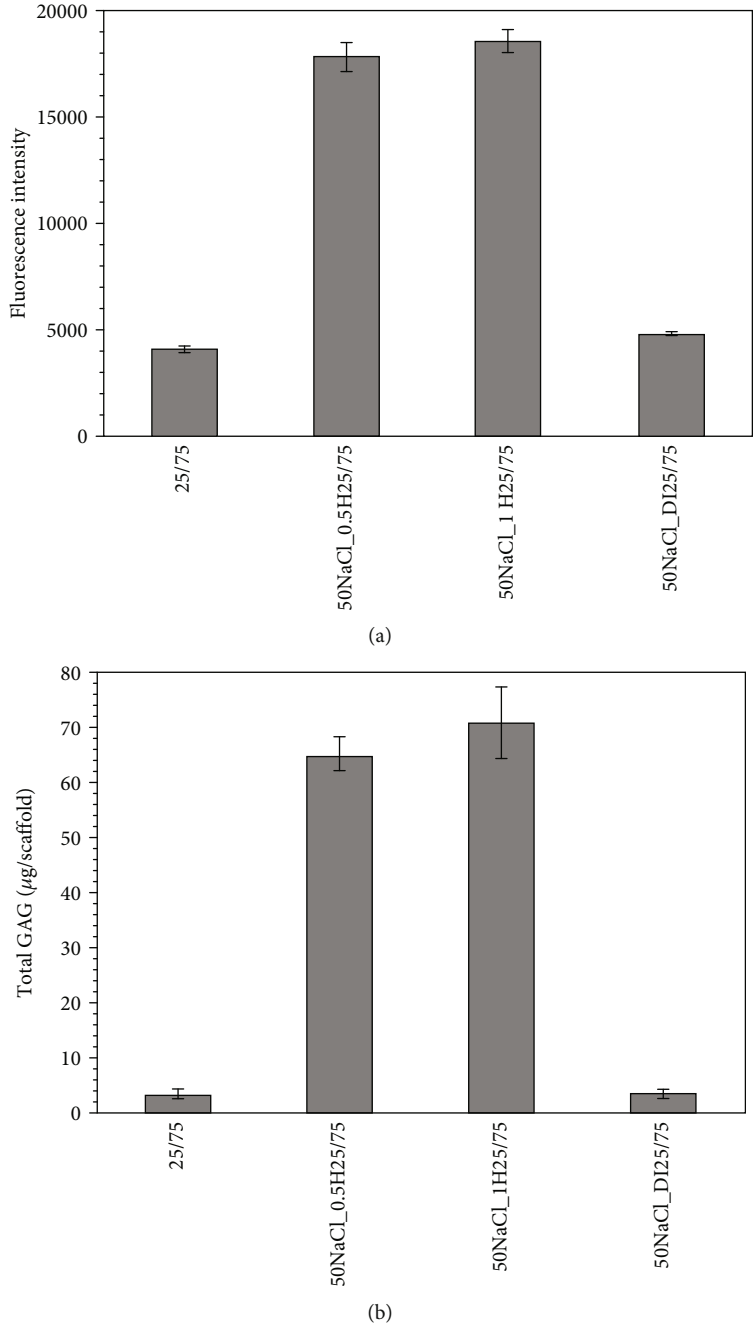


Figure 8: (a) Proliferation of porcine chondrocytes after cultured for 1 day and (b) amounts of extracted glycosaminoglycans after cultured for 28 days on the 25/75 PCL/PHBV scaffolds with and without local pores and with different kinds of leaching medium.

NaOH was considered to be a too high concentration because the obtained scaffold (50NaCl_3H25/75 PCL/PHBV) was a little disintegrated and broke into small pieces which cannot be handled further. The 50NaCl_1H25/75 PCL/PHBV was the best among these samples because it had high numbers of LP and not disintegrated.

3.3.1. Contact Angles of Scaffolds. Water contact angles of these scaffolds were also determined. From Table 5, the contact angles of scaffold without LP (25/75 PCL/PHBV) decreased with time and became zero at 11 min. Those scaffolds with LP exhibited zero degree of contact angles since the initial time ($t = 0$) which indicated the high hydrophilicity of the scaffolds. The scaffolds with LP which use deionized water as leaching medium were also prepared for being as a control sample. The sample is designated as 50NaCl_DI25/75 PCL/PHBV. Its water contact angle decreased with time and became zero at 6 min, which suggested that the hydrophilicity was better than the scaffold without LP but poorer than the scaffolds with LP which use NaOH as a leaching medium.

