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Fabrication and characterization of nanoscale-roughened PCL/collagen micro/nanofibers treated with plasma for tissue regeneration

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Abstract

A surface-modified electrospun poly(ε-caprolactone) (PCL)/collagen fibrous mat was fabricated with a simple coating and the selective plasma-treatment method using a template with 800-nm holes. A homogeneous nanoscale pattern (376 ± 40 nm) was successfully obtained on the collagen region coated on the PCL micro/nanofibers. The mechanical and *in vitro* cellular activities (cell proliferation and osteogenic activities) were evaluated and compared with controls, including electrospun PCL fibers coated with the same collagen component, to characterize the effectiveness of the coated collagen component roughened with the nanoscale. Significant increases in cell viability of about 1.4-fold and calcium deposition of about 1.3-fold were observed on the plasma-etched PCL/collagen fibrous mats compared to those on the PCL/collagen fibrous mats. These results show the newly designed fibrous biomedical scaffold can induce significant cellular activities at the interface between cells and the topological boundary with regenerating tissues.

Introduction

Chemical and physical surface modifications of biomedical scaffolds have been widely investigated for tissue engineering because the surface properties of bio-substitutes can be directly related to the determining the biocompatibility between the biological environment and cellular activities.[1, 2] It has been reported that the Various scales of micro- and nano-surface topography affect a variety of cellular responses (initial cell adhesion, proliferation, and differentiation).[3, 4] According to previous studies, the microsized surface-pattern on biomaterials influences cell morphology and the cytoskeleton, whereas hierarchical or nanosized surface patterns affect cell proliferation and differentiation.[5, 6, 7] Additionally, a nanosized surface pattern significantly stimulated human mesenchymal stem cells (MSCs), resulting in bone mineral production *in vitro*.[8]

Several methods have been proposed to increase scaffold surface bioactivity, such as chemical modifications using sodium hydroxide,[9] topological surface modifications using selective laser sintering,[10] surface modifications using irradiation with ion beams,[11] and chemical and physical modifications using plasma treatments.[12, 13, 14, 15] Of these techniques, the plasma process has been widely used because the method allows modification of only the surface properties with minimal sacrifice of the bulk properties of the treated materials. In addition, no toxic chemical solvents are used. Although various chemicals used in surface modification processes to prepare tissue engineering scaffolds can be removed, very small amounts of residual solvent can terminate *in vitro* or *in vivo* cellular activities.[16, 17] In addition, the plasma treatment has been effective for modifying synthetic polymers, including poly(ε-caprolactone) (PCL) and poly(ethylene oxide terephthalate)/poly(butylene terephthalate) by presenting the required chemical functionalities on the polymer surface and enhancing hydrophilicity and permeability, which are pre-requisite properties of biomedical scaffolds for cell attachment, proliferation, and differentiation on the surfaces.[18] Several groups have shown outstanding results for synthetic biopolymers treated with plasma.[12, 13, 14, 15] According to Yildirm et al, oxygen plasma-treated PCL scaffolds fabricated using the solid-freeform-fabrication technique showed significantly increased cellular activities, including alkaline phosphatase (ALP) activity and osteocalcin secretion from mouse osteoblast cells.[12] Furthermore, electrospun nanofibers of poly(ethylene oxide terephthalate)/poly(butylene terephthalate) were treated with oxygen plasma by Nandakumar et al., and demonstrated outstanding osteogenic differentiation of human mesenchymal stem cells.[13] Also, our group invented a new plasma treatment using nano-sized anodic aluminum oxide (AAO) templates (100 and 800 nm) to obtain a homogeneous nano-sized surface roughness on electrospun nonwoven PCL fibers and melt-plotted PCL struts.[14, 19] The PCL scaffolds with a nanosized pattern (430 ± 63 nm) significantly increased initial cell attachment, proliferation, and even osteogenic differentiation of osteoblast-like-cells (MG63) compared to those of conventional plasma treated scaffolds, which did not have a roughened structure.

However, the mechanical properties of the plasma-treated PCL fibers with a roughened structure had decreased about 10% compared to those of the un-treated fibrous PCL scaffolds due to

the highly roughed surface.[19] Moreover, although the plasma-roughened PCL scaffolds provided significantly improved cellular activities compared to the untreated surface, the plasma-treated PCL scaffolds were absent of cell-recognizing signals.

In general, to improve various synthetic scaffolds and make them more conducive to cellular responses, A protein-based surface-coating has been used to obtain highly bioactive interfaces that allow for efficient cell adhesion and sustain differentiated phenotypes.[20, 21] Thus, a variety of protein components from the extracellular matrix (ECM) (collagen, gelatin, and fibronectin etc.) have been conjugated on plasma-modified surfaces to increase cellular responses, such as cell attachment and proliferation.[22, 23]

In general, PCL has several advantages, including flexible mechanical and degradation properties and biocompatibility, and it can alter the surface by modifying hydrophobicity using various surface modification methods with preferred functional groups.[24] Collagen has been extensively used to increase interactions between cells and materials because it provides both mechanical support to tissues and cell-binding sites (Arg-Gly-Asp (RGD) and GFOGER).[25, 26, 27] In this study, we propose a newly modified surface for electrospun PCL/collagen fibers, which was obtained by an electrospinning/coating/selective-plasma-treatment process. We applied oxygen plasma treatment on the collagen-coated fibrous PCL to achieve a highly bioactive surface. We used plasma-etching supplemented with a nanosized AAO template (hole size = 800 nm) to obtain controlled roughness of the collagen region on the fibrous PCL surface. In our previous study,[28] cells (MG63)-imprinted surfaces showed the 702 ± 87 nm roughness, and in the roughed surface, meaningful ALP activity and calcium deposition were obtained, although the optimum surface roughness can differ by several parameters (cell-types and material stiffness etc.). Based on the reason, we selected the 800 nm template.

