Macromolecular Research, Vol. 22, No. 7, pp 746-752 (2014)
DOI 10.1007/s13233-014-2096-8

www.springer.com/13233
pISSN 1598-5032 eISSN 2092-7673

Hydrophobization of Silk Fibroin Nanofibrous Membranes by Fluorocarbon Plasma Treatment to Modulate Cell Adhesion and Proliferation Behavior

Minyoung Lee[1], Young-Gwang Ko[1], Jae Baek Lee[1], Won Ho Park[2], Donghwan Cho[1], and Oh Hyeong Kwon*[,1]

[1]Department of Polymer Science and Engineering, Kumoh National Institute of Technology, Gyeongbuk 730-701, Korea

[2]Department of Advanced Organic Materials and Textile System Engineering, Chungnam National University, Daejeon 305-764, Korea

Received January 21, 2014; Revised February 17, 2014; Accepted February 21, 2014

Abstract: Saturated fluorocarbon (CF_4) immobilized silk fibroin (SF) nanofibrous membranes were prepared and characterized for biomedical applications. Biocompatible barrier membranes that provide both hydrophobic and hydrophilic surface properties on each side are critical to prohibit soft tissue invasion into localized bone defect. As a barrier membrane, SF nanofibrous mat was fabricated by electrospinning method, and then subsequently modified with water vapor treatment for insolubilization in water and CF_4 gas plasma treatment for surface hydrophobization. Morphology of SF nanofibrous mats were observed by scanning electron microscopy. Conformational change of insolubilized SF nanofibers was confirmed by attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Immobilized fluorine atoms on CF_4 plasma treated SF nanofibrous membranes were detected using electron spectroscopy for chemical analysis (ESCA). Water contact angle of the SF nanofiber membrane surface was analyzed by varying plasma input power and time. Insolubilized SF nanofibrous membrane maintained nonwoven mat structure without deformation after water immersion. SF nanofibrous membranes showed significant increment of water contact angle from 99.7° to 141.2° by CF_4 gas plasma treatment. Fibroblasts on plasma untreated SF nanofibrous membranes were well attached and spread than a control tissue culture polystyrene dish. Fibroblasts on the CF_4 gas plasma treated SF nanofibrous membrane showed significantly lower proliferation behavior than plasma untreated SF nanofibrous membranes. Fluorocarbon immobilized SF nanofibrous barrier membrane will be useful for biomedical applications such as a guided bone regeneration.

Keywords: silk fibroin, electrospinning, nanofiber, CF_4 gas plasma treatment, barrier membrane.

Introduction

Silk is a fibrous nature protein produced by a variety of insects (such as raspy crickets, bees, ants, beetles, flies, spiders) including silkworm. Fibrous proteins such as silks and collagens are characterized by a highly repetitive primary sequence that leads to significant homogeneity in secondary structure, *i.e*., triple helices in the case of collagens and β-sheets in the case of many of the silks.[1] These types of proteins usually exhibit special mechanical properties, in contrast to the catalytic and molecular recognition functions of globular proteins. Because of these impressive mechanical properties, this family of proteins provides an important set of material options in the fields of controlled release, biomaterials and scaffolds for tissue engineering. Recently, many researchers have investigated silk proteins, mainly silk fibroin, as one of the candidate materials for biomedical applications, because it has several distinctive biological properties including biocompatibility, oxygen and water vapor permeability, biodegradability and minimal inflammatory reaction.[2,3] During decades of use, silk fibers have proven to be effective in many clinical applications particularly as sutures, tissue wall and membrane repairs, muscle ligament, blood vessel and nerve gadget restoration, and tooth, cartilage and bone reconstruction.[4-6]

Plasma can be used to modify the surface of certain substrate.[7-9] Partially ionized gas composed of electron, ions, photons, atoms and molecules with negative global electric charge is called plasma. Plasma, known as the fourth state of matter, is created by applying electrical fields to pure gas or gas mixtures in a vacuum chamber. The gas is then ionized and leads to a chemical reaction on the surface of the respective material. Plasma surface deposition is an effective method to modify the surface of natural polymers without changing their inherent properties. Plasma surface treatment on biomaterials provides a solution for the complicate shape surfaces

*Corresponding Author. E-mail: ohkwon@kumoh.ac.kr

modification with consistent, safe and simple process. In addition, wettability such as hydrophilicity, hydrophobicity and dirt repellency are expected by plasma. Plasma treatment can be applicable for hydrophobization of surface, overgrowth of cells as implants, prohibit cell growth as a membrane, enzyme immobilization for biomedical applications.