3.3.2. Proliferation of Cells and Total GAGs. Proliferation of porcine chondrocytes and the total GAGs extracted from

Table 6: SEM images of pristine 25/75 PCL/PHBV scaffold and 25/75 PCL/PHBV with surface modification and with local pores before and after 30 days of degradation under different conditions.

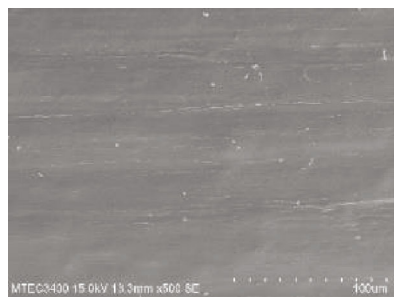
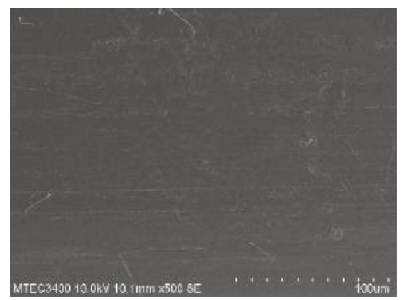
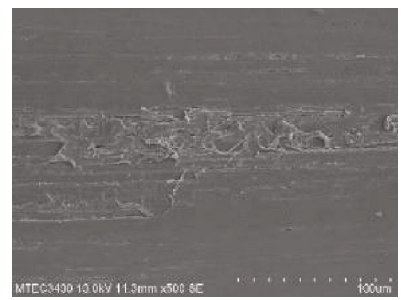
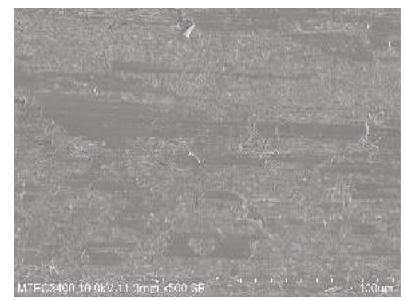
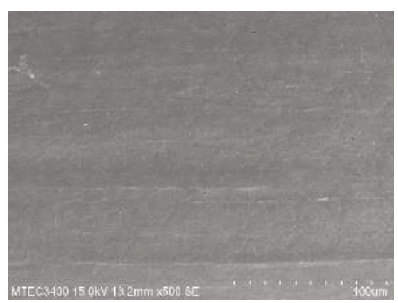
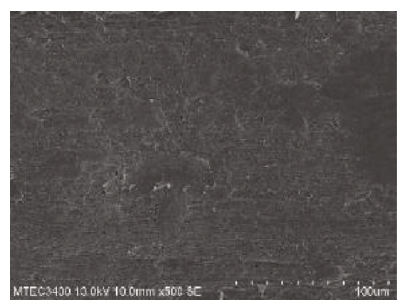
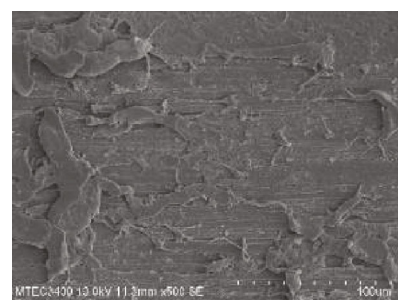
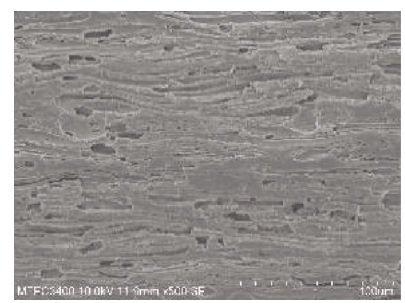
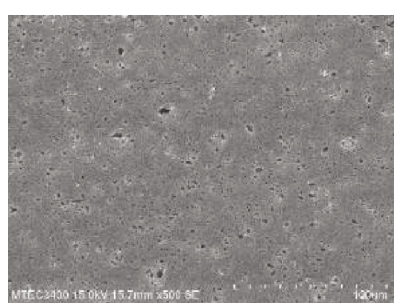
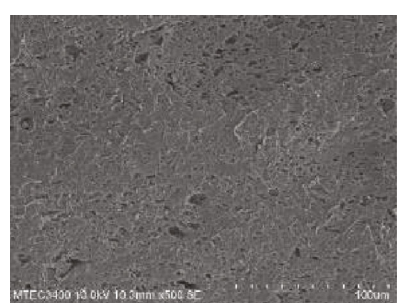
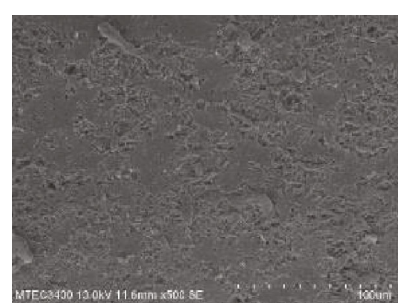
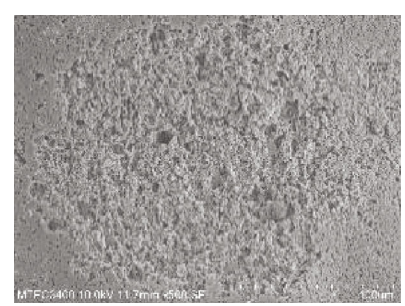
Sample	Day 0	Day 30		
		37°C	50°C	With enzyme, 37°C
25/75 PCL/PHBV				
1H25/75 PCL/PHBV				
50NaCl_ 1H25/75 PCL/PHBV				