By selecting the processing conditions of the coating/plasma-treatment, we achieved a nanosized regularly roughened collagen layer on the PCL micro/nanofibers. Various *in vitro* cellular activities of the fabricated PCL biomedical substitutes with micro-scale fibers and a nanoscale pattern were examined using osteoblast-like-cells (MG63) to assess its feasibility for use in a tissue regenerative scaffold. Our results suggest that the newly designed scaffold to be a useful biomaterial because of the synergistic effect between the hierarchical structure (micro/nanofibers and patterned nanosized-topology) and the chemical components (PCL and collagen).

Experimental

Materials

PCL (M_n = 90,000 g mol^{-1}) was obtained from Sigma-Aldrich (St. Louis, MO, USA). A PCL solution was prepared by dissolving 2 g PCL in 18 g of a solvent mixture of 80 wt% methylene chloride (Junsei, Tokyo, Japan) and 20 wt% dimethylformamide (Junsei) to generate an electrospun mat. Porcine type-I atelocollagen (Matrixen-PSP, Bioland, South Korea) was used to coat the PCL fibrous

mat. The collagen solution was dissolved in 0.05 M acetic acid (pH 3.2) at a fixed concentration of 0.5 wt%. The collagen was immersed in a 50 mM 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, M_w = 191.7 g/mol, Sigma) solution in 95% ethanol for 24 h at room temperature to cross-link the coated collagen on the scaffolds.

Plasma treatment of fibrous scaffolds

The PCL/collagen fibers were treated with oxygen plasma using LF plasma (CUTE–MP/R, Femto-Science Inc., Seoul, Korea). A low frequency of 50 kHz, power of 10 W, pressure of 5.3×10^{-1} Torr, and an oxygen flow rate of 10 standard cm^3/min (sccm) were used. It has been known that the oxygen plasma etching of polymer surfaces can be developed through the reaction of atomic oxygen with the surface carbon atoms, producing volatile reaction products.[29] The 20 × 20-mm^2 fibrous mat was placed in the plasma chamber and subjected to plasma treatment for various times. The temperature of the plasma chamber was < 37°C to avoid denaturing the collagen coated on the PCL. During the plasma process, an 800-nm template (AAO, Anodisc filter, Whatman International Ltd., Maidstone, England) was used as etching mask for the PCL fibrous mat to form a homogeneously roughened surface on the fibers. In addition, untreated PCL and PCL/collagen fibrous mats were used as controls. As another control, a conventional plasma-treated PCL/collagen fibrous mat was obtained using the following conditions, 10 W, 10 sccm, 10-min exposure time, and no AAO template.

Characterization of the scaffolds

To observe the morphology of the scaffolds, an optical microscope (BX FM-32; Olympus, Japan) connected to a digital camera, and scanning electron microscopy (SEM: SNE-3000M, SEC Inc., South Korea) were used.

To measure the water contact angle (WCA) of the PCL fibrous mat, one droplet (10 μL) of water was carefully dropped onto the surface of the mats, and the WCA was measured at 5 min.

To qualitatively measure the fiber surface roughness, surface roughness tester (Nanoview-m4151p, South Korea) and AFM (Nanowizard AFM, JPK Instruments, Germany) were used. In order to determine the roughness of the plasma treated fibers, 20 points on fiber surface were randomly selected and the roughness was measured.

For analyzing the chemical structures of the PCL and PCL/collagen fibrous mats, a Fourier-transform infrared (FT-IR) spectrometer (model 6700; Nicolet, West Point, PA, USA) was used. IR spectra represent the mean of 30 scans at 500-4000 cm^{-1} at a resolution of 8 cm^{-1}.

To measure the protein absorption ability, bicinchoninic acid (BCA) protein assay (Pierce Kit; Thermo Scientific, Waltham, MA, USA) was used. The samples were placed in 24-well plates containing minimum essential medium (Life Science, St. Petersburg, FL, USA), supplemented with 10% fetal bovine serum (Gemini Bio-Products, Sacramento, CA, USA) and 1% antibiotic/antimycotic

(Cellgro, Manassas, VA, USA) and incubated at 37°C for 1, 4, 12, and 24 h. Specimens were washed with PBS and lysed with 0.1% Triton X-100. An aliquot of the lysate (25 μL) was added to 200 μL of BCA working reagent, and the mixture was incubated for 30 min at 37°C. The absorbance at 562 nm was determined using a plate reader. Scaffolds incubated in serum-free medium were used as blanks. Protein adsorption was calculated as the mean ± the standard deviation (n = 5).