Guided bone regeneration (GBR) is a frequently used to treat bone defects. The principle of GBR is to create and maintain a segregated space by using a barrier membrane to prevent the invasion of fast growing epithelial and other soft tissues from migrating into the osseous defect, thereby allowing time for osteogenic cell populations originating from the parent bone.[4-6,10-13] In general, GBR membranes require flexibility to adapt optimally cover a bone defect, biomechanical strength to maintain the secluded space for bone formation and biocompatibility to facilitate the biomimic environment, and biodegradability to eliminate the need for membrane removal surgery. Especially, surface properties of GBR barrier membrane should be designed to prohibit ingrowth of soft tissues into bone regeneration space. Currently, a number of research are focus on manipulate advanced GBR membranes that provide not only physical barrier but also chemically surface modified membranes.

This research aims to develop biocompatible and biodegradable nanofibrous membranes, that is composed of a tissue compatible side and cell repellant side for biomedical applications. Electrospinning is one of the most suitable methods to fabricate a flexible nanofibrous membrane.[14-18] We have previously reported that cells on highly porous electrospun nanofibrous mat were well attached, spread, and proliferated much more than nonporous surfaces.[19,20] Nanofibrous mat accommodates attachment and proliferation of cells, and enables the efficient exchange of nutrient and metabolic wastes. To achieve this goal, we have fabricated highly biocompatible SF nanofibrous membranes by electrospinning method and then subsequently one side of membranes were modified to super hydrophobic by CF_4 gas plasma treatment. Biocompatible SF nanofibrous barrier membranes that provide both hydrophobic and hydrophilic surface properties on each side might have a positive effect to prohibit soft tissue invasion into localized bone defect in the field of GBR.

Experimental

Materials. Degummed silk fibroin (SF) was procured from Dasung Silk (Gyeongnam, Korea). Calcium chloride and ethanol were purchased from Daejung Chemicals and Metals (Gyeonggi, Korea) and used as received without further purification. A dialysis with cellulose tubular membrane having a molecular weight cutoff (MWCO) of 12 K was procured from Bumhan Commercial Co., Ltd (Seoul, Korea). Hexafluoro-2-propanol (HFIP) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Trypsin-EDTA solution, streptomycin, penicillin, and Dulbecco's modified Eagle's medium (DMEM) were bought from Gibco BRL (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was procured Sigma-Aldrich (St. Louis, USA).

Preparation of Silk Fibroin Nanofiber. Degummed silk fibroin (SF) were used for the preparation of SF nanofiber. SF was dissolved in $CaCl_2/CH_3CH_2OH/H_2O$ (1:2:8 in molar ratio) at 70 °C for 4 h. After dialysis with cellulose tubular membrane (MWCO, 12 K) against distilled water for 3 days, the aqueous fibroin solution was freeze-dried to get regenerated SF sponges. Regenerated SF sponges were dissolved in HFIP to obtain the spinning solution. The concentrations of SF solution were 4, 5, 6, and 7 wt%. To obtain uniform SF nanofibers by electrospinning, the electrospinning parameters, such as concentration of the spinning solution, applied voltage, flow rate, distance to the collector, were respectively set at 6 wt%, 20 kV, 2 mL/h and 10 cm. The electrospun nanofibers were dried under vacuum for overnight and treated by water vapor. Water vapor treatment was done in a desiccator at 37 °C for 4 h to make it insoluble in water. The water vapor treated SF nanofibers were washed with distilled water and dried under vacuum for overnight.

CF_4 Plasma Treatment of Silk Fibroin Nanofiber Membrane. The surface of SF nanofibers were hydrophobized by plasma treatement using CF_4 gas (Figure 1). CF_4 gas plasma treatment was carried out with SF nanofiber in a chamber (36 cm ×25 cm ×15 cm) connected to a two-stage rotary pump *via* liquid nitrogen cold trap. Water vapor treated SF nanofibers were placed on the electrode in the plasma chamber under nitrogen atmosphere. The chamber was evacuated to less than 10 Pa and CF_4 gas was admitted into the chamber. After pressure of the chamber being stabilized, plasma discharging was carried out by controlling electrical power at 50 W for 10 min. Upon the completion of surface modification by CF_4 gas, the gas feed was turned off and the chamber was vented to the atmosphere. Plasma treatment was carried out at room temperature.

Characterizations. The morphology of the electrospun SF nanofiber was observed with a scanning electron microscope (SEM: JSM-6380, JEOL Ltd., Japan) after sputter-coating with platinum. The average diameter of electrospun SF nanofibers was determined by the SEM and Image analyzer (TDISE Version 3.7.73). Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR: Vertex 80v, Hyperion 2000, Bruker Biospin, Germany) and ^{13}C cross-polarization magic-angle spinning nuclear magnetic resonance (CP/MAS NMR, Bruker, Germany) spectroscopy were determined to monitor the change of secondary structure of SF nanofiber before and after water vapor treatment. Surface modification of SF nanofiber by CF_4 gas was confirmed by electron spectroscopy for chemical analysis (ESCA; Quantera SXM, ULVAC-PHI, Japan). Water contact angles were determined by a sessile drop method at room temperature with a FACE contact angle meter (Model: Phoenix 300, SEO Co. Ltd, Korea). Each sample

was cut in size (1.0×1.0 cm) to measure water contact angles. All samples were measured 5 times and averaged. Contact angles were presented as a mean value (n=5) with a standard deviation.

