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Plasma-Treated Poly(lactic-*co*-glycolic acid) Nanofibers for Tissue Engineering

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Abstract: Nanofibers were prepared by electrospinning a solution of poly(lactic-*co*-glycolic acid) (PLGA) and their mean diameter was 340 nm. The PLGA nanofibers were treated with a plasma in the presence of either oxygen or ammonia gas to change their surface characteristics. The hydrophilicity of the electrospun PLGA nanofibers was significantly increased by the gas plasma treatment, as confirmed by contact angle measurements. XPS analysis demonstrated that the chemical composition of the PLGA nanofiber surface was influenced by the plasma treatment, resulting in an increase in the number of polar groups, which contributed to the enhanced surface hydrophilicity. The degradation behavior of the PLGA nanofibers was accelerated by the plasma treatment, and the adhesion and proliferation of mouse fibroblasts on the plasma-treated nanofibers were significantly enhanced. This approach to controlling the surface characteristics of nanofibers prepared from biocompatible polymers could be useful in the development of novel polymeric scaffolds for tissue engineering.

Keywords: plasma treatment, nanofiber, hydrophilicity, tissue engineering.

Introduction

Tissue or organ transplantation is a conventionally adapted treatment for patients who suffer organ failure or tissue loss due to accident or disease. However, transplantation is often limited due to shortage of available donors and immunological issues. Tissue engineering is a promising method to overcome these limitations and to provide man-made tissues or organs.[1-3] In this approach, the design and tailoring of polymer scaffolds is a critical factor for the successful production of man-made tissues, as they can be engineered using a combination of a patient's own cells and polymer scaffolds. In brief, tissue-specific cells are isolated from the patient via small biopsy and expanded *in vitro*. The cells are subsequently incorporated into three-dimensionally structured polymer scaffolds and transplanted back to the patient either by surgical implantation or in a minimally invasive manner using a syringe.[4]

Polymer scaffolds play a very important part in tissue engineering, taking on the function of the extracellular matrices in the body.[5,6] They are intended to bring cells together and regulate their function, thus controlling tissue formation and structure. Polymer scaffolds also allow the diffusion of nutrients, metabolites, and soluble factors to enhance tissue formation. Many polymers have been synthesized and used for tissue engineering applications. A copolymer of poly(lactic acid) and poly(glycolic acid) (e.g., PLGA) is one of the most widely used synthetic polymers in biomedical applications, including surgical sutures, implant materials, drug carriers, and scaffolds for tissue engineering, due to its biocompatibility and biodegradability.[7,8] Various methods have been reported up till now to fabricate porous polymeric scaffolds, including particulate-leaching,[9] phase separation,[10] gas foaming,[11] and emulsion freeze-drying.[12] Electrospinning has recently attracted attention due to its simplicity and the inexpensive features of the setup.[13] Polymeric nanofibers with various chemical and physical properties can be prepared by electrospinning and are potentially useful for filtration membranes, fiber-based sensors, and tissue engineering scaffolds.[14] Nanofibers have been considered useful in tissue engineering due to the large surface area that

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influences the adhesion, migration, and growth of cells.[15]

Although PLGA has been widely used as a tissue engineering scaffold, it has poor hydrophilicity and no natural cell recognition sites.[16] Various methods have been developed to modify the surface properties of polymers, including the hydrophilicity/hydrophobicity balance, surface free energy, and surface roughness.[17-19] Gas plasma treatment is a widely used method for chemical modification of polymers that can treat surfaces with complex shapes and generate desired functional groups such as hydroxyl and amino groups.[20] It has been reported that the surface characteristics of poly(L-lactic acid) (PLLA) films were dramatically changed after plasma treatment in the presence of various gases, and the adhesion force and affinity of mouse 3T3 fibroblasts on the PLLA films treated with ammonia plasma were greatly enhanced.[21,22]

In this study, nanofibers prepared by electrospinning a solution of PLGA were treated with plasma in the presence of either oxygen or ammonia gas. Changes in the surface characteristics of plasma-treated PLGA nanofibers, including surface hydrophilicity, chemical composition, and morphological changes, were investigated using contact angle method, X-ray photoelectron spectroscopy, and scanning electron microscopy. The degradation behavior and cellular responses to plasma-treated PLGA nanofiber matrices were also investigated to test their potential utility in tissue engineering applications.

