Effect of Microwave-Induced Argon Plasma on Electrospun Silk Fibroin/Wool Keratose (50/50) Scaffold

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Silk fibroin (SF) is a naturally occurring degradable fibrous protein with unique mechanical properties, excellent biocompatibility and processability. It has demonstrated strong potential for skeletal tissue engineering. Recent studies have mostly focused on nanofibrous SF (NSF) as a novel chondrogenic scaffold since its structure is very similar to collagen fibrous structure derived from natural extracellular matrix (ECM). However, less attention has been paid to the surface modification of NSF scaffold by microwave-induced argon plasma. Argon plasma has been used in many applications, such as pretreatment for plasma-induced grafting,

crosslinking surface macromolecules, removal of contamination on the surface, and ablation of material from the surface to remove a weak boundary layer and increase surface roughness for better adhesion. The present study was based on the hypothesis that a treatment of 3-D silk fibroin/wool keratose scaffold (SF/WK scaffold) by microwave-induced argon plasma would improve chondrogenic cell growth and new cartilage-specific ECM formation. It was found that plasma treatment could induce an essential modification on the surface of electrospun SF/WK scaffold. After argon plasma treatment, higher hydrophilicity of SF/WK scaffold was characterized by static water contact angle. Scanning electron microscopy showed that argon plasma treated SF/WK scaffold had larger pores of size and cylindrical shape whereas non-treated SF/WK scaffold had smaller pores of size and inverted conical shaped pores. The attachment and proliferation of normal human articular chondrocytes on the surface-modified SF/WK scaffold were significantly increased with a concomitant increase in the glycosaminoglycan synthesis. These results suggest that the porous SF/WK scaffold treated with microwave-induced plasma may be effective in enhancing the cellular behavior and chondrogenic differentiation of chondrocytes and have a further potential to be used in cartilage tissue engineering.

Keywords: Nanofibrous silk fibroin, microwave-induced argon plasma, chondrocytes, cartilage, scaffold

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I. INTRODUCTION

Silks are attractive biomaterials for bone tissue engineering because of their biocompatibility, mechanical properties and slow biodegradation. Silk-based biomaterials have previously been demonstrated to offer exceptional benefits over conventional synthetic and natural biomaterials in generating functional tissue replacements for various cells.[1,2] Also, they offer significant advantages for potential adipose tissue engineering applications; they have low immunogenicity [3], an absence of bioburdens [3] and slow degradation rates [4], show plasticity during processing [5], and

have impressive mechanical properties. [6]

Because silk scaffold in itself is not strong enough to bear weight and retain shape, gelatin or collagen were usually added to compose scaffold. [6] Electrospun nanofiber has received much interest as a novel scaffold because nanofibrous structure is very similar to collagen fibrous structure of natural extracellular matrix (ECM). Many recent studies have demonstrated that the cells seeded on the nanofibrous scaffold were well adhered and grown onto their surface due to their large specific areas. [7,8] However, nanofibrous scaffolds developed so far through electrospinning technique might have structural limits for cell proliferation. As the diameter of the electrospun nanofiber is very small and collected on a plane metal plate, the pore size is too small for the cells to grow inside and the shape is a 2-D non-woven sheet form. Hence, seeded cells can not migrate into inner space of the scaffold and the cells just grow on the surface of the scaffold. This means that it is not possible to regenerate a suitable 3-D tissue with this form of scaffold although the electrospun nanofibrous scaffold has a great advantage of large surface areas. Therefore, it is necessary to make 3-D scaffold of electrospun nanofibers with large pore sizes as well as large surface areas.

Plasma treatment has the advantage of modifying the surface region to a depth of only a few nanometers without affecting the bulk properties. A commonly used method is to introduce hydrogen peroxides at the surface either by inert gas plasma treatment followed by exposure to oxygen or by corona discharge treatment. [9] This

advantage can benefit the fabrication of scaffold with interpenetrating porous structure for tissue engineering. Argon plasma can also introduce oxygen functionality to the surface and the oxygen-modified surfaces lead to subsequent incorporation of polar groups, which improves wettability. [10] This facilitates adhesion and improves the biocompatibility of the material. Moreover, argon plasma treatment of hydrophobic materials (e.g. polyethylene) surfaces has been shown to produce a hydrophilic surface with decreased contact angle. [11,12]

The present study concentrated on investigating the effects of microwave-induced argon plasma treatment on attachment, proliferation and chondrogenic differentiation of human articular chondrocytes onto 3-D porous electrospun nanofibrous silk fibroin /wool keratose scaffold.

II. MATERIALS AND METHODS

1. Preparation of silk fibroin/wool keratose scaffold

Silk fibroin (SF) was obtained by soap-soda degumming. [13] Bombyx mori cocoons were boiled in an aqueous solution of marseillus soap 0.3 (wt. %) and sodium carbonate 0.2 (wt. %) for an hour to remove sericin (degumming) and then rinsed

with distilled water. Degummed SF was dissolved in a ternary solvent system of CaCl2/H2O/EtOH (mole ratio=1/8/2) solution at 85°C for 3 minutes and the solution was dialyzed against distilled water at room temperature for 3 days in order to remove salts (MWCO:12,000), then lyophilized. Wool keratose (WK) was obtained by oxidizing wool keratin with performic acid. Scoured Merino wool hairs were immersed in performic acid (HCOOOH) at 0˚C for 24 hours (liquor ratio=1:25). Then, soluble fraction was separated and dried in ambient temperature. All the other chemicals were used without further purification.