Cell Culture. The NIH3T3 fibroblasts were seeded onto the plasma treated and untreated nanofiber in 12-well plates at 5×10^4 cells/well. Seeded fibroblasts were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin G-streptomycin at 37 °C in 5% of CO_2 incubator. CF_4 gas plasma treated and untreated SF nanofibers cut in size of 20 mm diameter and used for the cell culture after sterilization using ultraviolet ray for overnight. After 2 h of culture, the medium was changed to remove unattached cells. Culture medium was changed every 2 days. Attached cell morphology and viability of fibroblasts were measured by SEM and MTT assay.

Results and Discussion

Morphology of Silk Fibroin Nanofiber. Nonwoven silk fibroin (SF) nanofibrous mats were fabricated by electrospinning of SF-HFIP solutions. To fabricate consistent nanofibrous mat, morphology of SF nanofiber was surveyed by altering SF concentration (5-7 wt%), electric voltage (10-20 kV), feed rate (0.5-2.0 mL/h) of SF-HFIP solution and tip to collect distance (8-12 cm) of electrospining condition (Supplementary information 1). Among these electrospinning parameters, concentration of SF-HFIP solution was the most critical parameter in fabrication of nanofibers. Beaded fiber was observed under 5 wt% SF-HFIP solution, while 7 wt% showed thick and heterogeneous fiber diameter (Figure 2). A 6 wt% SF-HFIP solution showed randomly aligned relatively homogeneous unimodal nanofibers with mean 747 nm in diameter. Based on image analysis data, electrospinning condition of 10 kV, 2.0 mL/h, 8 cm with 6 wt% SF-HFIP soultion was chosen to fabricate relatively consistent SF nanofibrous mats for further experiment.

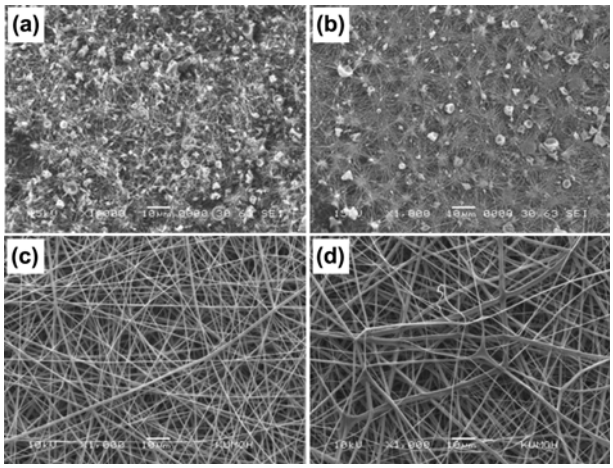

Figure 2. SEM micrographs of electrospun SF nanofibers fabricated from different concentration of (a) 4 wt%, (b) 5 wt%, (c) 6 wt%, and (d) 7 wt% of SF-HFIP solutions, respectively. Other condition such as applied voltage, tip-to-collector distance, flow rate were set as 10 kV, 10 cm, 2.0 mL/h, respectively.

Stabilization of Nonwoven SF Nanofibrous Mat. Electrospun SF nanofibrous mat was easily soluble in water. Thus, insolubilization of SF nanofibrous mats required for biomedical applications.[11-13] The SF nanofiber mats were treated with ethanol vapor, methanol vapor and water vapor at 40 °C, respectively. Methanol and ethanol vapor treated SF mats were brittle and collapsed due to the excessive crystallization of fibroin chain. However, water vapor treated SF mat kept flexibility without serious deformation of fibrous morphology (Figure 3(b)). The water vapor treated silk fibroin nanofibrous mat showed densely stacked network structure after water immersion (Figure 3(c)). After water immersion, mean diameter of nanofi-

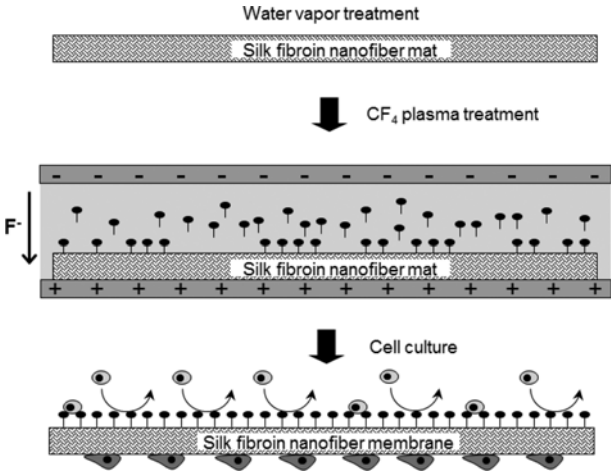

Figure 1. Schematic diagram of a CF_4 gas plasma treated SF nanofibrous membrane.