Experimental

Materials. Poly(lactic-*co*-glycolic acid) (PLGA) (50/50) and 1,1,1,3,3,3-hexafluoro-2-isopropyl alcohol (HFIP) were purchased from Purac (USA) and Acros (USA), respectively, and used without further purification. 3-[4,5-dimethythiazol-2-yl]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega (USA).

Preparation of PLGA Nanofibers. PLGA nanofibers were prepared by electrospinning a 9% (w/v) PLGA solution in HFIP. Nanofibers were collected on a target drum that was placed at a distance of 8 cm from the syringe tip (inner diameter 0.0838 mm). A voltage of 17 kV was applied to the collecting target by a high voltage power supply, and the flow rate of the solution was 4 mL/min. Nanofibers were dried in a vacuum for 24 h at room temperature to remove the remaining solvent.

Plasma Treatment. Electrospun PLGA nanofibers were treated with plasma using a Miniplasma-station (Plasmart, Korea) under either oxygen or ammonia gas. The chamber was evacuated to less than 10 mTorr before it was filled with gas, followed by generation of glow-discharged plasma for a predetermined time.

Contact Angle Measurement. The contact angle of water droplets contacting the samples was measured using a DSA100 Drop Shape Analyzer System (KRÜSS, Germany). Deionized water was used, and ten independent measurements were averaged.

Scanning Electron Microscopy. A scanning electron microscope (Hitachi S-2460N, Japan) was used to investigate the morphology of gold-coated PLGA nanofibers before and after plasma treatment. Mean diameter of the nanofibers was determined by image analysis of SEM images (Scope Eye II).

X-Ray Photoelectron Spectroscopy. The surface chemical composition of PLGA nanofibers before and after plasma treatment was investigated using X-ray photoelectron spectroscopy (XPS). XPS spectra of plasma-treated samples were acquired on an ESCALAB 250 XPS spectrometer (VG Scientific, USA). High-resolution spectra of C1s, O1s, and N1s peaks were also recorded and used to quantify the chemical composition of polar groups on the surface by deconvolution and curve-fitting of the peaks.

***In Vitro* Degradation.** Non-treated and plasma-treated PLGA nanofibers were cut into 1 × 1 cm^2 squares, immersed in a PBS solution (Gibco, USA), and incubated at 37 °C under a 5% CO_2 atmosphere. Samples were removed at each time point, washed thoroughly with fresh PBS solution, and rinsed twice with distilled water. Rinsed samples were lyophilized and weighed. Changes in weight were measured and normalized to initial values before degradation. Degradation experiments were performed under sterile conditions.

Cell Culture. Mouse NIH 3T3 fibroblasts were supplied by the Korean Cell Line Bank (KCLB), and cultured in Dulbecco's modified eagle's medium (DMEM; Gibco, USA) containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) at 37 °C under a 5% CO_2 atmosphere. PLGA nanofibers were pre-treated with DMEM overnight, then cells were seeded on the surface of nanofibers at the density of 5×10^4 cells/well and incubated at 37 °C under a 5% CO_2 atmosphere. Photographs of fibroblasts adhered to the nanofibers were taken using a fluorescence microscope (Olympus, Japan). Cell viability was evaluated by an MTS assay. Culture medium was removed at each time point, and cells were rinsed with DMEM. An MTS solution (1.9 mg/mL MTS and 300 μM phenazine ethosulfate in Dulbecco's phosphate buffered saline, pH 6.0) was added to each well containing 0.5 mL of DMEM and cells were then incubated 37 °C for 2 h, followed by measurement of an optical density at 490 nm (BIORAD 680, USA).