Table 7: Amounts of weight loss and ratios of area under peak A : B from IR spectra of pristine 25/75 PCL/PHBV scaffold and 25/75 PCL/PHBV with surface modification and with local pores at 30 days of degradation under different conditions.

Sample	Weight loss (%)			Ratio of area under peak A : B		
	37°C	50°C	With enzyme, 37°C	37°C	50°C	With enzyme, 37°C
25/75 PCL/PHBV	0.16 ± 0.05	0.75 ± 0.07	12.61 ± 2.78	8.44	7.93	7.05
1H25/75 PCL/PHBV	0.37 ± 0.10	2.01 ± 0.05	18.89 ± 3.10	7.58	7.48	6.56
50NaCl_1H25/75 PCL/PHBV	0.74 ± 0.08	2.96 ± 0.09	49.18 ± 4.12	6.97	6.47	5.58

the culture on the 25/75 PCL/PHBV scaffolds with and without LP were studied. Figure 8(a) shows the proliferation of cells at 1 day of culture. The proliferation of cells on the scaffold with LP which used deionized water as leaching medium (50NaCl_DI25/75 PCL/PHBV) was close to that of the scaffold without LP (25/75 PCL/PHBV). Interestingly, the scaffolds with LP which used NaOH as a leaching medium (50NaCl_0.5H25/75 and 50NaCl_1H25/75 PCL/PHBV) provided greater proliferation of cells than the one without LP. Figure 8(b) revealed the total GAGs extracted from the culture on these scaffolds for 28 days. The trend of total GAGs was quite similar to that of the proliferation. The 50NaCl_0.5H25/75 and 50NaCl_1H25/75 PCL/PHBV scaffolds exhibited higher amounts of GAGs than the 25/75 and 50NaCl_DI25/75 PCL/PHBV. This result suggested that both LP structure and hydrophilicity of scaffolds were important factors to promote the biological activities of cells which can be observed from the proliferation and total GAGs. The only LP structure without surface modification with NaOH (i.e., 50NaCl_DI25/75 PCL/PHBV) could not help improve the biological properties of scaffolds. Lastly, comparing between 2 types of scaffolds with LP which use NaOH as a leaching medium, it was found that the 50NaCl_1H25/75 PCL/PHBV exhibited higher amount of GAGs ($71 \pm 6\,\mu$g/scaffold) than that of the 50NaCl_0.5H25/75 PCL/PHBV ($65 \pm 3\,\mu$g/scaffold). Conclusively, the 50NaCl_1H25/75 PCL/PHBV revealed the best properties among these samples.

