

Surface Modification of Polycaprolactone Scaffolds by Plasma Treatment for Chondrocyte Culture

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Abstract. The surface modification of polycaprolactone scaffolds by low pressure oxygen (O₂) plasma for chondrocyte culture was studied. Two different starting PCL scaffolds were employed: polycaprolactone (PCL) and alkaline hydrolyzed polycaprolactone (HPCL) scaffolds which were fabricated by a high pressure supercritical CO₂ technique. The surface properties of the plasma-treated scaffolds and the chondrocyte-scaffold responses were evaluated in comparison with their starting scaffolds. The surface morphology, hydrophilicity and chemical composition were evaluated by scanning electron microscopy (SEM), water contact angle measurement, and X-ray photoelectron spectroscopy (XPS), respectively. The scanning electron micrographs revealed the rough surfaces of both plasma-treated scaffolds. The measured water contact angles on the plasma-treated scaffolds appeared much smaller than those on the untreated scaffolds; the smallest contact angle was found on the plasma-treated HPCL scaffold. The O/C atomic ratio was also found to increase in both plasma-treated scaffolds. The porcine chondrocytes cultured on each scaffold for given days (up to 21 days) were assessed for their proliferation and cartilage-specific gene expression. The proliferation results demonstrated that the number of chondrocytes cultured on each scaffold at day 21 was insignificantly different. However, the RT-PCR results revealed that the type II collagen gene expression was observed only in the chondrocytes cultured on the plasma-treated HPCL scaffold.

Keywords: Polycaprolactone, Scaffolds, Supercritical CO₂, Plasma treatment, Chondrocytes

1. Introduction

Recently, biodegradable aliphatic polyesters, e.g., poly(lactic acid) (PLA), poly(glycolic acid) (PGA), polyhydroxybutyrate (PHB) and polycaprolactone (PCL), have been extensively studied as tissue-engineered scaffolds in bone and cartilage regeneration, owing to their good mechanical properties, processability, non-cytotoxicity and adjustable biodegradation [1-2]. However, their rather hydrophobic surfaces can give rise to inefficient cell attachment and cell growth. In recent year, surface modifications of polyesters have been attempted [3-4]. In general, wet-chemical treatments do not only induce irregular polymeric surface etching, but also leave harmful chemical residues on the scaffolds. Plasma treatment has been alternatively used since it rarely involves chemical reagents hazardous to cells and biological tissues and causes changes in the beneficial bulk properties of the materials [5-6].

The objective of this work was, hence, to study the surface modification of PCL scaffolds by low pressure plasma treatment with O₂ gas for chondrocyte culture. Two different starting PCL scaffolds were employed: PCL scaffolds fabricated by a high pressure supercritical CO₂ technique using unmodified PCL pellets (coded as PCL) and NaOH-hydrolyzed PCL pellets (coded as HPCL). The two resulting plasma-treated scaffolds, coded as plasma-treated PCL and plasma-treated HPCL, were characterized in comparison with their starting scaffolds for their surface morphology, hydrophilicity and atomic composition using scanning electron microscopy (SEM), contact angle measurement and X-ray photoelectron spectroscopy

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(XPS), respectively. In addition, the responses of chondrocytes to the scaffolds were examined, in terms of cell proliferation and cartilage-specific gene expression using Alamar blue assay and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, respectively.

2. Materials and Methods

2.1. Preparation of Porous PCL and HPCL Scaffolds

Polycaprolactone (PCL) ($M_w = 80,000$ g/mol) was supplied from Sigma-Aldrich Corporation in a pellet form. Hydrolyzed polycaprolactone (HPCL) pellets were prepared by the hydrolysis of PCL pellets with 6N NaOH at 50°C for 5 h. The alkaline-treated pellets were washed thoroughly in de-ionized water and freeze-dried overnight. Typically, both PCL and HPCL samples were dried overnight at room temperature under vacuum prior to the fabrication. 5 g of each dried sample was loaded into a cylindrical vessel which was heated at 60°C for 10 min. Subsequently, the vessel was filled with CO₂ at 15 MPa and soaked for 3 h. At the end of the process, the whole system was rapidly depressurized at 1.2 cc/sec. Finally, the porous polycaprolactone (PCL) and hydrolyzed polycaprolactone (HPCL) scaffolds with an average pore size in the range of 150-250 μm were resulted.

2.2. Plasma Modification of PCL and HPCL Scaffolds

Plasma treatment was carried out on a low pressure RF discharge (model PDC-002, Harrick) which is sustained in pure O₂. The PCL and HPCL scaffolds were placed on the glass plate positioned approximately 7.5 cm below the plasma reactor in a direction orthogonal to the plasma source head. The chamber was evacuated below a pressure of 205 mTorr and then filled with pure oxygen. The scaffolds were treated at 30W for 30 min.

