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Effects of surface topography, hydrophilicity and chemistry of surface-treated PCL scaffolds on chondrocyte infiltration and ECM production

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Abstract

The interaction of chondrocytes with two surface-treated polycaprolactone (PCL) scaffolds was examined. The surface treatments of PCL scaffolds were performed by alkaline hydrolysis and followed by oxygen plasma treatment. The hydrolysis of PCL was conducted either prior to scaffold fabrication, yielding pre-hydrolyzed PCL, or after scaffold fabrication, yielding post-hydrolyzed PCL. Both hydrolyzed scaffolds were subsequently subjected to plasma treatment. The resulting scaffolds were denoted as plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds, respectively. The surface morphology, wettability and chemical composition of the surface-treated scaffolds were investigated. The histological results revealed that the chondrocytes infiltrated more thoroughly inside the plasma-treated pre-HPCL scaffold.

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Keywords: Polycaprolactone; Hydrolysis; Plasma treatment; Topography; Hydrophilicity; Chondrocytes; Infiltration; Extracellular matrix.

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1. Introduction

Biodegradable polyesters, e.g., poly(lactic acid) (PLA), poly(glycolic acid) (PGA), polycaprolactone (PCL), and their copolymers, have been extensively studied and developed for their potential uses as cell/tissue culture substrates in tissue engineering [1]. PCL is, in particular, a semi-crystalline linear polyester. It possesses intriguing properties suitable for cartilage tissue engineering applications, such as good biodegradability, biocompatibility, mechanical strength and flexibility [2]. Nevertheless, its low surface wettability due to its rather hydrophobicity adversely affects cell attachment and proliferation. In recent years, several surface treatments of polyesters have been attempted to increase their surface hydrophilicity in order to improve the cell-material interfaces [3, 4]. Such methods as alkaline hydrolysis and plasma treatment of polymeric scaffolds were proved to significantly improve cell adhesion and enhance cell proliferation and functions [5-7].

In this study, surface-treated PCL scaffolds were prepared and subsequently characterized. The sequential surface treatments of the PCL scaffolds were first performed by alkaline hydrolysis and then followed by low pressure oxygen plasma treatment. The resultant scaffolds were characterized in comparison with their starting scaffold for their surface morphology, hydrophilicity and atomic composition using scanning electron microscopy (SEM), contact angle measurement, and X-ray photoelectron spectroscopy (XPS), respectively. The interaction of porcine chondrocytes with the scaffolds was assessed for their proliferation and cartilage-specific gene expression using an Alamar blue assay and RT-PCR analysis, respectively. The cell infiltration and extracellular matrix (ECM) production were also investigated by histological analysis.

2. Materials and Methods

2.1. Materials

Polycaprolactone (PCL) (*Mw*=80,000 g/mol) was supplied by Sigma-Aldrich Corporation in a pellet form.

2.2. Fabrication of porous PCL and pre-HPCL scaffolds by a high pressure supercritical CO2 technique

PCL pellets were hydrolyzed with 6 M NaOH at 50 ^{o}C for 5 h to yield hydrolyzed PCL (HPCL) pellets. Both starting PCL and HPCL pellets were vacuum dried overnight at room temperature prior to the fabrication. 5 g of dried PCL or HPCL sample was loaded into a cylindrical vessel which was heated at 60 ^{o}C for 10 min, filled with CO_2 at 15 MPa for 3 h, and finally depressurized at 1.2 cc/sec, yielding PCL or pre-HPCL scaffold, respectively. The scaffolds with pore sizes in the range of 150-250 μm were fabricated in this study.

2.3. Surface treatments of PCL scaffolds by hydrolysis and plasma treatment

The PCL scaffold was subsequently hydrolyzed with 1 M NaOH at 45 ^{o}C for 6 h, yielding post-HPCL scaffold. Plasma treatment was carried out on a low pressure RF discharge (model PDC-002, Harrick) which was sustained in pure oxygen. The pre-HPCL and post-HPCL scaffolds were placed in the plasma chamber, which was evacuated below a pressure of 205 mTorr and then filled with pure oxygen, and treated at 30W for 30 min, yielding plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds, respectively.