3.4. Degradation of PCL/PHBV Blend Scaffolds. Degradation behaviors of the 25/75 PCL/PHBV with and without LP were studied after immersion of scaffolds in phosphate buffer (pH 7.4). Three types of samples including (1) the scaffold without LP and without surface treatment (25/75 PCL/PHBV), (2) the scaffold without LP but with surface treatment (1H25/75 PCL/PHBV), and (3) the scaffold with LP and with surface treatment (50NaCl_1H25/75 PCL/PHBV) were investigated. The experiments were conducted at 37°C or 50°C. Another experiment was studied under enzymatic condition at 37°C in which lipase was added in the immersive medium. Table 6 shows SEM images of fresh scaffolds (Day 0) and the scaffolds at Day 30 of degradation at 37°C or 50°C without enzyme and 37°C with enzyme. For all types of scaffold, the surface became coarser after degradation. The higher temperature of degradation and especially the enzymatic degradation, the surfaces of scaffolds were rougher. The scaffold with LP and surface treatment (50NaCl_1H25/75 PCL/PHBV) revealed remarkably rough and disintegrate surface after enzymatic degradation.

The amounts of weight loss after degradation were also determined. For each sample, the degree of weight loss increased with increasing temperature. With using an enzyme in degradation, the degree of weight loss was drastically increased (see Table 7). PCL and PHBV molecules might be shortened and some small molecules by-products were removed according to hydrolysis of the ester groups [16, 17]. Comparing among the different types of scaffolds at either 37°C or 50°C, the weight loss of each scaffolds was not much different. However, under enzymatic condition, the weight loss of the 50NaCl_1H25/75PCL/PHBV was noticeably higher than others. According to the high hydrophilicity of surface resulted from surface treatment and the existence of LP which provided higher surface area, the rate of degradation of this scaffold was greater than others.

The change of important functional groups of the scaffolds after degradation was observed from the FT-IR spectra. Figure 9 shows the FT-IR spectra of the 25/75 PCL/PHBV, the 1H25/75 PCL/PHBV, and the 50NaCl_1H25/75 PCL/PHBV after 30 days of degradation in phosphate buffer at 37°C and 50°C, respectively. The spectra of these scaffolds after 30 days of enzymatic degradation at 37°C were also investigated which can be accessed in the Supplementary Material (see Figure S1). The peaks at 1056, 1177 (peak A), and 1730 cm^{-1} (peak B) contributed to C_{sp3}-O, C_{sp2}-O, and C=O stretching in the ester functional group, respectively [31]. The chain scission from hydrolysis of ester which occurs at carbonyl carbon and oxygen atoms (C_{sp2}-O bond) was confirmed by the lowering of the area under curve of the peak (A). However, the C=O bond does not involve in the hydrolysis of ester; therefore, the intensity of peak B seemed to be constant. The ratio of peak A : B was determined (see Table 7) to indicate the degree of hydrolysis. For each type of scaffold, the ratios of peak A : B decreased with increasing temperature of degradation and further decreased under enzymatic degradation. Decreasing of ratio of peak A : B suggested that the degradation through hydrolysis has occurred. Comparing among different types of scaffolds, the 50NaCl_1H25/75PCL/PHBV showed lower ratio of peak A : B than those of 1H25/75 PCL/PHBV and 25/75 PCL/PHBV, respectively. This result revealed that the scaffold with high surface hydrophilicity and with LP had higher rate of degradation than those without surface treatment and without LP.

From the surface morphology observed from SEM images, the degree of weight loss, and the FT-IR spectra, it was found