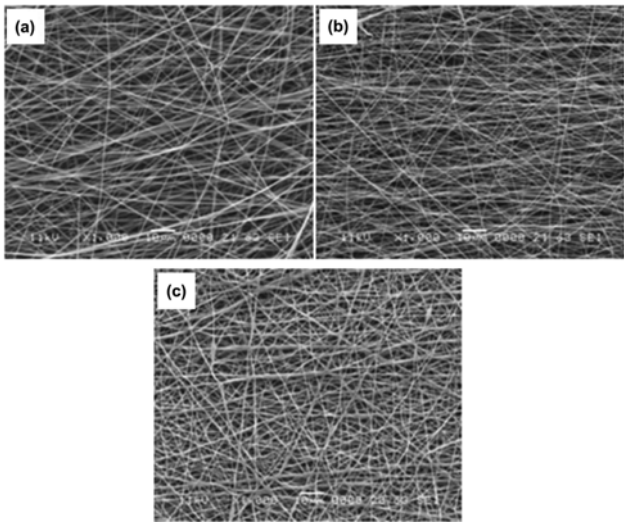

Figure 3. SEM micrographs of SF nanofibers before (a), after water vapor treatment (b), and after immersion in water (c), respectively.

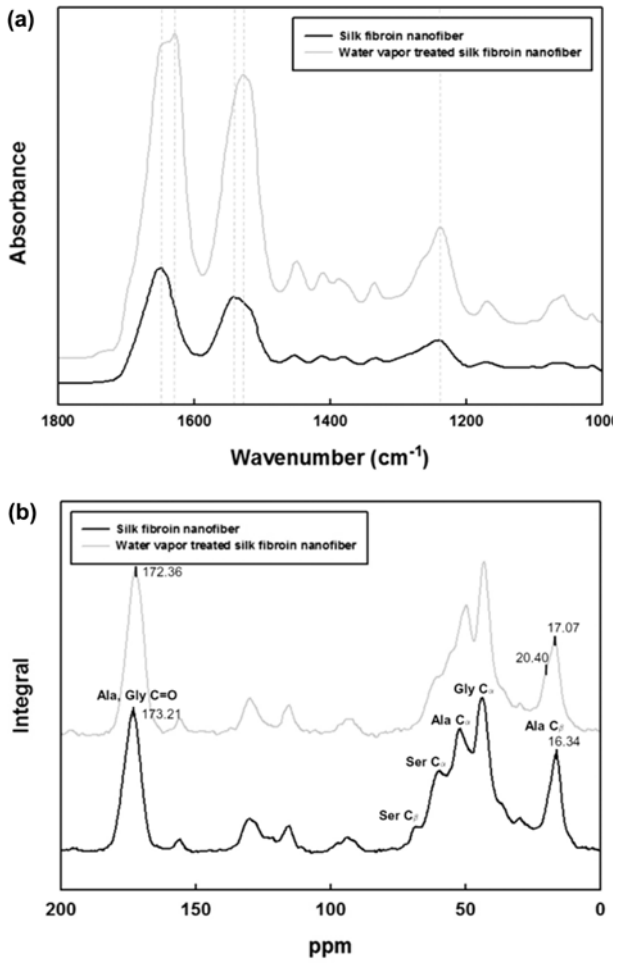

Figure 4. ATR-FTIR (a) and ^{13}C NMR (b) spectra of SF nanofibrous mats.

ber was slightly increased to about 800 nm. Stabilized and insolubilized SF nanofibrous mat facilitates sequential procedure of surface modification and cell culture.

Conformational Change of SF Nanofiber. Major conformations of silk fibroin are composed with random coil, α-helix and β-sheet. Organic solvents such as methanol or ethanol and water induce crystallization of silk fibroin by converting random coil to β-sheet form.[11-13] Conformational change of the water vapor treated SF mats could be compared using ATR-FTIR spectroscopy (Figure 4(a)). The characteristic absorption band of SF are found in the 1655 cm^{-1} (amide I), 1544 cm^{-1} (amide II) and 1240 cm^{-1} (amide III) which is random coil conformation. Absorption band of water vapor treated SF mat has shifted to 1630 cm^{-1} (amide I), 1528 cm^{-1} (amide II) of β-sheet conformation. We assume that water vapor induced hydrogen bond of silk fibroin molecules with rearranged crystal structure.