To evaluate the mechanical properties of the scaffolds, a universal tensile machine (Toptech 2000; Chemilab, South Korea) in tensile mode was used. Samples measuring 10×20 mm were prepared. Data were acquired in five independent experiments. All data are presented as means with standard deviation (SD). The specimen thickness were measured and averaged at three different points. The stress-strain curves of the scaffolds were recorded at a stretching speed of 0.5 mm s^{-1} at room temperature.

In vitro cell culture

The fabricated fibrous scaffold (diameter = 5 mm) was sterilized with 70% ethanol and ultraviolet (UV) light, and then placed in culture medium overnight. MG63 cells (ATCC number CRL-1427, ATCC, Manassas, VA) were used to evaluate cellular behavior on the scaffolds. The cells were cultured in minimum essential medium (Life Science) supplemented with 10% fetal bovine serum (Gemini Bio-Products) and 1% antibiotic/antimycotic (Cellgro). The cells were cultured up to passage 12 and collected by treatment with trypsin-EDTA (ethylenediaminetetraacetic acid). The cells were seeded onto the surfaces at a density of 1×10^5 cells per sample and incubated in an atmosphere of 5% CO_2 at 37°C. The medium was changed every 2 days.

Proliferation of viable cells

The MTT assay was used to analyze the viability of the cells (Cell Proliferation Kit I; Boehringer Mannheim, Mannheim, Germany) after culturing for 1, 3, and 7 days. This assay is based on the cleavage of the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases in viable cells to produce purple formazan crystals. Cells on the scaffold were incubated with 0.5 mg mL^{-1} MTT for 4 h at 37°C. Absorbance was measured at 570 nm using a microplate reader (EL800; Bio-Tek Instruments, Winooski, VT, USA). Five samples from each incubation period were tested, and each test was performed in triplicate.

Live/dead and DAPI/phalloidin analysis

After 6 h cell-culture, the fibrous scaffolds were exposed to 0.15 mM calcein AM and 2 mM ethidium homodimer-1 for 45 min in an incubator to permit observation of live and dead cells. The stained specimens were visualized under a microscope (TE2000-S, Nikon, Japan) equipped with an epifluorescence attachment and a SPOT RT digital camera (SPOT Imaging Solutions). Stained images

were captured, in which green and red colors indicated live and dead cells, respectively.

Also, after 6 h of cell culture, the fibrous scaffolds were analyzed with diamidino-2-phenylindole (DAPI; dilution ratio of 1:100, Invitrogen, Carlsbad, CA, USA) staining. Fluorescence staining was performed to characterize the nuclei of the cells (Invitrogen, Carlsbad, CA, USA). Alexa Fluor 568 Phalloidin (dilution ratio of 1:100, Invitrogen) was used to visualize the actin cytoskeleton of the cells in the scaffolds by fluorescence microscopy (CKX41; Olympus).

ALP activities and alizarin red S staining

After 14 day cell-culture, for alkaline phosphatase (ALP) staining, cells were washed with PBS twice and equilibrated with AP buffer (100 mM Tris-Cl, pH 9.5, 100 mM NaCl, and 10 mM $MgCl_2$); NBT and BCIP staining solutions were then applied for 30 min. The reaction was stopped by addition of PBS containing 20 mM EDTA. The osteogenic medium was refreshed every 3 days.

Calcium mineralization was determined by alizarin red S staining of MG63 cells in 24-well plates. The cells were cultured in MEM containing 50 μg mL^{-1} vitamin C and 10 mM β-glycerophosphate. Then, the cells were washed three times with PBS, fixed in 70% (v/v) cold ethanol (4°C) for 1 h, and air-dried. The ethanol-fixed specimens were stained with 40 mM alizarin red S (pH 4.2) for 1 h and washed three times with purified water. Specimens were then destained with 10% cetylpyridinium chloride in 10 mM sodium phosphate buffer (pH 7.0) for 15 min. An optical microscope was used to observe the extent of staining, and the OD was measured at 562 nm using a Spectra III UV microplate reader. The optical densities (OD) of calcium deposition were normalized to the total protein content. All values are expressed as means ± SD (n = 5).

Total protein content

The total protein content was measured using the bicinchoninic acid (BCA) protein assay (Pierce Kit; Thermo Scientific). The scaffolds were assayed after culturing for 7 and 14 days. Specimens were washed with PBS and lysed with 1-mL 0.1% Triton X-100. An aliquot of the lysate (25 μL) was added to 200-μL BCA working reagent, and the mixture was incubated for 30 min at 37°C. The protein concentration was determined from the absorbance at 562 nm using a plate reader.

Statistical analyses

All data are presented as means ± standard deviation. Statistical analyses were carried out using the SPSS software (SPSS, Inc., Chicago, IL, USA). Single-factor analysis of variance was used. A value of $p < 0.05$ was considered to indicate statistical significance.