2.4. Characterization

A scanning electron microscope (Hitachi S-3400N) was used for the observation of the internal pore morphology of the scaffolds. The samples were fractured and coated with gold by a sputter-coater. The hydrophilicity of the 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, Ramé-Hart, USA). The surface chemical composition of the scaffolds was investigated by using an X-ray photoelectron spectrometer (AXIS ULTRA^{DLD}, Kratos analytical, Manchester UK.) using a hemispherical electrostatic energy analyzer and Al

K_α (1.4 keV) X-ray source. The base pressure in the XPS analysis chamber was about $5x10^{-9}$ torr. The photoelectrons were detected with a hemispherical analyzer positioned at an angle of 45^o with respect to the normal to the sample surface.

2.5. Cell Culture

The sterilized 8 mm scaffold discs were placed into 24-well culture plates. Each scaffold was seeded with porcine chondrocytes at concentration of 1.0×10^6 cells/specimen and then incubated for 3 h to allow the cells to attach. Afterwards, the cell-seeded scaffolds were cultured in a 24-well culture plate under 5% CO_2 atmosphere at 37 ^{o}C for 21 days. The culture medium was regularly replaced every 2 days.

2.6. Cell Proliferation Assay

Cell proliferation on each scaffold was assessed by an Alamar blue assay, which is based on the detection of metabolic activity of the cultured cells. After a 21-day incubation period, the chondrocytes cultured on each scaffold were further incubated in a medium containing resazurin dye for 4 h. The fluorescence intensity, directly related to the cell proliferation, of aliquots (200 μl) of each medium was subsequently read at 530/590 nm.

2.7. RNA Extraction and RT-PCR Analysis

In brief, after a 21-day culture period, total RNA was extracted from the chondrocytes cultured on each scaffold using TRIZOL reagent (Invitrogen) by following the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized from 2 μg of RNA using Prime RT Master synthesis Kit (GeNet Bio) in 20 μl reaction. PCR analysis was performed to determine the expression of cartilage-specific genes, i.e., type II collagen and aggrecan. The mRNA level of 18S rRNA was used as an internal control. The PCR products were identified by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining. The band intensity of type II collagen and aggrecan was assessed using Image J program. The data were normalized by the 18S rRNA level in each sample.

2.8. Histological Analysis

After 21-day culture, the cells were fixed with 2% paraformaldehyde solution, embedded in paraffin, and sectioned (7 μm thickness). The sections were stained with hematoxylin and eosin (H&E) to observe cell infiltration and extracellular matrix production, respectively.

3. Results and Discussion

3.1. Characterization

The SEM images of internal pore surfaces of each scaffold are revealed in Fig. 1. The surfaces of both plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds were noticeably rough (Fig. 1(b-c)), whereas the surface of the untreated PCL scaffold appeared fairly smooth (Fig. 1(a)). The internal pore surface of the plasma-treated post-HPCL scaffold was, however, much rougher than that of the plasma-treated pre-HPCL scaffold. The different surface topography between the plasma-treated pre- HPCL and plasma-treated post-HPCL scaffolds was resulted from the different stages in conducting the alkaline hydrolysis of the PCL scaffolds: before versus after scaffold fabrication.

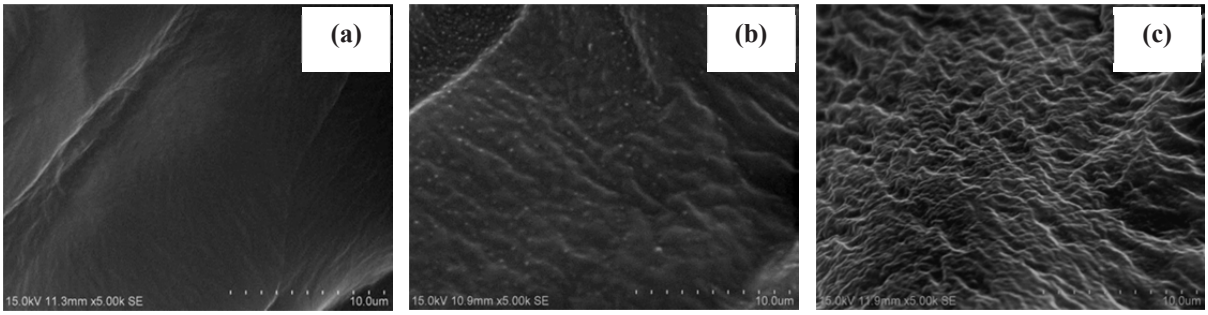

Fig. 1. SEM images of internal pore surfaces of scaffolds: (a) PCL; (b) plasma-treated pre-HPCL and (c) plasma-treated post-HPCL (original magnification x5000).