2.3. Characterization

Scanning electron microscopy (SEM, Hitachi S-3400N) was used for the observation of the internal pore morphology of all PCL and HPCL scaffolds before and after plasma treatment. The hydrophilicity of all scaffolds was comparatively evaluated by means of water contact angle measurement using a sessile drop technique with an optical bench-type contact angle goniometer (model 100-00-220, Rame-Hart, USA). The chemical composition of the scaffold surface layers was determined using an X-ray photoelectron spectroscopy (XPS).

2.4. Cell Proliferation Assay

The sterilized scaffold discs (8 mm in diameter and 2 mm in thickness) were placed into 24-well culture plates. Each sample was seeded with porcine chondrocytes at concentration of 1.5×10^6 cells/specimen. The cell-seeded scaffolds were incubated under 5% CO₂ atmosphere at 37°C for 7, 14 and 21 days. The culture medium was regularly replaced every 3 days. Cell proliferation on each scaffold was assessed by Alamar blue assay, which is based on the detection of metabolic activity of cells. Typically, after those given incubation periods, the cultured cells were further incubated in a medium containing resazurin dye for 4 h. Aliquots of 200 μl of each medium from each scaffold were read at 530/590 nm for fluorescence intensity measurement.

2.5. RNA Extraction and Semiquantitative RT-PCR

In brief, after a 21-day culture period, total RNA was extracted from the cultured chondrocytes using TRIZOL reagent (Invitrogen) following the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized from 2 μg of RNA using the Prime RT Master synthesis Kit (GeNet Bio) in a 20 μl reaction. PCR analysis was conducted to determine the cartilage-specific gene expression. The mRNA levels of 18s rRNA were used as internal controls. The PCR products were identified by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining.

3. Results and Discussion

As revealed in Figure 1(a) and 1(b), the surfaces of the PCL and HPCL scaffolds appeared fairly smooth. After plasma treatment, the surfaces of the resulting PCL and HPCL scaffolds turned rough, as shown in

Figure 1(c) and 1(d), respectively, owing to the surface etching caused by both crosslinking and chain scission in the polymer upon the plasma treatment [5].

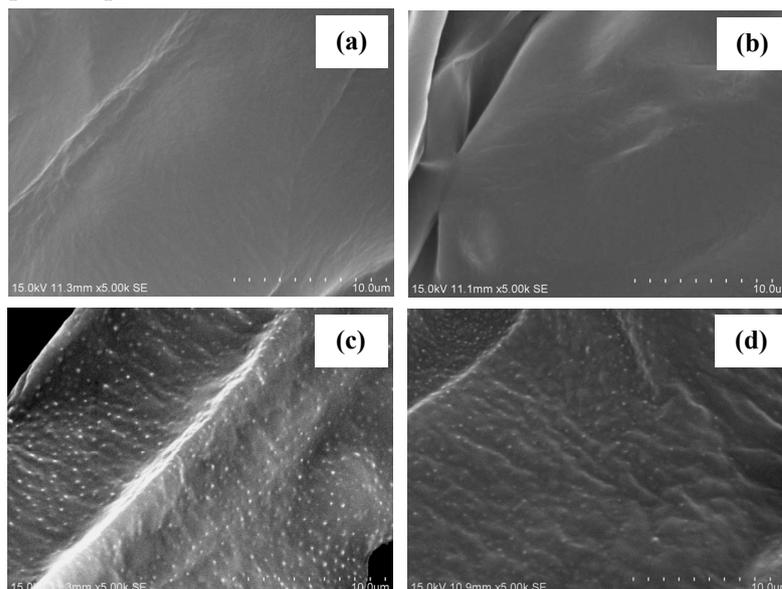


Fig. 1: SEM images of internal pore surfaces of scaffolds: (a) PCL, (b) HPCL, (c) plasma-treated PCL, and (d) plasma-treated HPCL, original magnification x5000.

To evaluate the hydrophilicity of the scaffolds, the water contact angle on the surface of each scaffold was measured by a sessile drop technique; the results are demonstrated in Table 1. Considerable decreases in water contact angles from $129.97 \pm 0.40^\circ$ to $48.03 \pm 12.89^\circ$ and from $101.73 \pm 6.54^\circ$ to 0° were observed on the PCL and HPCL scaffolds comparing between before and after the plasma treatment, respectively. It was previously suggested that, under the O_2 plasma treatment, the scaffolds became more etched, and an increase in the concentration of polar components, e.g., $-C-O-$, $>C=O$, and $-COOH$, on the surfaces was resulted [6]. Interestingly, a relatively much smaller water contact angle was observed on the plasma-treated HPCL scaffold, compared with that on the plasma-treated PCL scaffold. The greater hydrophilicity of the plasma-treated HPCL might be attributed to the additional carboxylate ($-COO^-$) and hydroxyl ($-OH$) groups on the HPCL chain termini, which was resulted from the alkaline hydrolysis

Table 1: Water contact angles measured on various PCL scaffolds.