^{13}C CP/MAS NMR spectroscopy provides additional evidence to confirm the conformational changes of the silk fibroin proteins from the random coil to the β-sheet conformation, as the chemical shifts of the carbon atoms in silk proteins. The formation of β-sheet can be identified by the C chemical shifts of Gly (glycine), Ser (serine), and Ala (alanine) that are indicative of β-sheet conformation in Figure 4(b). Ala is a major constituent of SF, and the chemical shift of the methyl groups of the Ala residues (Ala C_β), are representative of the conformational status of the silk protein. Chemical shifts of the Ala C_β region in the SF nanofibers are shown in Figure 4(b). The chemical shift of Ala C_β, and Ala C=O carbons in SF nanofibers was 16.34 ppm, 173.21 ppm, respectively, indicating that the Ala residues of the SF nanofibers were mainly in the random coil conformation. The water vapor treated SF nanofiber, however, showed shifted Ala C_β at 17.07 ppm with a shoulder at 20.40 ppm and Ala C=O at 172.36 ppm, which can be assigned to Ala C_β in the SF nanofibers. This finding clearly indicates that the Ala residues in the SF nanofiber chains take the β-sheet conformation by water vapor treatment.

Fluorocarbon Immobilization on a SF Nanofibrous Mat Surface. The CF_4 gas plasma was treated on one side of SF nanofibrous membranes by varying input power (10-50 W) and time (100-600 s) (Table I). Structural deformation of silk fibroin nanofibrous mats by CF_4 gas plasma treatment was not observed. Fluorocarbon plasma treatment can make a surface hydrophobic. Water contact angle of surface modified SF nanofibrous mats was measured to analyze the effect of CF_4 plasma treatment. CF_4-immobilized surfaces exhibited dramatically increasing contact angles by increasing applied plasma power, while applied time showed negligible contact angle changes. This result indicates that intensity of input power affect surface hydrophobicity. Water contact angle of SF nanofibrous membrane surface was increased dramatically from 99.7° to 141° after CF_4 gas plasma treatment with 50 W of input power for 600 s.

To confirm immobilization of fluorocarbon molecules on SF nanofibrous mat surface, elemental analysis was carried out using ESCA. In Figure 5, SF nanofibrous mat showed 63.2% of C, 16.4% of N and 30.3% of O atomic composition. And CF_4 gas plasma treated SF nanofibrous mat showed

Table I. Water Contact Angle of Silk Fibroin Nanofibrous Membranes Before and After Plasma Treatment[a]

CF_4 Plasma Treatment		Water Contact Angle (°)
Applied Power (W)	Time (s)	
-	-	99.70 ± 2.12
10	100	116.14 ± 3.15
20	100	125.18 ± 1.42
40	100	134.44 ± 4.30
50	100	136.64 ± 5.29
50	300	137.44 ± 8.88
50	600	141.18 ± 2.33

[a]mean±SD, n=5.