The water contact angles on the scaffolds measured by a sessile drop technique are listed as a function of time in Table 1. The hydrophilicity of the PCL scaffolds was enhanced significantly after surface treatments by alkaline hydrolysis and plasma treatment. At 30-sec measurement, the water contact angles at 118.10±3.70^{o}, 0^{o} and 35.80±1.00^{o} were observed on the PCL, plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds, respectively, suggesting that surface treatments induced polar components, e.g., -C-O-, >C=O, and –COOH, on the polymeric chains. The greater hydrophilicity of the plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds was also attributed to the additional carboxylate (-COO^{-}) and hydroxyl (-OH) groups on the HPCL chain termini, which were resulted from the alkaline hydrolysis and plasma treatment.

Table 1. Water contact angles on various PCL scaffolds measured as a function of time.

Exp #	Sample code	Water contact angle (X^{o})			
		0 sec	10 sec	30 sec	60 sec
1	PCL	120.20±2.43	119.80±2.14	118.10±3.70	117.60±3.40
2	Plasma-treated pre-HPCL	42.55±1.06	8.35±11.81	0** (15 sec)	-
3	Plasma-treated post-HPCL	41.07±1.24	36.70±5.16	35.80±1.00	0**

* The test was carried out in triplicate.
**The sample surface was completely wetted.

Furthermore, the water contact angles on the plasma-treated pre-HPCL and post-HPCL scaffolds decreased with an increasing measuring time. The plasma-treated pre-HPCL scaffold was, however, wetted more readily than the plasma-treated post-HPCL. The former expressed a zero-degree contact angle at 15 sec, while the latter did at 60 sec. The hydrolysis of PCL prior to the scaffold fabrication and the subsequent plasma treatment of the fabricated scaffold rendered the greater and more homogeneous hydrophilic characteristics of the plasma-treated pre-HPCL scaffold.

The surface chemical composition of the scaffolds was determined by XPS measurement; the results are demonstrated in Table 2. The O/C atomic ratios of the surface-treated scaffolds were noticeably higher than that of the starting PCL scaffold, due to the presence of newly generated oxygen-containing groups on their surfaces. These results were in good agreement with those obtained from the water contact angle measurement. Interestingly, the plasma-treated pre-HPCL scaffold exhibited a greater O/C atomic ratio (50.28±2.02%) than the plasma-treated post-HPCL scaffold (37.83±4.83%). This explained why the plasma-treated pre-HPCL scaffold was wholly wetted more quickly than the plasma-treated post-HPCL scaffold. The surface hydrophilicity and wettability of the PCL scaffold were enhanced prominently when it was initially hydrolyzed prior to the scaffold fabrication and subsequently plasma-treated after fabrication.

Table 2. Surface chemical composition and O/C atomic ratio detected on the surfaces of PCL scaffolds.

Exp #	Sample code	Chemical composition (%)		O/C atomic ratio (%)
		Carbon	Oxygen	
1	PCL	77.32±1.03	22.69±1.03	29.36±1.72
2	Plasma-treated pre-HPCL	66.55±0.89	33.45±0.89	50.28±2.02
3	Plasma-treated post-HPCL	72.60±2.55	27.40±2.55	37.83±4.83

* The test was carried out in duplicate.

3.2. Cell Proliferation

The proliferation of porcine chondrocytes on each scaffold was determined by using an Alamar blue assay. As revealed in Fig. 2, the number of cells on the surface-treated PCL scaffolds was greater than that of the untreated PCL scaffold. Nevertheless, there was no significant difference in the cell number found between the plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds after 21-day culture peroid.