Results and discussion

Plasma processing conditions to obtain a roughened PCL/collagen fiber surface

We used a simple dipping process with a 0.5 wt% collagen solution to fabricate the electrospun PCL

micro/nanofibers coated with type-I collagen. Before dipping the electrospun fibers, the fibrous mat was treated with the plasma conditions (10 W, 10 min, and 10 sccm) to add the hydrophilic property to the hydrophobic PCL fibers. The WCA of PCL fibrous mat was 106 ± 3° at 5 min, while for the plasma treated PCL fibrous mat the WCA was 11 ± 2° at 5 min. After coating the collagen on the PCL fibrous mat, the mat was dried in a freeze-dryer at −75°C for 12 h. The mass ratio of fibrous PCL mat to coated collagen was 1/0.15 ± 0.07. Figure 1 (a,b) shows scanning electron microscopic (SEM) images of the electrospun PCL and freeze-dried PCL/collagen fibers. The coated collagen was well embedded in the PCL/collagen fibrous mat.

In general, as plasma exposure time, power applied, and oxygen flow rate influence the etching efficiency of fibrous mats, various plasma exposure times (0–5 h) and power (10–50 W) under a constant oxygen flow rate (10 sccm) were used to assess the effect of roughness (R_a) on the PCL/collagen fibers. We have shown previously that the PCL fiber nanosized patterns can be highly dependent on using the AAO template,[19] so we used the 800-nm AAO template during this plasma process.

Figure 1 (c, d) shows the SEM micrographs for the various plasma exposure periods (10 min, 2 h, and 4 h) of the roughened PCL/collagen fibrous mat surface with and without the template, which is a general plasma treatment method. Plasma power and oxygen flow rate were fixed at 10 W and 10 sccm, respectively, to obtain the roughened PCL/collagen fiber surface. In the SEM and three-dimensional optical images, A homogeneously roughened surface of the micro/nanofibers treated with the template was obtained after a 4-h exposure time (Fig. 1c), whereas the etched fibrous PCL/collagen surface, which was treated without the AAO template, showed a less-developed surface pattern after 2 h and a melted surface was detected at the 4-h exposure time (Fig. 1d). As shown previously,[19] using the template during plasma treatment of PCL/collagen fibers increased uniform etching performance compared to that of the general plasma process. Figure 1(e, f) shows the roughness (R_a) of the PCL/collagen fibers for the different plasma exposure times (constant power, 10 W) and power (under constant exposure of 4 h). As shown in the results, increasing exposure time, increased the roughness, but < 4 h resulted in slightly melted fibers. However, plasma power did not significantly affect PCL/collagen surface roughness.

Based on these results, we set the condition of the plasma treatment as 10 W and 4 h to obtain a PCL/collagen roughened surface (R_a = 631 ± 52 nm).

Tensile property

In general, Scaffolds must have the appropriate mechanical properties to endure the environmental stressors in host tissues. These mechanical properties can also affect the morphology and activity of cultured cells; e.g., the scaffold elastic modulus not only influences contractility in the actin-cytoskeleton,[30] but also controls the of mitogen-activated protein kinase (MAPK) and the extracellular signal regulated kinase (ERK) activities, resulting in osteogenic differentiation.[31]

The mechanical properties before and after plasma treatment were similar for a short plasma treatment time. However, mechanical properties degrade if the exposure time to the plasma etching process is too long. A uniaxial tensile test was performed to evaluate the mechanical properties of the plasma-treated PCL/collagen.

Figure 2 (a,b) shows the stress–strain curves, Young's modulus, and maximum stress, respectively, of the electrospun PCL and plasma-treated PCL fibers (PCL-P), which were treated under the PCL/collagen (10 W, 4 h, and 10 sccm) and the plasma-treated PCL/collagen (PCL/Col-P) conditions. As expected, a clear difference (about 8% for Young's modulus) was observed between the electrospun PCL and PCL-P fibers. However, the differences in the modulus and maximum stress between the PCL and PCL/Col-P fibers were negligible ($p < 0.05$). This occurred because the etched region of the PCL/Col-P fibers was coated with collagen, not electrospun PCL fiber. Figure 2 (c, d) shows the SEM images of plasma-etched PCL/Col-P using uncross-linked collagen before and after dissolving the collagen component. As shown by the PCL/Col-P dissolved collagen, the PCL fiber surface was completely smooth, indicating that the electrospun PCL fibers were not rough. Based on these results, the PCL/Col-P tensile properties were similar to those of the pure PCL fibrous mat.

Fourier-transform infrared (FT-IR) and protein absorption

Figure 2(e) displays the FT-IR spectra of the pure PCL, PCL/Col (before PCL/collagen fiber plasma treatment), and PCL/Col-P. In the spectrum, The amide-I band at 1630 cm^{-1} and the amide-II band at 1555 cm^{-1} of coated collagen are visible in PCL/Col and PCL/Col-P, but not in pure PCL. The IR spectra show that the peak positions are similar before and after the plasma treatment. We confirmed that collagen was positioned appropriately on the plasma-treated PCL/Col-P fibrous mat.