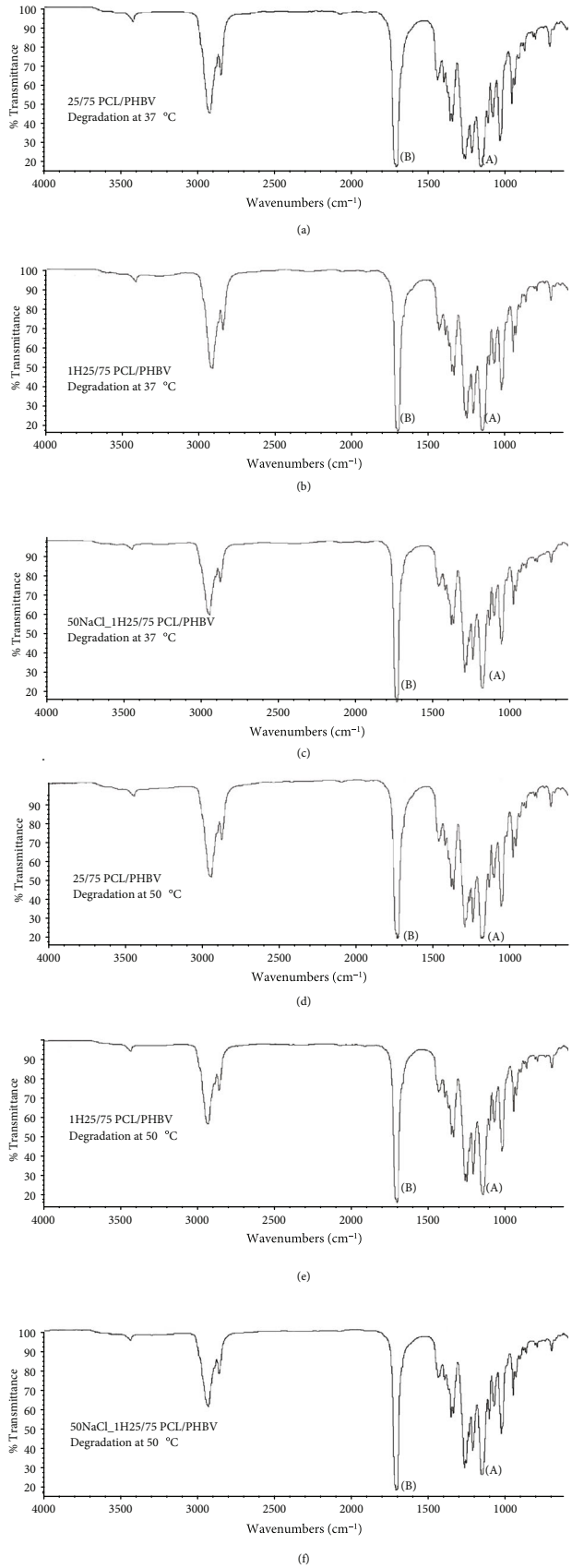


Figure 9: Infrared spectra of (a) 25/75, (b) 1H25/75, and (c) 50NaCl_1H25/75 PCL/PHBV after 30 days of degradation in phosphate buffer at 37°C and the same scaffolds in (d), (e), and (f) at 50°C, respectively.

Table 8: Compressive strength of pristine 25/75 PCL/PHBV scaffold and 25/75 PCL/PHBV with surface modification and with local pores before and after 30 days of degradation in phosphate buffer under different conditions.

Sample	Compressive strength (MPa)			
	Day 0	Day 30		
		37 ˚C	50 ˚C	With enzyme, 37 ˚C
25/75 PCL/PHBV	14.25 ± 0.87	10.76 ± 0.57	9.66 ± 0.80	7.79 ± 0.38
1H25/75 PCL/PHBV	11.44 ± 0.59	8.98 ± 0.76	8.07 ± 0.80	6.27 ± 0.35
50NaCl_1H25/75 PCL/PHBV	9.76 ± 0.44	7.63 ± 0.31	6.53 ± 0.30	4.64 ± 0.45

that the scaffold with high surface hydrophilicity and with existence of LP could help improve the rate of degradation.

Furthermore, the compressive strength of the scaffolds before and after degradation in phosphate buffer (pH 7.4) was determined and presented in Table 8. The mechanical properties of the scaffold are important aspects to indicate whether they match the intended tissue or tolerate physiological forces in organs, especially in bone and cartilage scaffoldings which are load-bearing applications. For the pristine scaffolds (Day 0), the compressive strength of 25/75 PCL/PHBV was higher than those of 1H25/75 PCL/PHBV, and 50NaCl_1H25/75PCL/PHBV, respectively. The results could be implied that the surface modification and the presence of LP debilitated mechanical property of the scaffolds. For all conditions of degradation (i.e., 37˚C, 50˚C, and 37˚C with enzyme), the same trends were observed. Consider the effect of degradation temperature, the compressive strength of the scaffolds was lower with increasing temperature from 37˚C to 50˚C. These values were further decreased under the enzymatic degradation. Evidently, this result was consistent with the amount of weight loss and the results from the FT-IR spectra that the scaffold with high surface hydrophilicity and with presence of LP exhibited more degradation. Even though, the compressive strengths of all scaffolds were decreased after 30 days of degradation, these values still were in a range of the compressive strength of human articular cartilage i.e., 3-17 MPa for the knee and hip cartilage with the thickness in a range of about 1-2 mm [32]. All scaffolds, especially the scaffold with surface modification and LP (50NaCl_1H25/75PCL/PHBV), revealed the potential for use as cartilage scaffolds according to their sufficiently high mechanical property and good biological property.

