Surface Characteristics of Plasma-Treated PLGA Nanofibers

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Summary: PLGA nanofibers were prepared by an electrospinning method (mean diameter, 340 nm) and treated with plasma in the presence of either oxygen or ammonia gas to modify the surface of the nanofibers. The surface hydrophilicity of electrospun PLGA nanofibers was greatly increased by plasma treatment, which was confirmed by contact angle measurement. XPS analysis demonstrates that the number of polar groups on the surface of PLGA nanofibers after plasma treatment was increased, and this was considered to contribute to the enhanced surface hydrophilicity of the nanofibers. This approach to controlling the surface properties and structures of nanofibers could be useful in the design and tailoring of novel synthetic extracellular matrices for tissue engineering applications.

Keywords: hydrophilicity; nanofiber; plasma; tissue engineering

Introduction

Tissue or organ transplantation is a conventionally adapted treatment for patients who suffer the loss or failure of an organ or tissue. However, this method is often limited due to the shortage of available donors and to immunological issues associated with infectious diseases. Tissue engineering is one recent and exciting approach that can overcome the limitations of organ transplantation and provide man-made tissues or organs for the patients.[1–3] In this approach, tissues or organs 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 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.[4]

In tissue engineering approaches, a polymer scaffold is intended to bring cells together and regulate the function of the cells, thus controlling the tissue structure. The polymer scaffold also allows the diffusion of nutrients, metabolites, and soluble factors, implementing the general roles of extracellular matrices of tissues in the body.[5,6] Many polymers to date have been designed and synthesized for tissue engineering applications. A copolymer of poly(lactic acid) and poly(glycolic acid) (PLGA) is one of the most widely used synthetic polymers in biomedical applications. PLGA has been widely used in the areas of surgical sutures, implant materials, drug carriers, and scaffolds for tissue engineering, due to its biocompatibility and biodegradability.[7,8] However, PLGA has poor hydrophilicity, and no natural cell recognition sites exist on the surface of PLGA.[9]

Various methods have been developed to modify the surface properties of polymers such as the balance of hydrophilicity/hydrophobicity, surface free energy, and surface roughness.[10–12] Gas plasma treatment is a frequently used method for the

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chemical modification of a polymer that can treat a surface with a complex shape and can generate desired functional groups such as hydroxyl and amino groups on the surface of the polymer.[13] It was 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 was greatly enhanced.[14,15]

Electrospinning has recently attracted much attention due to its simplicity and the inexpensive features of the setup. Electrospinning enables one to produce polymeric nanofibers, which can be potentially used for filtration membranes, fiber-based sensors, and tissue engineering scaffolds.[16] Nanofibers have been considered useful in tissue engineering due to the large surface area that influences the adhesion, migration, and growth of cells.[17]

In this study, nanofibers were prepared by electrospinning a solution of PLGA, which can be potentially useful for tissue engineering applications. The PLGA nanofibers were treated with plasma in the presence of oxygen or ammonia gas in order to alter their surface characteristics. Changes in the surface characteristics of the PLGA nanofibers, including the surface hydrophilicity, chemical composition, and morphological changes, were investigated using a contact angle method, X-ray photoelectron spectroscopy, and scanning electron microscopy, respectively.

Experimental Part

Materials

Poly(lactide-*co*-glycolide) (PLGA) and 1,1,1,3,3,3-hexafluoro-2-isopropyl alcohol (HFIP) were purchased from Purac and Acros, respectively, and used without further purification.

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. The nanofibers were dried in a vacuum for 24 hr 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). Deionized water was used for the measurement, and ten independent measurements were performed and averaged.

Scanning Electron Microscopy

A scanning electron microscope (Hitachi S-2350) was used to investigate the morphology of gold-coated PLGA nanofibers before and after plasma treatment. The mean diameter of the nanofibers was determined by image analysis of SEM pictures (Scope Eye II).

X-Ray Photoelectron Spectroscopy

The surface chemical composition of PLGA nanofibers before and after plasma treatment was investigated by X-ray Photoelectron Spectroscopy (XPS). XPS spectra of the plasma-treated samples were acquired on an ESCALAB 250 XPS spectrometer (VG Scientific). 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 of the plasma-treated

nanofibers by deconvolution and curve-fitting of the peaks.