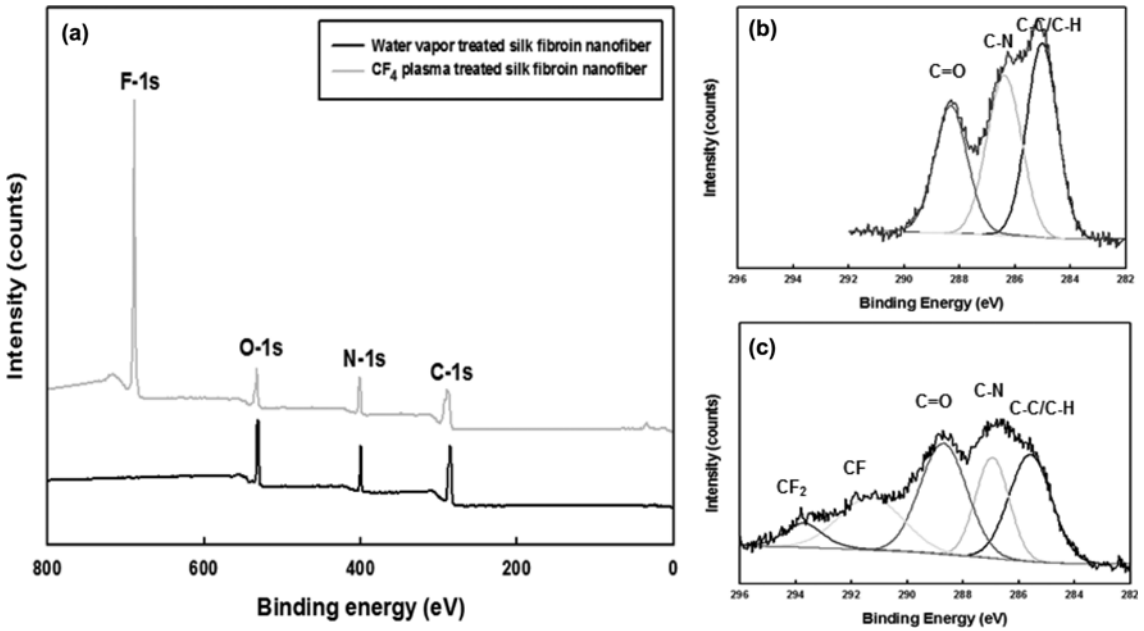

Figure 5. ESCA spectra of SF nanofibers (a). C-1s core level spectra of SF nanofibrous mats before (b) and after (c) CF_4 gas plasma treatment.

43.0% of C, 10.0% of N and 9.4% of O and 37.58% of F atomic composition. Also CF and CF_2 peaks were observed at C1s core level scan spectra of CF_4 gas plasma treated SF nanofibrous mat, while no CF and CF_2 peaks were detected on water vapor treated SF nanofibrous mat (Figure 5(b), (c)). From these results, we confirmed that fluorocarbon was successfully immobilized on SF nanofibrous mat surfaces by CF_4 gas plasma treatment.

Cell Culture on SF Nanofibrous Membranes. To demonstrate biocompatibility of surface modified SF nanofibrous membranes, fibroblasts were cultured on each tissue culture polystyrene dish (TCPS), plasma untreated SF nanofibrous membrane and CF_4 gas plasma treated SF nanofibrous membrane, thereafter cell proliferation behavior was analyzed by MTT assay (Figure 6). Initial cell attachment and spreading on CF_4 gas plasma treated SF nanofibrous membrane surfaces was lower than plasma untreated SF nanofibrous surfaces. Subsequently, the CF_4 gas plasma treated SF nanofibrous membrane showed significantly lower cell proliferation behavior than TCPS and plasma untreated SF nanofibrous mat surface after 7 days of culture. Fibroblasts were well attached and proliferated on plasma untreated SF nanofibrous membranes. Surface topography is very important to cell adhesion. Nanofibrous matrixes may allow pseudopodia to anchor more tightly, and this mode of anchorage could contribute to the adhesion strength of cells to nanofibrous matrixes. Generally, anchorage-dependent tissue cells attach more easily to a surface with a certain degree of roughness than to a smooth surface, probably because these cells are apt to make focal contacts by pseudopodia on the surface of irregular nanofibrous matrixes.[20]

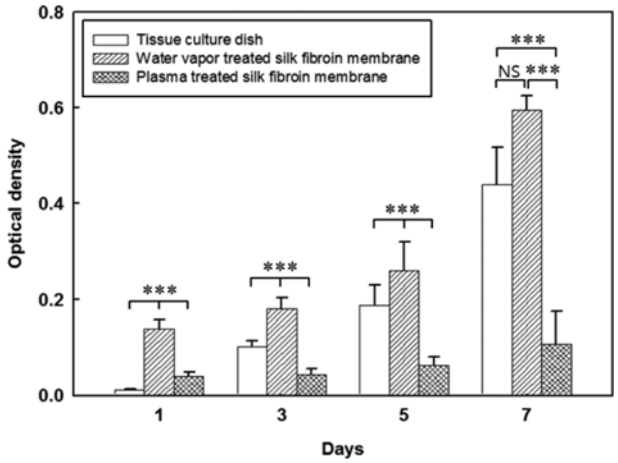

Figure 6. Cell proliferation behavior on each surface. Data are shown as mean±S.D. ***p<0.001, NS, not significant.

Nevertheless, attachment and proliferation of fibroblasts were highly suppressed on CF_4 gas plasma treated SF nanofibrous membrane. These results probably due to the surface hydrophobicity of CF_4 gas plasma treated SF nanofibrous membrane. Generally, the dependence of cell growth on the water contact angle of substrate was similar to that of the adhesion. When the surface topography is smooth and flat, fibroblasts could proliferate at the highest rate when cultured on the substrate with a contact angle around 70 degrees, which was also the most favorable for cell adhesion.[21-23] When water droplet is put on the substrate surface, wetting is suppressed on rough surface especially on the surface of microgroove, resulted in increment of water contact angles. Even though water contact angle was overestimated due to a rough