Results and Discussion

Nanofibers were prepared by electrospinning a PLGA solution. The mean diameter of the PLGA nanofibers was 340±22 nm (mean±standard deviation), with a unimodal size distribution as determined by image analysis (Figure 1(a)). PLGA nanofibers were then treated with plasma in the presence of oxygen or ammonia gas to modify the surface proper-

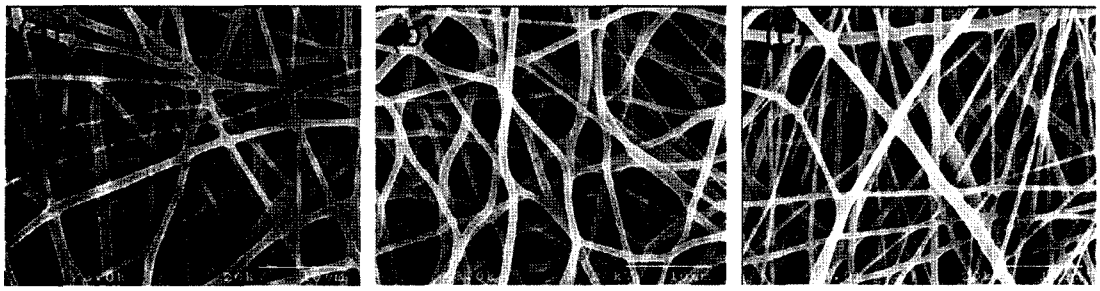

Figure 1. SEM images of (a) non-treated, (b) oxygen plasma-treated, and (c) ammonia plasma-treated PLGA nanofibers for 30 sec.

ties of the nanofibers, as plasma treatment can induce significant changes in the polymer surface, even after a short treatment time. Morphological changes were observed by SEM. No significant changes in the morphology of PLGA nanofibers were observed when exposed to plasma for 30 sec (Figures 1(b) and 1(c)). PLGA nanofibers exposed to oxygen plasma for 300 sec slightly lost their nanofibrous structure (data not shown). The dimensions and morphology of PLGA nanofibers, however, were not significantly influenced by ammonia plasma treatment for 300 sec (data not shown).

To investigate changes in surface hydrophilicity during plasma treatment, we measured the contact angles of plasma-treated PLGA nanofibers. Hydrophilic characteristics of PLGA nanofibers were substantially increased after plasma treatment in the presence of both oxygen and ammonia (Figure 2). The contact angle of water droplets contacting non-treated PLGA nanofibers was 134°, indicating extreme hydrophobic characteristics, as expected. This value decreased to 115° and 45° after treatment with oxygen plasma and ammonia plasma, respectively (treatment time=300 sec). This reduction in water contact angle clearly indicates increased surface hydrophilicity, which may have been caused by the introduction of new polar groups, such as hydroxyl and amino groups, on the surface. The ammonia gas-plasma method more effectively enhanced the surface hydrophilicity of PLGA nanofibers.

Changes in the chemical compositions of PLGA nanofiber surfaces before and after plasma treatment were investigated by XPS (Figures 3 and 4). The O/C content was slightly increased after oxygen plasma treatment, and a new N1s peak was observed after treatment with ammonia plasma, indicating newly formed N-containing functional groups such as amines. Chemical compositions of the plasma-treated nanofibers were quantified by deconvolution and curve-fitting of XPS spectra. Spectra were fitted with a Gaussian function, and O1s spectra of oxygen plasma-treated samples were resolved into two components, C-O and C=O. After oxygen