Results and Discussion

PLGA nanofibers were prepared by an electrospinning method in order to mimic the nanofibrous structure of extracellular matrices of tissues in the body. Although various methods have been reported to produce porous polymer scaffolds for tissue engineering applications, nanofibers also have attracted much attention for their tissue regeneration and wound healing applications.[17] PLGA, one of the most extensively used biomedical polymers, was dissolved in HFIP, and nanofibers were prepared by electrospinning the solution. The mean diameter of the PLGA nanofibers was 340 nm with unimodal size distribution (Figure 1), as determined by image analysis.

Plasma treatment induces significant changes on the surface of polymers, even after a short treatment time. PLGA nanofibers were treated with plasma in the presence of either oxygen or ammonia gas to modify the surface properties of the nanofibers. The morphological changes in the plasma-treated PLGA nanofibers were observed by SEM (Figure 2). PLGA nanofibers exposed to oxygen plasma for 180 s slightly lost their nanofibrous structures (Figure 2c). However, the dimension and morphology of the PLGA nanofibers were not significantly influenced by ammonia plasma treatment (Figure 2e).

The contact angle of plasma-treated PLGA nanofibers was measured to investigate the surface hydrophilicity of the nanofibers. The hydrophilic characteristics of both the oxygen plasma-treated and ammonia plasma-treated PLGA nanofibers were dramatically increased in parallel with treatment time (Figure 3). The contact angle of water droplets contacting non-treated PLGA nanofibers was 139°, indicating the quite hydrophobic properties of the non-treated PLGA. This value was decreased to 112° and 47° after treatment with oxygen plasma and ammonia plasma, respectively (treatment time = 180 s). The reduction of the water contact angle clearly indicates the increased surface hydrophilicity, which may have been caused by the introduction of new polar groups on the surface of the PLGA nanofibers.

We next performed the XPS analysis to investigate the changes in chemical composition of the PLGA nanofiber surfaces before and after plasma treatment. The XPS spectra of PLGA nanofibers before

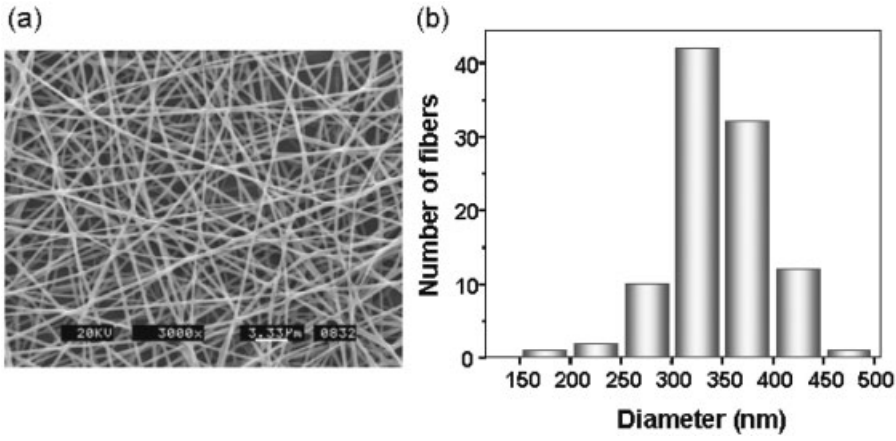

Figure 1.
(a) SEM image and (b) diameter distribution of electrospun PLGA nanofibers.