The protein absorption ability of a scaffold is closely related to its roughness, chemical composition, and hydrophobicity, and influences initial cell-attachment due to the absorption ability of various proteins, such as fibrinogen, fibronectin, vitronectin, and immunoglobulin.[15]

Figure 2(f) shows the ability of pure PCL, PCL-P, PCL/Col, and PCL/Col-P fibrous mats to absorb proteins 1-, 4-, 12-, and 24-h periods. Interestingly, PCL-P and PCL/Col showed similar ability, and significantly higher protein absorption than that of pure PCL, but the PCL/Col-P fibrous mat had greater protein absorption ability than the other fibrous mats. These results indicate that the synergistic effect of the coated collagen component and roughened collagen surface increased protein interactions. Based on the results, we suggest that the PCL/Col-P mat induces significantly higher initial cell attachment than do the PCL, PCL-P, and PCL/Col fibrous mats.

In vitro *cellular activities*

To assess cellular activities *in vitro*, electrospun PCL, PCL-P, PCL/Col, PCL/Col-SP mats, which were plasma treated (plasma exposure time, 10 min and power, 10 W), and PCL/Col-P were prepared. The 3D roughness profile of the scaffolds was measured using atomic force microscopy AFM (Fig.

3a–e), and the results were converted to roughness values (R_a) (Fig. 3f). The plasma-roughened surfaces (PCL-P and PCL/Col-P) were consistently patterned, and the roughness values of PCL-P and PCL/Col-P were similar.

We conducted live/dead assays using osteoblast-like cells (MG63) to demonstrate cell viability on the five scaffolds. The test was performed after 6 h of cell culture. Fluorescence images of the scaffolds are presented in Figure 3(a–e). Green and red designate live and dead cells, respectively. Most of the attached cells were viable on all scaffolds. However, the number of viable cells on the PCL/Col-P scaffolds was higher than that on the other fibrous scaffolds.

Another fluorescence image shows the stained nuclei (blue)/F-actin (red) on the scaffolds after 6 h of cell culture (Fig. 3(a–e)). Similar to the live/dead result, the PCL/Col-P scaffold showed significantly greater stretched F-actin behavior compared to the other scaffolds. The number of nuclei and F-actin area percentage were measured (Fig. 3(g, h)). As expected, PCL/Col-P had significantly higher cell attachment compared to PCL/Col-SP ($p < 0.05$) and greater actin activation compared to the other scaffolds. SEM images show the morphology of cells attached/proliferated on the scaffolds after 6 h of culture. These results indicate that the PCL/Col-P scaffold provided the optimum environmental conditions for initial cell adhesion and proliferation due to the synergistic effect of the coated collagen component and the nanosized surface-topography of collagen region. According to several researchers, nano-structured surfaces can influence cell functions, including proliferation, differentiation, and alignment.[32, 33] The topological cue on a substrate can stimulate the osteoblastic differentiation, resulting in longer adhesion between cells and substrate and inducing cytoskeleton tension.[8] The indirect mechanotransductive reaction can cause various cellular responses. Kubo et al. reported that cytoplasmic localization of the focal adhesion protein (vinculin) was observed in bone marrow-derived osteoblasts on micro/nano-topological surfaces with 300-nm nodules, while weak appearance in the cells on the pure microsized surfaces. In the surfaces with 300-nm nodules, significant ALP activities were observed compared to that of pure microsized surface.[34] The result indicated that the application of nano-topological structure in the substrate can completely influence cell contact, providing more appropriate spaces for cell proliferation and differentiation.[35, 36]

The number of viable cells on the fibrous scaffolds was determined using the MTT assay after culture for 1, 3, and 7 days (Fig. 4(a)). As expected, the PCL/Col-P scaffold held the highest number of cells. This result was in good agreement with the number of nuclei in Fig. 3(g). In addition, coated collagen on the PCL fibers shows considerably higher on cell proliferation than the plasma-etching effect on pure PCL (PCL-P) ($p < 0.05$). The general fibrous mat (PCL/Col-SP) plasma treatment did not promote cellular proliferation, but the plasma-etched PCL/Col-P had a synergistic effect derived from the collagen component and the topological pattern.

Optical images of ALP activity and Alizarin Red-S staining of scaffolds cultured for 14 days were obtained to qualitatively compare ALP activity and calcium deposition on the fibrous scaffolds, (Fig. 4(b, c)). ALP activity and calcium mineralization were significantly higher in the PCL/Col-P

scaffolds than in the other types ($p < 0.05$), demonstrating more active mineralization in the PCL/Col-P scaffolds.

Calcium deposition in the fibrous scaffolds was evaluated quantitatively (Fig. 4(d)). The values were normalized to total protein content, and the PCL scaffold was set to 100%. Calcium deposition on the PCL/Col-P scaffolds on days 7 and 14 were significantly higher than on the other scaffolds, indicating that the plasma-etching fibrous PCL/collagen mat provided a more suitable chemical and topological environment for cell proliferation and osteogenic activity than did the other scaffolds.