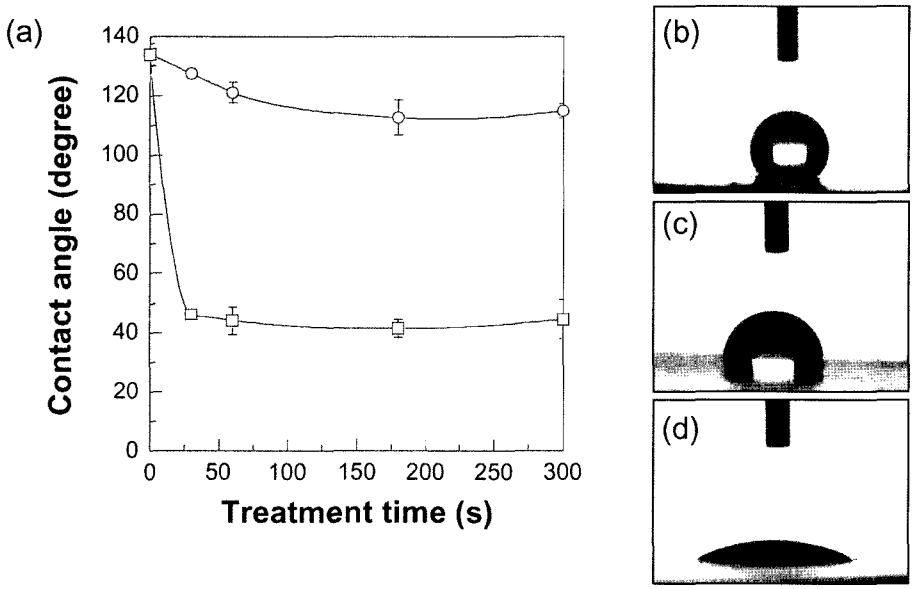

Figure 2. (a) Changes in the contact angles of water droplets taken immediately after contacting PLGA nanofibers treated with oxygen (○) or ammonia (□) plasma as a function of treatment time. Photographs of water droplets were also taken immediately after contacting, (b) non-treated, (c) oxygen plasma-treated, and (d) ammonia plasma-treated PLGA nanofibers for 300 sec.

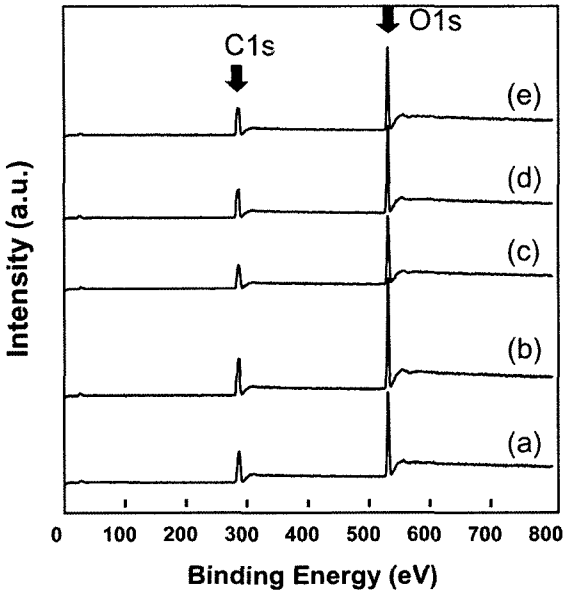

Figure 3. XPS spectra of PLGA nanofibers treated with oxygen plasma for (a) 0, (b) 30, (c) 60, (d) 180, and (c) 300 sec.