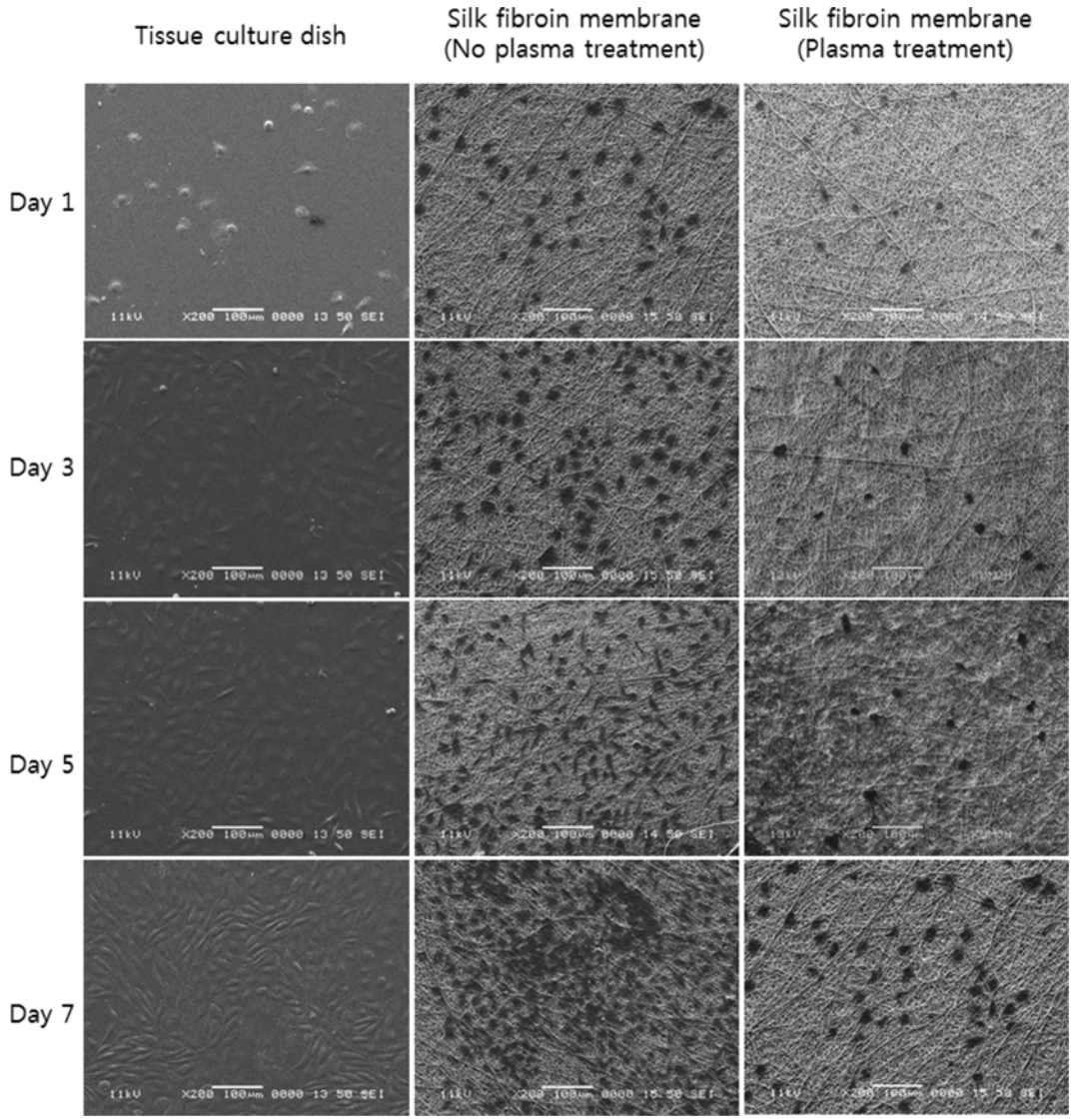

Figure 7. SEM micrographs of cell proliferation on each surface.

nanofibrous membrane surface, CF_4 gas plasma treated SF nanofibrous membrane showed too high water contact angle (141°) to attach and proliferate fibroblasts. SEM microphotographs of cultured cells also assisted the result of quantitative proliferation data (Figure 7). Seldom fibroblasts were observed on the CF_4 gas plasma treated SF nanofibrous membrane compared to plasma untreated SF nanofibrous membrane. Fluorocarbon immobilized super hydrophobic SF nanofibrous membrane surface strongly prevent not only initial cell attachment but also cell proliferation.