Conclusion

In this study, nanoscale roughened biomedical scaffolds consisting of electrospun PCL micro/nanofibers coated with type-I collagen were prepared with a versatile coating and a selective plasma-etching method. The plasma-etched PCL/collagen scaffolds had homogeneous nanosized surface roughness (376 ± 40 nm), similar tensile properties, and were highly effective in terms of absorbing proteins compared to those composed of pure electrospun PCL/collagen. The scaffolds significantly improved initial cell adhesion and proliferation and osteogenic activities due to the synergistic effect of the coated collagen component and the nanoscale-roughened topology in the coated collagen. Based on these results, we propose our new biomimetic scaffold for use in regeneration of various soft and hard tissues.

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Figures

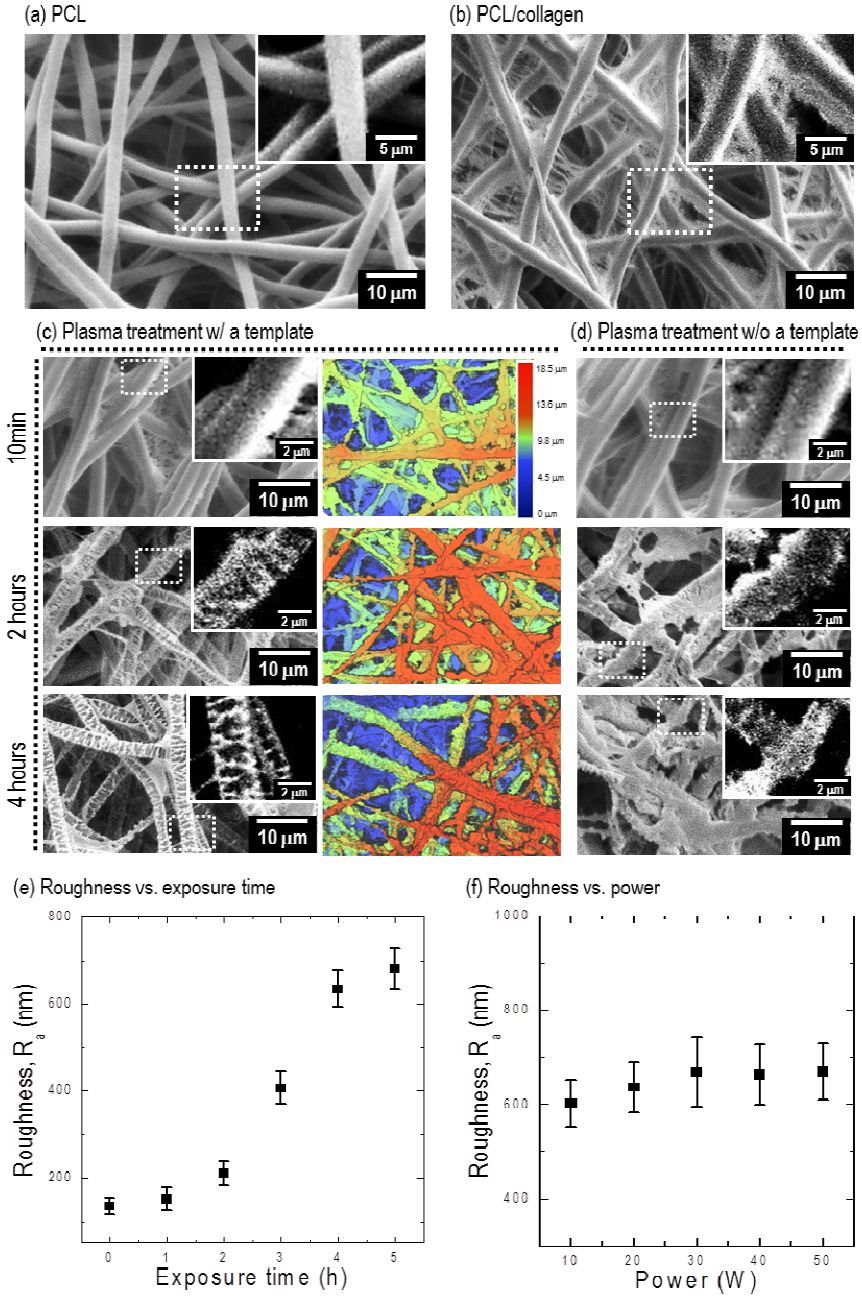


Figure 1. Scanning electron microscopy (SEM) images of (a) electrospun poly(ε-caprolactone) (PCL) fibers and (b) freeze-dried PCL fibers coated with collagen. (c) SEM images of PCL/collagen fibers treated with plasma-etching (with a template) for various plasma exposure times. (d) SEM images of PCL/collagen fibers treated with the plasma process without a template. Roughness values (R_a) of PCL/collagen-treated fibers with the plasma (with a template) for (e) various plasma exposure times and (f) power.