4. Conclusions

The polymer blend scaffolds of PCL/PHBV were fabricated from fused deposition modeling (FDM). The effects of filament feeding speed and hatch speed on filament diameters and pore sizes were studied. Various ratios of PCL : PHBV blending including 100/0, 75/25, 50/50, and 25/75 were used. The scaffolds with NaOH surface treatment had high hydrophilicity which was observed from their water contact angles. From indirect cytotoxicity testing, all scaffolds were nontoxic to cells even if they were immersed in phosphate buffer for 150 days. For use as cartilage scaffolds, the proliferation of porcine chondrocytes and total GAGs extracted from the cultures on the surface treated scaffolds with different blending ratios were investigated. The surface-treated scaffold with a blending ratio of 25/75 (1H25/75 PCL/PHBV) exhibited the highest proliferation and total GAGs. The effect of local pores (LP) on the scaffolds was also studied. The scaffolds with LP were prepared from FDM along with salt leaching technique using NaCl as porogens. Sizes of LP were in a range of 1-5 μm. The scaffolds were treated by different concentrations of NaOH including 0.5, 1, and 3 M. All scaffolds with LP and with surface treatment had high hydrophilicity. However, it was found that a 3 M NaOH was a too high concentration in which the scaffold was disintegrated. The scaffolds with LP and with 1 M NaOH surface treatment (50NaCl_1H25/75 PCL/PHBV) exhibited the highest proliferation of cells and total GAGs (71 μg/scaffold at Day 28). The results suggested that this scaffold was a good candidate for use as a cartilage scaffold. Furthermore, the degradation behaviors of the scaffolds were studied. The nonsurface treated, surface treated without LP, and surface treated with LP scaffolds were degraded in phosphate buffer (pH 7.4) for 30 days at 37˚C and 50˚C for non-enzymatic condition and at 37˚C for enzymatic condition. The surface treated with LP scaffold (50NaCl_1H25/75 PCL/PHBV) showed the highest amount of weight loss, followed by the surface treated without LP (1H25/75 PCL/PHBV), and the nonsurface-treated scaffolds (25/75 PCL/PHBV), respectively. Supposedly, the degradation of PCL and PHBV undergoes hydrolysis of the ester functional group which was confirmed by the disappearance of the C_{sp2}-O bond in the ester group as observed from the FT-IR spectra. The compressive strengths of the scaffolds before and after degradation were sufficiently high with regard to those values of the human articular cartilage. Altogether, the results demonstrated that the surface treated with LP scaffold possessed excellent properties and exhibited potential for use as a cartilage scaffold.

Data Availability

The data of more infrared spectra used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors have no conflict of interest.

Acknowledgments

The authors acknowledge the funding from the Faculty of Engineering and the research unit in polymer rheology and processing, Thammasat University.

Supplementary Materials

Figure S1: infrared spectra of (a) 25/75, (b) 1H25/75, and (c) 50NaCl_1H25/75 PCL/PHBV after 30 days of degradation in phosphate buffer at 37°C and the same scaffolds in (d), (e), and (f) at 50°C, and (g), (h), and (i) with enzyme at 37°C. *(Supplementary Materials)*

References

[1] M. Bhattacharjee, J. Coburn, M. Centola et al., "Tissue engineering strategies to study cartilage development, degeneration and regeneration," *Advanced Drug Delivery Reviews*, vol. 84, pp. 107–122, 2015.

[2] N. Fazal and N. Latief, "Bombyx mori derived scaffolds and their use in cartilage regeneration: a systematic review," *Osteoarthritis and Cartilage*, vol. 26, no. 12, pp. 1583–1594, 2018.

[3] S. Manjunath Kamath, S. K. Rao, D. Jaison et al., "Melatonin delivery from PCL scaffold enhances glycosaminoglycans deposition in human chondrocytes - bioactive scaffold model for cartilage regeneration," *Process Biochemistry*, vol. 99, pp. 36–47, 2020.