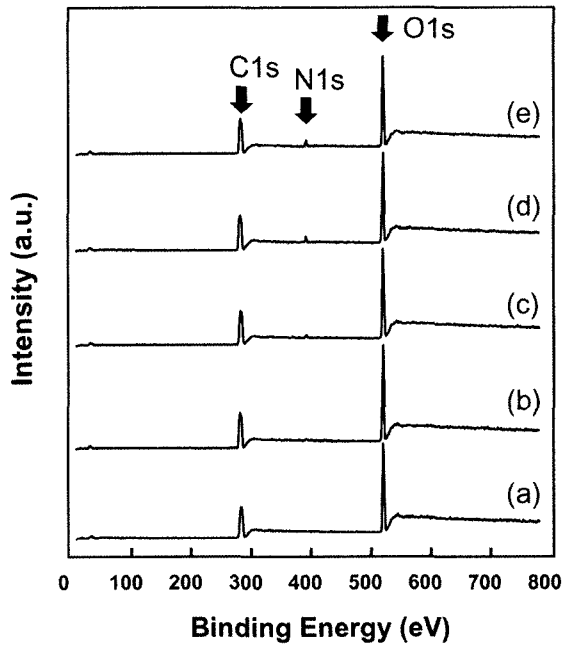

Figure 4. XPS spectra of PLGA nanofibers treated with ammonia plasma for (a) 0, (b) 30, (c) 60, (d) 180, and (c) 300 sec.

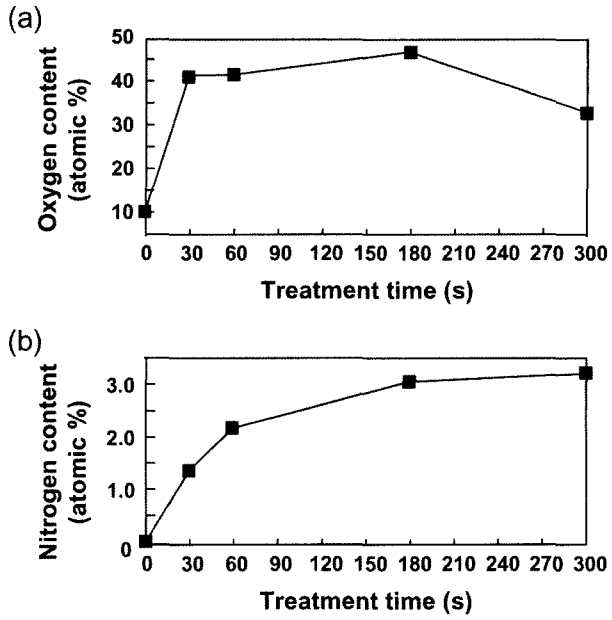

Figure 5. Atomic percent of (a) oxygen as C-O bond of oxygen plasma-treated PLGA nanofibers and (b) nitrogen of ammonia plasma-treated PLGA nanofibers.

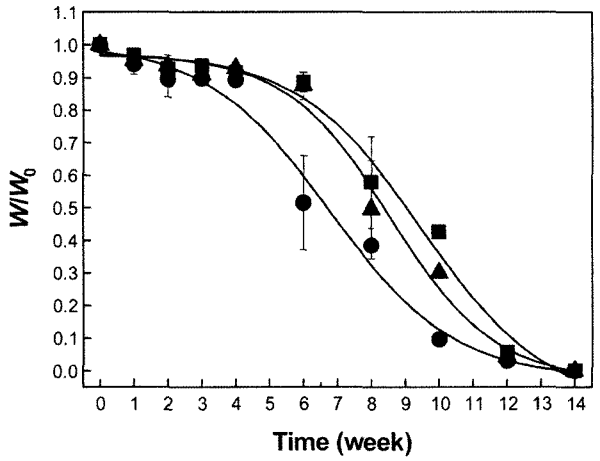

Figure 6. *In vitro* degradation behavior of non-treated (■), oxygen plasma-treated (●), and ammonia plasma-treated (▲) PLGA nanofibers. PLGA nanofibers were treated with plasma for 30 sec and incubated in PBS solution at 37 °C under 5% CO_2 atmosphere.

plasma treatment, the atomic percentage of oxygen with a single bond to carbon (C-O) significantly increased compared to non-treated PLGA nanofibers (Figure 5(a)). This can be attributed to formation of hydroxyl or peroxyl groups on the surface of PLGA nanofibers after oxygen plasma treatment. Interestingly, PLGA nanofibers had an abundance of nitrogen atoms, up to a 3.2% increase, after ammonia plasma treatment (Figure 5(b)).