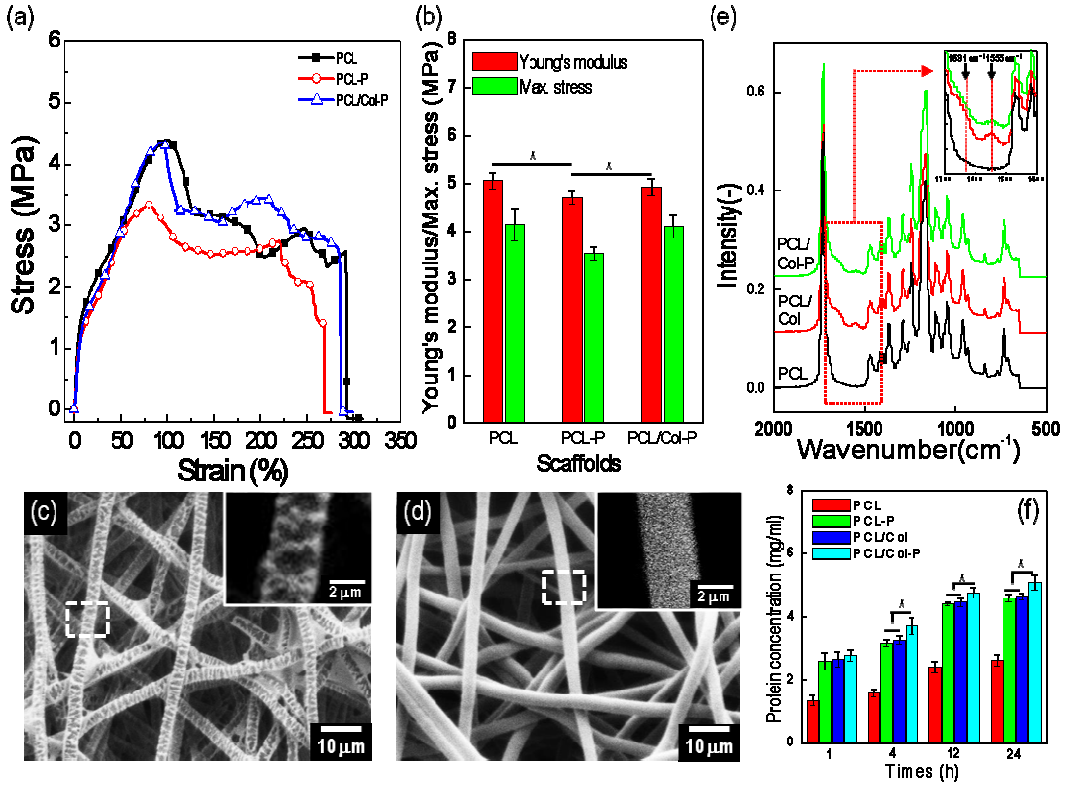


Figure 2. (a) Stress-strain curves and (b) Young's modulus, and maximum stress for poly(ε-caprolactone) (PCL), plasma-treated PCL fibers (PCL-P), and plasma-treated PCL/collagen (PCL/Col-P). Scanning electron microscopic (SEM) images (c) before and (d) after dissolving the collagen coating on a PCL/Col-P fibrous mat. (e) Fourier-transform infrared spectra for PCL, PCL/Col, and PCL/Col-P. (f) PCL, PCL/Col, and PCL/Col-P protein absorption values over various time periods. Asterisks indicate significant differences.