[4] F. Sharifi, S. Irani, G. Azadegan, M. Pezeshki-Modaress, M. Zandi, and M. Saeed, "Co-electrospun gelatin-chondroitin sulfate/polycaprolactone nanofibrous scaffolds for cartilage tissue engineering," *Bioactive Carbohydrates and Dietary Fibre*, vol. 22, p. 100215, 2020.

[5] T. Xu, Q. Yao, J. M. Miszuk et al., "Tailoring weight ratio of PCL/PLA in electrospun three-dimensional nanofibrous scaffolds and the effect on osteogenic differentiation of stem cells," *Colloids Surfaces B Biointerfaces*, vol. 171, pp. 31–39, 2018.

[6] M. Generali, D. Kehl, A. K. Capulli, K. K. Parker, S. P. Hoerstrup, and B. Weber, "Comparative analysis of polyglycolic acid-based hybrid polymer starter matrices for _in vitro_ tissue engineering," *Colloids Surfaces B Biointerfaces*, vol. 158, pp. 203–212, 2017.

[7] I. Rajzer, A. Kurowska, A. Jabłoński et al., "Layered gelatin/PLLA scaffolds fabricated by electrospinning and 3D printing- for nasal cartilages and subchondral bone reconstruction," *Materials and Design*, vol. 155, pp. 297–306, 2018.

[8] E. Venugopal, K. S. Sahanand, A. Bhattacharyya, and S. Rajendran, "Electrospun PCL nanofibers blended with Wattakaka volubilis active phytochemicals for bone and cartilage tissue engineering," *Nanomedicine Nanotechnology, Biologie et Médecine*, vol. 21, p. 102044, 2019.

[9] W. Kosorn, M. Sakulsumbat, P. Uppanan et al., "PCL/PHBV blended three dimensional scaffolds fabricated by fused deposition modeling and responses of chondrocytes to the scaffolds," *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, vol. 105, no. 5, pp. 1141–1150, 2017.

[10] H. Eslami, H. Azimi Lisar, T. S. Jafarzadeh Kashi et al., "Poly(-lactic-co-glycolic acid)(PLGA)/TiO_2 nanotube bioactive composite as a novel scaffold for bone tissue engineering: in vitro and in vivo studies," *Biologicals*, vol. 53, pp. 51–62, 2018.

[11] C. Wang, N. Feng, F. Chang et al., "Cartilage repair: injectable cholesterol-enhanced stereocomplex polylactide thermogel loading chondrocytes for optimized cartilage regeneration (Adv. Healthcare Mater. 14/2019)," *Advanced Healthcare Materials*, vol. 8, no. 14, article 1970056, 2019.

[12] H. Liu, Y. Cheng, J. Chen et al., "Component effect of stem cell-loaded thermosensitive polypeptide hydrogels on cartilage repair," *Acta Biomaterialia*, vol. 73, pp. 103–111, 2018.

[13] M. Parvizifard and S. Karbasi, "Physical, mechanical and biological performance of PHB-chitosan/MWCNTs nanocomposite coating deposited on bioglass based scaffold: potential application in bone tissue engineering," *International Journal of Biological Macromolecules*, vol. 152, pp. 645–662, 2020.

[14] J. Wu, J. Sun, and J. Liu, "Evaluation of PHBV/calcium silicate composite scaffolds for cartilage tissue engineering," *Applied Surface Science*, vol. 317, pp. 278–283, 2014.

[15] R. Liang, J. Zhao, B. Li et al., "Implantable and degradable antioxidant poly(ε-caprolactone)-lignin nanofiber membrane for effective osteoarthritis treatment," *Biomaterials*, vol. 230, no. 1, p. 119601, 2020.

[16] K. Shi, J. Jing, L. Song, T. Su, and Z. Wang, "Enzymatic hydrolysis of polyester: degradation of poly(ε-caprolactone) by _Candida antarctica_ lipase and _Fusarium solani_ cutinase," *International Journal of Biological Macromolecules*, vol. 144, pp. 183–189, 2020.

[17] M. F. Shockley and A. H. Muliana, "Modeling temporal and spatial changes during hydrolytic degradation and erosion in biodegradable polymers," *Polymer Degradation and Stability*, vol. 180, p. 109298, 2020.