Conclusions

Silk fibroin (SF) nanofibrous nonwoven mats were fabricated using electrospinning technique. Water vapor treatment of silk fibroin nanofibrous membrane made it insoluble in water. Nanofibrous structure and dimensional integrity of silk fibroin nanofiber was maintained in aqueous solution. Conformational change in secondary structure of silk fibroin was appeared from random coil to β-sheet by water vapor treatment. The surface of silk fibroin nanofibrous membranes was hydrophobized by CF_4 gas plasma treatment. Saturated fluorocarbon was immobilized successfully on silk fibroin nanofibrous mats. CF_4 gas plasma treated silk fibroin surface with 50 W power input for 600 seconds induces highest water contact angle. Saturated fluorocarbon immobilized silk fibroin surface showed significantly lower proliferation of fibroblasts compared with plasma untreated silk fibroin nanofiber surface. Hydrophobization of SF nanofiber surface by CF_4 gas plasma treatment could modulate cell adhesion and proliferation behavior and expand their utility for biomedical applications. Biocompatible SF nanofibrous membranes that provide both

hydrophobic and hydrophilic surface properties on each side might be a promising barrier materials to prohibit soft tissue invasion into localized bone defect in the field of GBR.

Acknowledgment. This work was supported by the National Research Foundation of Korea (NSF) grant funded by the Korea government (MSIP) (NRF-2012M2A2A6035747).

Supporting Information: Information is available regarding the SEM images of electrospun nanofibers under the different spinning conditions. The materials are available *via* the Internet at http://www.springer.com/13233.

References

(1) G. H. Altman, F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, J. Chen, H. Lu, J. Richmond, and D. L. Kaplan, *Biomaterials*, **24**, 401 (2003).
(2) M. Retzepi and N. Donos, *Clin. Oral Implants Res.*, **21**, 567 (2010).
(3) K.-H. Kim, L. Jeong, H.-N. Park, S.-Y. Shin, W.-H. Park, S.-C. Lee, T.-I. Kim, Y.-J. Park, Y.-J. Seol, Y.-M. Lee , Y. Ku, I.-C. Rhyu, S.-B. Han, and C.-P. Chung, *J. Biotechnol.*, **120**, 327 (2005).
(4) B.-M. Min, L. Jeong, K. Y. Lee, and W. H. Park, *Macromol. Biosci.*, **6**, 285 (2006).
(5) L. Jeong, K. Y. Lee, J. W. Liu, and W. H. Park, *Int. J. Biol. Macromol.*, **38**, 140 (2006).
(6) C. Zaharia, M.-R. Tudora, P. O. Stanescu, E. Vasile, and C. Cincu, *J. Optoelect. Adv. Mater.*, **14**, 163 (2012).
(7) Y. Iriyama, T. Yasuda, D. L. Cho, and H. Yasuda, *J. Appl. Polym. Sci.*, **39**, 249 (1990).
(8) N. Hayashi, S. Ihara, S. Satoh, and C. Yamabe, *Rep. Fac. Sci. Engrg. Saga Univ.*, **30**, No. 2 (2001).
(9) O. Kylián, M. Petr, A. Serov, P. Solař, O Polonskyi, J. Hanuš, A. Choukourov, and H. Biederman, *Vacuum*, **100**, 57 (2014).
(10) B.-M. Min, G. Lee, S. H. Kim, Y. S. Nam, T. S. Lee, and W. H. Park, *Biomaterials*, **25**,1289 (2004).
(11) K. E. Park, S. Y. Jung, S. J. Lee, B.-M. Min, and W. H. Park, *Int. J. Biol. Macromol.*, **38**, 165 (2006).
(12) J. P. Chen, S.-H. Chen, and G.-J. Lai, *Nanoscale Res. Lett.*, **7**, 1 (2012).
(13) K. Yamada, Y. Tsuboi, and A. Itaya, *Thin Solid Films*, **440**, 208 (2003).
(14) G. E. Wnek, M. E. Carr, D. G. Simpson, and G. L. Bowlin, *Nanoletters*, **3**, 213 (2003).
(15) H. Fong, I. Chun, and D. H. Reneker, *Polymer*, **40**, 4585 (1999).
(16) X. M. Mo, C. Y. Xu, M. Kotaki, and S. Ramakrishna, *Biomaterials*, **25**, 1883 (2004).
(17) X. Zong, K. Kim, D. Fang, S. Ran, B. S. Hsiao, and B. Chu, *Polymer*, **43**, 4403 (2002).
(18) H. Yoshimoto, Y. M. Shin, H. Terai, and J. P. Vacanti, *Biomaterials*, **24**, 2077 (2003).
(19) I. S. Lee, O. H. Kwon, W. Meng, I. K. Kang, and Y. Ito, *Macromol. Res.*, **12**, 374 (2004).
(20) O. H. Kwon, I. S. Lee, Y.-G. Ko, W. Meng, K. H. Jung, I. K. Kang, and Y. Ito, *Biomed. Mater.*, **2**, S52 (2007).
(21) Y. Tamada and Y. Ikada. *J. Colloid Interface Sci.*, **155**, 334 (1993).
(22) Y. Tamada and Y. Ikada. *Polymer*, **34**, 2208 (1993).
(23) Y. Tamada and Y. Ikada. *J. Biomed. Mater. Res.*, **28**, 783 (1994).