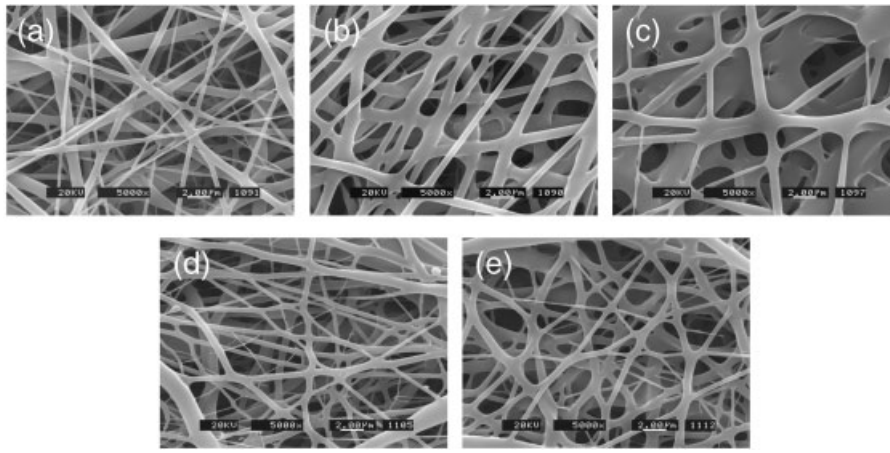

Figure 2.
SEM images of (a) non-treated, (b,c) oxygen plasma-treated, and (d,e) ammonia plasma-treated PLGA nanofibers. Treatment time was varied from (b,d) 30 to (c,e) 180 s.

and after plasma treatment are shown in Figure 4 and 5. XPS analysis demonstrates that the O/C content was slightly increased after oxygen plasma treatment. However, the intensity of carbon with a single bond to oxygen (C–O) significantly increased, compared with that of non-treated PLGA nanofibers (data not shown). This can be attributed to the formation of hydroxyl or peroxyl groups on the surface of PLGA nanofibers after oxygen plasma treatment. Interestingly, the surface of the PLGA nanofibers was abundant with nitrogen atoms after ammonia plasma treatment (Figure 5). A new N1s peak was observed after ammonia plasma treatment, indicating newly formed N-containing functional groups such as amines. The N/C content of PLGA nanofibers increased from 0.02 to 0.05 after ammonia plasma treatment.

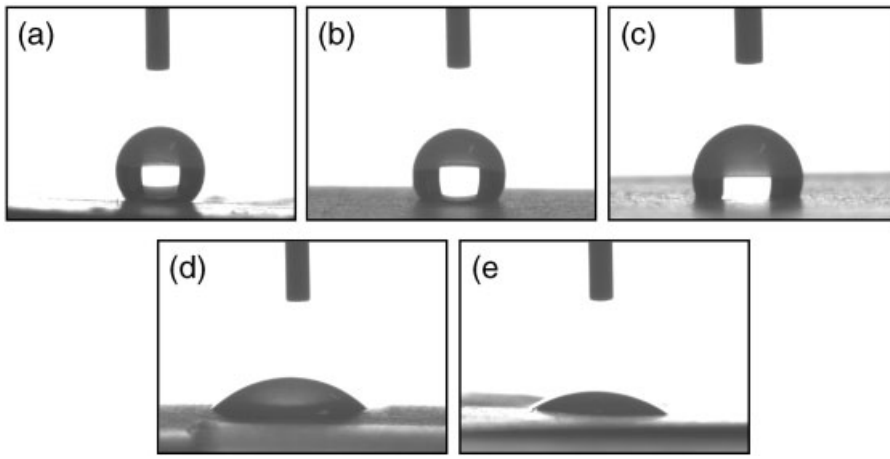

Figure 3.
Photographs of water droplets taken immediately after contacting (a) non-treated, (b,c) oxygen plasma-treated, and (d,e) ammonia plasma-treated PLGA nanofibers. Treatment time was varied from (b,d) 30 to (c,e) 180 s.

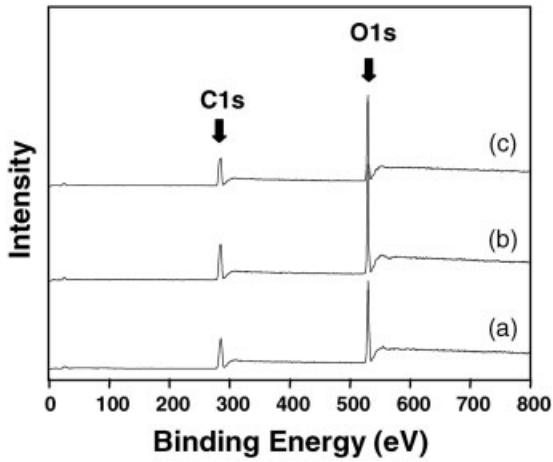

Figure 4.
XPS spectra of PLGA nanofibers treated with oxygen plasma for (a) 0, (b) 30, and (c) 180 s.