[18] A. Baptista-Perianes, S. M. Malmonge, M. M. O. Simbara, and A. R. Santos Junior, "In vitro evaluation of PHBV / PCL blends for bone tissue engineering," *Materials Research*, vol. 22, no. 6, article ???, 2019.

[19] T. Limongi, L. Lizzul, A. Giugni et al., "Laboratory injection molder for the fabrication of polymeric porous poly- epsilon-caprolactone scaffolds for preliminary mesenchymal stem cells tissue engineering applications," *Microelectronic Engineering*, vol. 175, pp. 12–16, 2017.

[20] K. A. van Kampen, E. Olaret, I. C. Stancu, L. Moroni, and C. Mota, "Controllable four axis extrusion-based additive manufacturing system for the fabrication of tubular scaffolds with tailorable mechanical properties," *Materials Science and Engineering: C*, vol. 119, p. 111472, 2021.

[21] J. Borowiec, J. Hampl, M. Gebinoga et al., "Thermoforming techniques for manufacturing porous scaffolds for application in 3D cell cultivation," *Materials Science and Engineering: C*, vol. 49, pp. 509–516, 2015.

[22] Y. Shi, T. Pan, W. Zhu, C. Yan, and Z. Xia, "Artificial bone scaffolds of coral imitation prepared by selective laser sintering," *Journal of the Mechanical Behavior of Biomedical Material*, vol. 104, article 103664, 2020.

[23] S. H. Diermann, M. Lu, Y. Zhao, L. J. Vandi, M. Dargusch, and H. Huang, "Synthesis, microstructure, and mechanical behaviour of a unique porous PHBV scaffold manufactured using selective laser sintering," *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 84, pp. 151–160, 2018.

[24] C. Gayer, J. Ritter, M. Bullemer et al., "Development of a solvent-free polylactide/calcium carbonate composite for selective laser sintering of bone tissue engineering scaffolds," *Materials Science and Engineering: C*, vol. 101, pp. 660–673, 2019.

[25] A. Kovalcik, L. Sangroniz, M. Kalina et al., "Properties of scaffolds prepared by fused deposition modeling of poly(hydroxyalkanoates)," *International Journal of Biological Macromolecules*, vol. 161, pp. 364–376, 2020.

[26] C. S. Moura, J. C. Silva, S. Faria et al., "Chondrogenic differentiation of mesenchymal stem/stromal cells on 3D porous poly (ε-caprolactone) scaffolds: effects of material alkaline treatment and chondroitin sulfate supplementation," *Journal of Bioscience and Bioengineering*, vol. 129, no. 6, pp. 756–764, 2020.

[27] F. Yang, C. Chen, Q. Zhou et al., "Laser beam melting 3D printing of Ti6Al4V based porous structured dental implants: fabrication, biocompatibility analysis and photoelastic study," *Scientific Reports*, vol. 7, no. 1, p. 45360, 2017.

[28] A. Matsiko, J. P. Gleeson, and F. J. O'Brien, "Scaffold mean pore size influences mesenchymal stem cell chondrogenic differentiation and matrix deposition," *Tissue Engineering. Part A*, vol. 21, no. 3–4, pp. 486–497, 2015.

[29] C. L. Jones, J. Liu, and D. Xu, "Structure, biosynthesis, and function of glycosaminoglycans," *Comprehensive Natural Products II: Chemistry and Biology*, vol. 6, pp. 407–427, 2010.

[30] F. Krichen, Z. Ghlissi, R. B. Abdallah et al., "Glycosaminoglycans from grey triggerfish and smooth hound skins: rheological, anti-inflammatory and wound healing properties," *International Journal of Biological Macromolecules*, vol. 118, no. Part A, pp. 965–975, 2018.

[31] B. Phil, *Advanced Organic Spectroscopy Tools for Beginning Organic Spectroscopists: Using Simulated Spectra to Learn how to Solve Complicated Organic Structures*, California State Polytechnique University, Pomona, 1st edition, 2015.

[32] D. E. Shepherd and B. B. Seedhom, "Thickness of human articular cartilage in joints of the lower limb," *Annals of the Rheumatic Diseases*, vol. 58, no. 1, pp. 27–34, 1999.