Exp #	Sample code	Water contact angle (X°)*
1	PCL	129.97 ± 0.40
2	HPCL	101.73 ± 6.54
3	Plasma-treated PCL	48.03 ± 12.89
4	Plasma-treated HPCL	0^{**}

*tested onto three different sites of each sample.

**The sample surface was completely wettable.

The atomic composition of the PCL and HPCL scaffolds before and after O_2 plasma treatment was determined by XPS measurement. As demonstrated in Table 2, increases in the O/C atomic ratio in the plasma-treated scaffolds were clearly observed, indicating the presence of some newly generated oxygen-containing groups on the surfaces of the plasma-treated PCL and plasma-treated HPCL scaffolds. The plasma-treated HPCL scaffold apparently possessed a slightly lower O/C atomic ratio than the plasma-treated PCL scaffold, while the water contact angle observed on this scaffold was quite smaller. Upon alkaline hydrolysis, some ester groups in PCL chains degraded into $-COOH$ and $-OH$ groups, leading to less

oxidation of the HPCL scaffold during the plasma treatment. As a consequence, fewer oxygen-containing functional groups were detected in the plasma-treated HPCL scaffold.

Table 2: Chemical composition and O/C atomic ratio detected on the surfaces of various PCL scaffolds.

Exp #	Sample code	Chemical composition (%)			O/C atomic ratio (%)
		Carbon	Oxygen	Silicon	
1	PCL	78.04	21.96	-	28
2	HPCL	75.28	23.02	1.70	31
3	Plasma-treated PCL	71.92	27.42	0.66	38
4	Plasma-treated HPCL	74.45	25.55	-	34

Alamar blue assay was performed to evaluate the chondrocyte proliferation at different culture periods, i.e., 7, 14 and 21 days. As demonstrated in Figure 2, the proliferation of the cultured chondrocytes exhibited a strong tendency to increase from day 7 to day 21. Only the chondrocytes cultured on the PCL scaffold had a lower cell proliferation once the culture period prolonged to 21 days. However, the number of cultured cells observed on all scaffolds at day 21 was not substantially different.

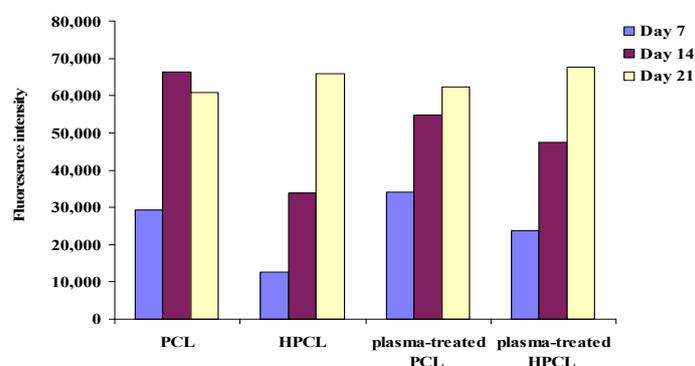


Fig. 2: Cell proliferation on various PCL scaffolds at day 7, day 14, and day 21.

The mRNA expression of type II collagen, a specific marker of cartilage ECM, was evaluated by RT-PCR at a 21-day incubation period. As revealed in Figure 3, type II collagen gene expression was observed only in the chondrocytes cultured on the plasma-treated HPCL scaffold. The homogeneous and high hydrophilicity of the porous scaffold resulted from both alkaline hydrolysis and plasma treatment played a significant role on the function of the porcine chondrocytes by providing good cellular infiltration into the entire three dimensional scaffold. The cartilage-specific gene expression could then be promoted.

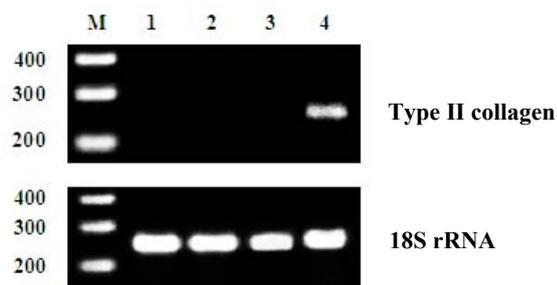


Fig. 3: mRNA expression of type II collagen in chondrocytes cultured on various PCL scaffolds at a 21-day incubation period; (lane 1) PCL, (lane 2) HPCL, (lane 3) plasma-treated PCL, (lane 4) plasma-treated HPCL, and M= marker.

4. Conclusions

The low pressure oxygen plasma treatment of polycaprolactone (PCL) and alkaline hydrolyzed polycaprolactone (HPCL) scaffolds resulted in the surface roughness, increased surface hydrophilicity, and generation of oxygen-containing functional groups at the surfaces of the scaffolds. The proliferation of porcine chondrocytes cultured on each scaffold appeared insignificantly different at a 21-day culture period. The RT-PCR results, however, revealed that the type II collagen gene expression was found only in the chondrocytes cultured on the plasma-treated HPCL scaffold.

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6. References

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