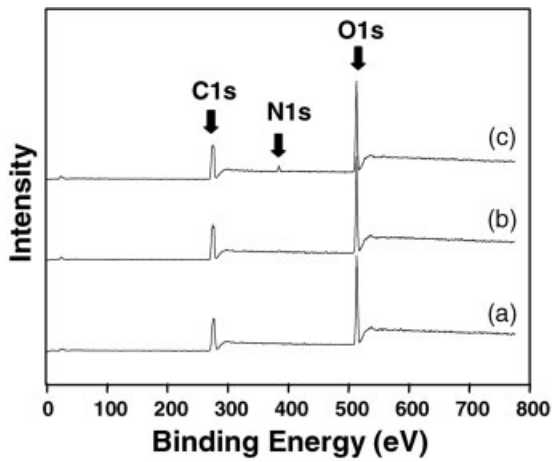

Figure 5.
XPS spectra of PLGA nanofibers treated with ammonia plasma for (a) 0, (b) 30, and (c) 180 s.

Conclusions

The surface characteristics of PLGA nanofibers were greatly influenced by plasma treatment in the presence of either oxygen or ammonia gas. The hydrophilicity and the content of polar groups on the surface of PLGA nanofibers were significantly increased after treatment with either oxygen plasma or ammonia plasma. This approach to controlling the hydrophilicity and chemical composition of nanofibers can be useful in the design and tailoring of novel synthetic extracellular matrices that can regulate the adhesion and growth of cells to be engineered in tissue engineering approaches.

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[1] R. Langer, J. P. Vacanti, *Science* **1993**, *260*, 920.
[2] D. J. Mooney, A. G. Mikos, *Sci. Am.* **1999**, *280*, 60.
[3] L. G. Griffith, G. Naughton, *Science* **2002**, *295*, 1009.

[4] K. Y. Lee, D. J. Mooney, *Chem. Rev.* **2001**, *101*, 1869.
[5] M. P. Lutolf, J. A. Hubbell, *Nature Biotechnol.* **2005**, *23*, 47.
[6] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, "*Molecular Biology of the Cell*", Garland Publishing, New York 1994, p. 971.
[7] L. D. Harris, B.-S. Kim, D. J. Mooney, *J. Biomed. Mater. Res.* **1998**, *42*, 396.
[8] J. Khandare, T. Minko, *Prog. Polym. Sci.* **2006**, *31*, 359.
[9] Y. Q. Wang, X. Qu, J. Lu, C. F. Zhu, L. J. Wan, J. L. Yang, J. Z. Bei, S. G. Wang, *Biomaterials* **2004**, *25*, 4777.
[10] K. Webb, V. Hlady, P. A. Tresco, *J. Biomed. Mater. Res.* **1998**, *41*, 422.
[11] R. Daw, S. Candan, A. J. Beck, A. J. Devlin, I. M. Brook, S. MacNeil, R. A. Dawson, R. D. Short, *Biomaterials* **1998**, *19*, 1717.
[12] R. Bos, H. C. van der Mei, H. J. Busscher, *FEMS Microbiol.* **1999**, *23*, 179.
[13] J. R. Hollahan, B. B. Stafford, R. D. Falb, S. T. Payne, *J. Appl. Polym. Sci.* **1969**, *13*, 807.
[14] J. Yang, G. X. Shi, J. Z. Bei, S. G. Wang, Y. L. Cao, Q. X. Shang, G. G. Yang, W. J. Wang, *J. Biomed. Mater. Res.* **2002**, *62*, 438.
[15] Y. Wan, J. Yang, J. Yang, J. Bei, S. Wang, *Biomaterials* **2003**, *24*, 3757.
[16] Q. P. Pham, U. Sharma, A. G. Mikos, *Tissue Eng.* **2006**, *12*, 1197.
[17] J. Venugopal, S. Ramakrishna, *Appl. Biochem. Biotechnol.* **2005**, *125*, 147.