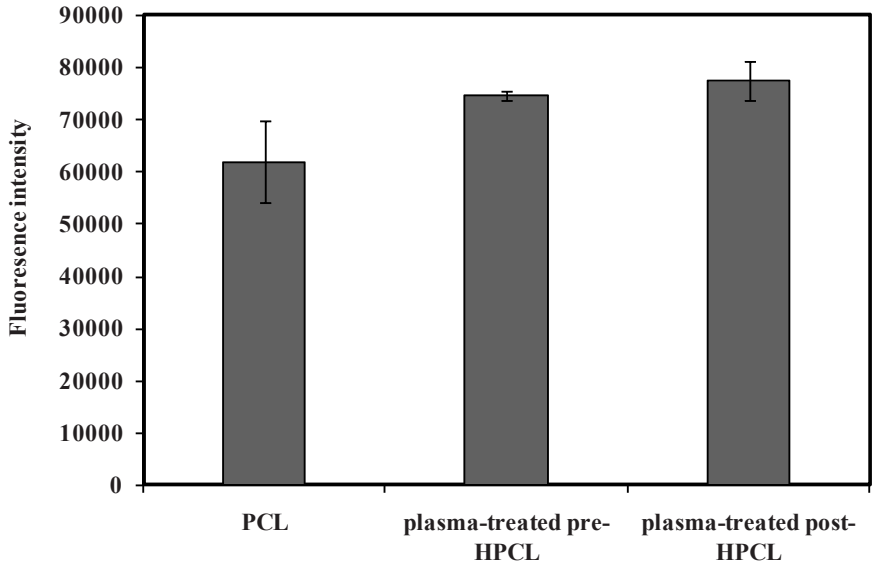

Fig. 2. Cell proliferation on various PCL scaffolds at a 21-day culture period

3.3. Cartilage-Specific Gene Expression

Fig. 3 demonstrates the expression of cartilage-specific gene in the chondrocytes cultured on various PCL scaffolds after a 21-day culture period. The considerable expression of type II collagen and aggrecan mRNA was found on the surface-treated PCL scaffolds; no gene expression was detected on the untreated PCL scaffold. This suggested that the improved surface properties, in terms of surface hydrophilicity, roughness and chemical compositions, resulted from both alkaline hydrolysis and low pressure oxygen plasma treatment provided a favorable environment for the chondrocytes to maintain their cellular phenotype and function more properly. There were, however, no significant differences in expression levels of both genes observed between the plasma-treated pre-HPCL and plasma-treated post-HPCL scaffolds.

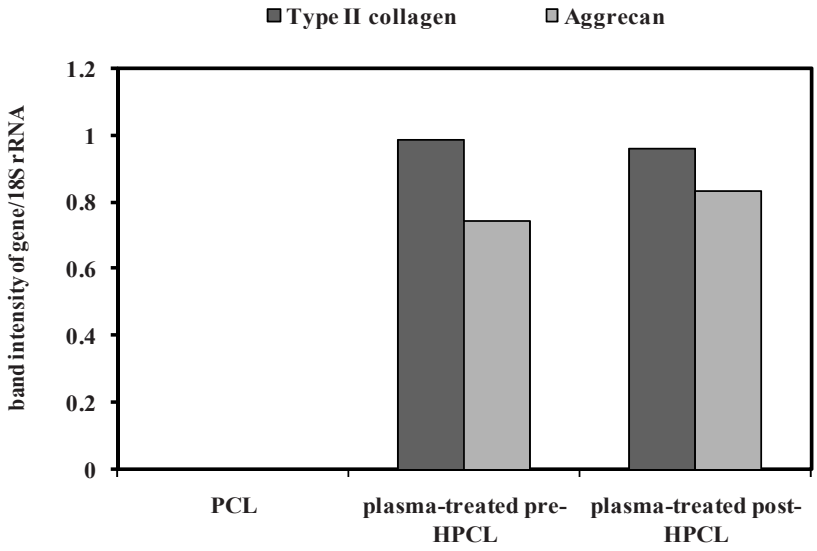

Fig. 3. Band intensity of mRNA expression of type II collagen and aggrecan secreted from porcine chondrocytes cultured on various PCL scaffolds at a 21-day culture period.