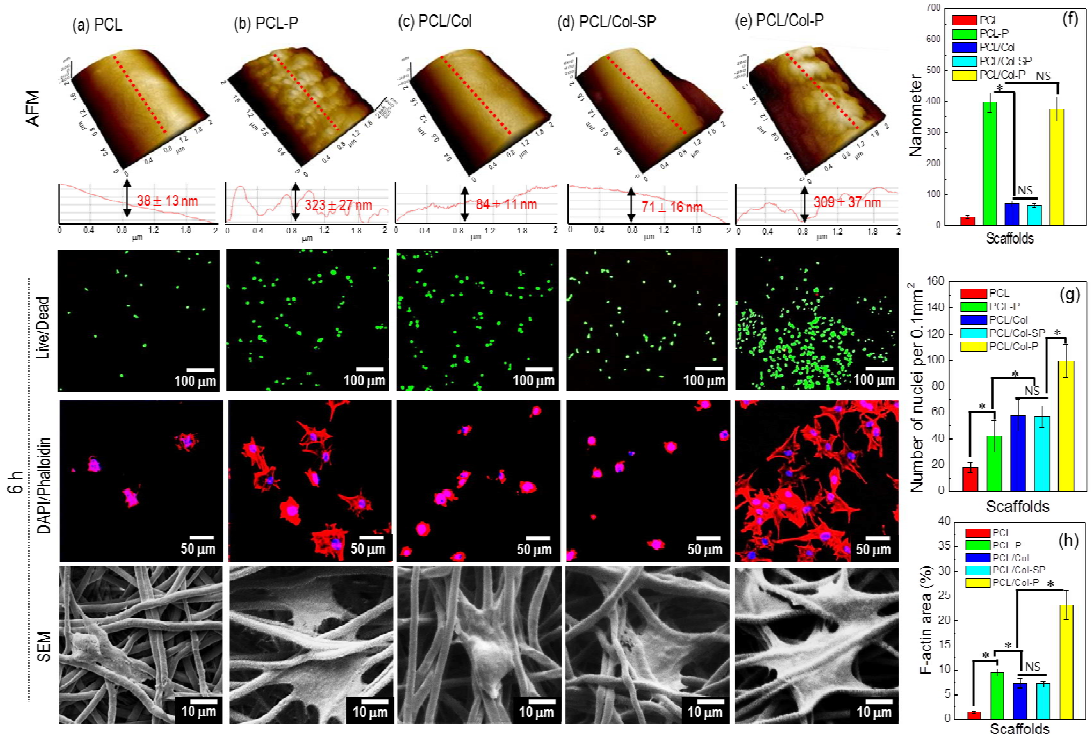


Figure 3. Surface roughness of the scaffolds as determined by atomic force microscopy. Live/dead, DAPI/phalloidin, and scanning electron microscopy (SEM) images after 6-h cell culture of (a) poly(ε-caprolactone) (PCL), (b) plasma-treated PCL fibers (PCL-P), (c) PCL/Col, (d) plasma-treated PCL/collagen (PCL/Col-SP), and (e) PCL/Col-P. (f) Surface roughness (R_a) of the fibers. Data are presented as means ± standard deviation (SD) (n = 10). (g) Number of nuclei and (h) the F-actin area from the DAPI/phalloidin images after a 6 h culture. Data are presented as means ± SD (n = 10).

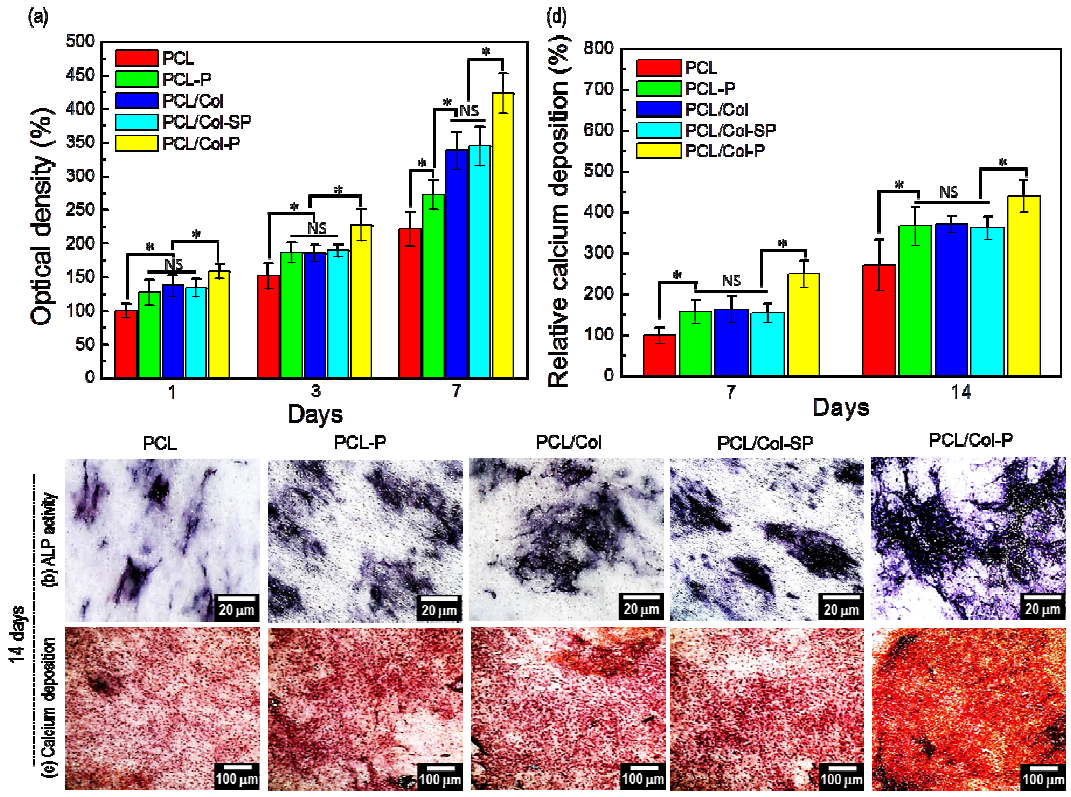
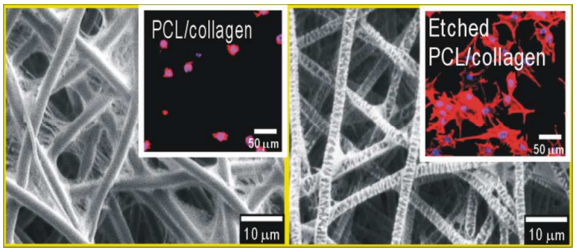


Figure 4. (b) Cell proliferation as determined by MTT assay. Optical images of (b) alkaline phosphatase (ALP) activity and (c) Alizarin Red-S staining for poly(ε-caprolactone) (PCL), plasma-treated PCL fibers (PCL-P), PCL/Col, plasma-treated PCL/collagen (PCL/Col-SP), and PCL/Col-P after 14 days of cell culture. (d) Calcium deposition on MG63 cells in the fibrous mats from 7–14 days. Asterisks and NS indicate significant differences and non-significance, resepctively.



A nanoscale roughened poly(ε-caprolactone)/collagen fibrous mat was fabricated with the selective plasma-treatment method using a template with 800-nm holes.