The effect of plasma treatment on the degradation behavior of PLGA nanofibers was investigated next. PLGA nanofibers treated with gas plasma for 30 sec were incubated in PBS at 37 °C under a 5% CO_2 atmosphere for 14 weeks. Both oxygen and ammonia plasma-treated nanofibers degraded much faster than non-treated ones, likely due to their

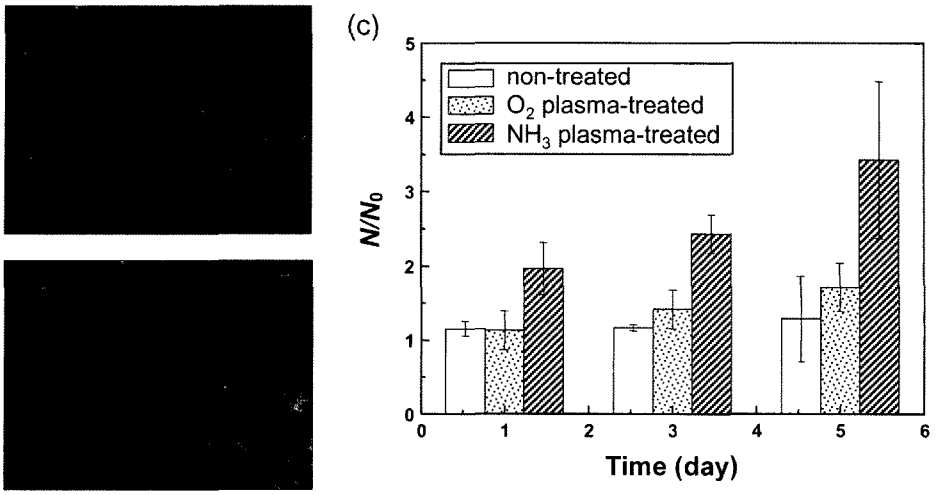

Figure 7. Photomicrographs of 3T3 fibroblasts adherent to the surface of (a) non-treated and (b) ammonia plasma-treated PLGA nanofibers. Cells were stained with octadecylrhodamine B, and original pictures were taken after 24 h of culture at 100× magnification. (c) Growth of 3T3 fibroblasts after seeding on various PLGA nanofibers for 5 days.

increased hydrophilic characteristics (Figure 6). Controlled degradation of polymer scaffolds is critical in tissue engineering, as it is desirable to coordinate the degradation rate of the scaffolds with the rate of new tissue formation.[23-25] Degradation behavior of PLGA nanofibers was influenced by plasma treatment, likely due to improved hydrophilicity of the nanofibers.

NIH 3T3 fibroblasts were seeded on PLGA nanofibers and their adhesion and proliferation were investigated in order to test the nanofibers as a tissue engineering scaffold. Cellular adhesion to PLGA nanofibers was significantly improved by plasma treatment, especially by ammonia plasma treatment (Figure 7). Cells seeded on ammonia plasma-treated nanofibers proliferated significantly more than those on non-treated and oxygen plasma-treated nanofibers. The growth rate of 3T3 cells increased from 0.04 day^{-1} on non-treated nanofibers to 0.15 day^{-1} on oxygen-treated and 0.32 day^{-1} on ammonia plasma-treated nanofibers. Ammonia plasma treatment significantly influenced the adhesion and proliferation of 3T3 cells on PLGA nanofibers. Enhanced hydrophilicity of PLGA nanofibers could be useful in tissue engineering approaches to control fibroblast phenotypes that are critical for tissue formation.

Conclusions

Treatment of PLGA nanofibers with either oxygen plasma or ammonia plasma significantly decreased the hydrophobicity and increased the number of polar groups on the nanofiber surface. Adhesion and growth of mouse fibroblasts on PLGA nanofibers were also substantially improved by plasma treatment in the presence of oxygen or ammonia gas. This approach to controlling the hydrophilicity and chemical composition of nanofiber surfaces may be useful in the development of novel synthetic extracellular matrices for tissue engineering.

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