3.4. Cell Infiltration and Extracellular Matrix Production

The results from the histological analysis revealed that the chondrocytes could adhere and proliferate more effectively on the surfaces of the surface-treated PCL scaffolds than those on the untreated PCL scaffold, as seen in Fig. 4. Moreover, the formation of extracellular matrix was observed more intensely on the surface-treated scaffolds. Apparently, the chondrocytes cultured on the plasma-treated pre-HPCL scaffold infiltrated into the inner scaffold more thoroughly than those on the plasma-treated post-HPCL scaffold. This was attributed to the difference in the hydrophilic characteristics of the scaffolds. The greater and more homogeneous surface hydrophilicity and wettability of the plasma-treated pre-HPCL scaffold facilitated the cell infiltration through the inner scaffold more profoundly, and consequently, a higher ECM content was produced and distributed throughout the plasma-treated pre-HPCL scaffold. The surface topography, hydrophilicity and chemical composition of the scaffolds played a significant role in the interaction between the porcine chondrocytes and the PCL scaffolds.

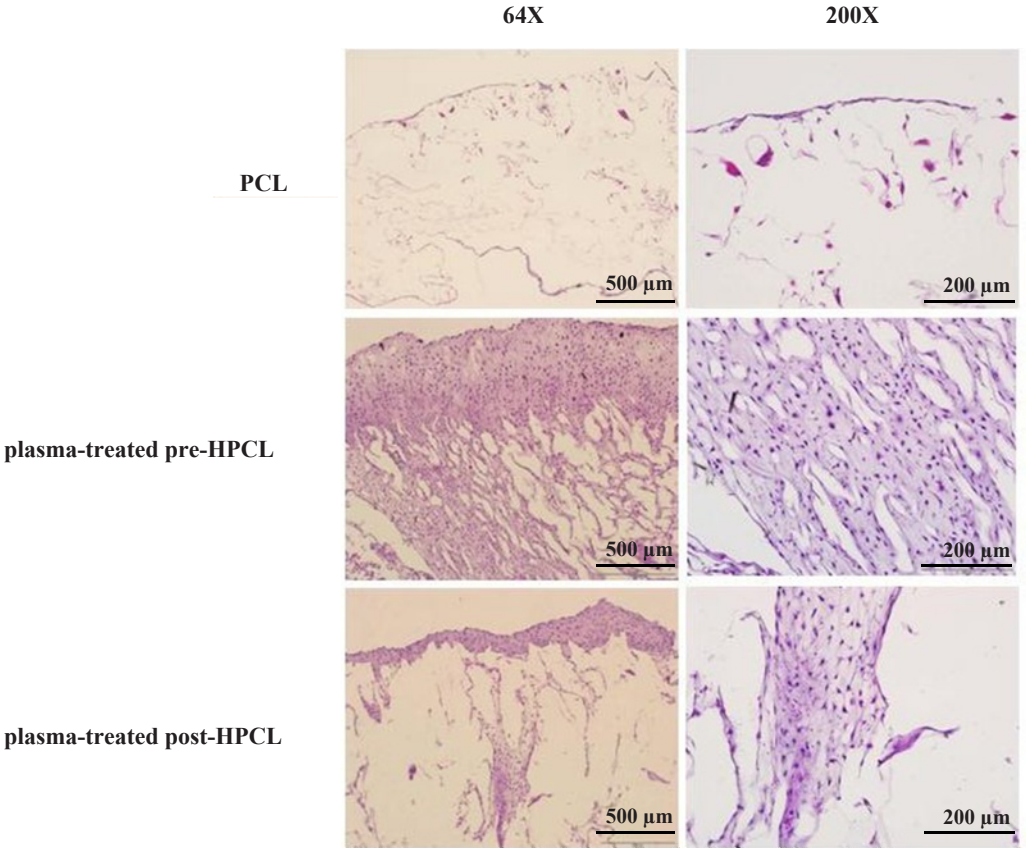

Fig. 4. Histological appearances of H&E stained cross-sections of various PCL scaffolds at a 21-day culture period.

4. Conclusion

The surface roughness, wettability and chemical composition of PCL scaffolds were drastically altered by the sequential surface treatments: alkaline hydrolysis and oxygen plasma treatment. The surface-treated scaffolds were found to promote the proliferation and the type II collagen and aggrecan gene expression of the porcine chondrocytes. However, the cells cultured on the plasma-treated pre-HPCL scaffold infiltrated through the scaffold more thoroughly and produced more ECM than those cultured on the plasma-treated post-HPCL scaffold. These results demonstrated that the greater and more homogeneous hydrophilicity of the scaffold provided the more favorable environment for the chondrocytes to grow and function more effectively.

Acknowledgements

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