2. Fabrication of 3-D porous SF/WK scaffold

3-D porous nanofibrous silk fibroin (NSF) scaffold was fabricated via electrospinning followed by a salt-leaching method. Dope was prepared by dissolving the prepared SF in 98% formic acid for 4 hours at 12% of a final concentration. Impurities and bubbles in the solution were removed by filtration and brief vacuum. For electrospinning, the dope was put into a 10 ml syringe with a 22G stainless steel syringe needle connected to a high voltage power supply (CPS-60 k02v1, Chungpa EMT Co., Ltd., Seoul, Korea). The dope flow rate was accurately controlled by a metering pump (KD Scientific Inc., Holliston, MA). A grounded rolling metal drum

was used as a collector for sheet-like NSF scaffold fabrication. The electrospinning was carried out at room temperature and 60% humidity. Electric potential and distance to collector were fixed at 12 kV and 10 cm, respectively. For fabricating porous scaffold, electrospun NSF dispersion was collected in the methanol bath by replacing it with 1,4-dioxane.(Fig. 1) At this time, NaCl particles (300~350 nm) were added to the dispersion as porogens. After mild stirring, the mixture was poured into a glass vessel (cylindrical shape) and then lyophilized at −40°C. It was then cross-linked with glutaraldehyde vapor in a sealed chamber for 1 day and immersed in 0.1 M glycine in 0.2M sodium carbonate buffer (pH 9.2) for 1 day to neutralize toxicity of glutaraldehyde. After several washes in phosphate-buffered saline, the product was lyophilized at −40°C again. The final product was considered as a porous SF/WK scaffold and cut into a disk (9 mm in diameter and 0.15 mm in thickness). Structural characteristics of electrospun silk fibroin/wool keratose (50/50) scaffold were shown in Table 1.

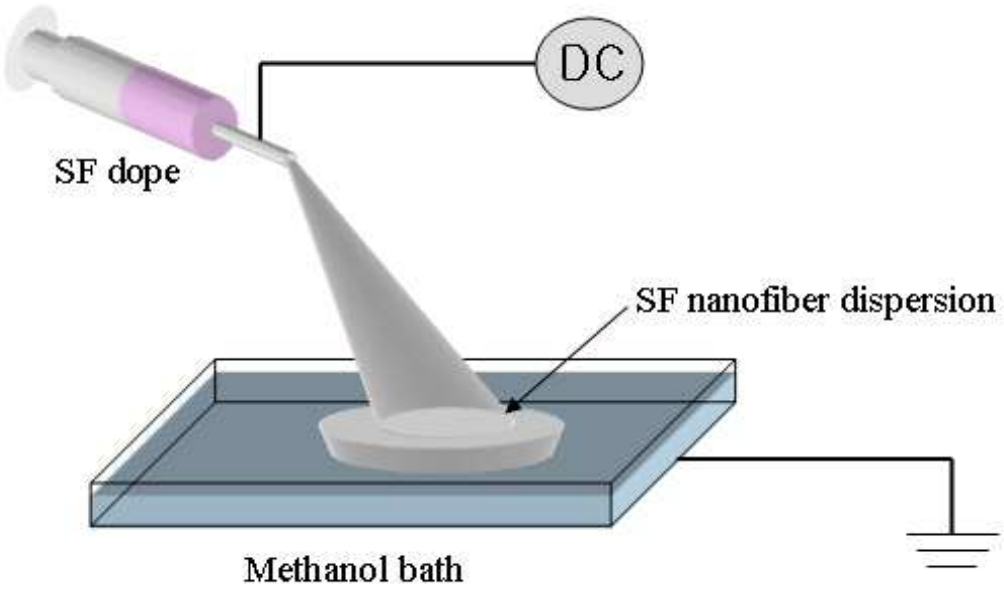

Figure 1. Scheme of electrospinning apparatus for manufacturing 3-D SF nanofibrous scaffold.

Table 1. Structural characteristics of electrospun silk fibroin/wool keratose (50/50) scaffold.

Material	SF/WK scaffold
Diameter (mm)	9
Thickness (mm)	1.5
Fiber diameter (nm)	459 ± 155
Pore size (μm)	390-550
Porosity (%)	91 ± 1.7

3. Surface modification of SF/WK scaffold by microwave-induced argon plasma

Scaffolds were treated with a 2.45 GHz, waveguide-based, microwave-induced argon plasma system at atmospheric pressure for 12.4 seconds as previously described.[14] this system consists of a 1 kW magnetron power supply, a WR-284 copper waveguide and an applicator including a tuning and a nozzle section. Argon was used as a working gas for this plasma system, and the gas flow rate was approximately 100 l/min at 8 kgf/cm^2.

4. Scanning electron microscopy (SEM)

The surface morphology of 3-D porous SF/WK scaffold treated without or with microwave-induced argon plasma was observed under a scanning electron microscope (Hitachi S-4700, Tokyo, Japan). The scaffold was mounted and sputter-coated with gold/platinum using an ion coater (E1010, Hitachi, Japan) and then observed at an accelerating voltage of 15 kV.

5. Water contact angle measurement

In order to examine the effects of plasma treatment on the hydrophilicity of SF/WK scaffold according to the argon plasma treatment, the surface was characterized by static water contact angle measurements using the sessile drop method. For the sessile drop measurement, approximately ten water droplets were placed on the dry surface of each composite. The contact angles of water both of the composites were detected at room temperature using a SEO contact angle analyzer (Phoenix 300A, Surface Electro Optics Co. Ltd., Suwon, Korea) equipped with a special optical system and a charge-coupled device camera.

6. Cell cultures and conditions

Neonatal human knee articular chondrocytes (nHAC-kn, Lonza, Walkersville, MD) were cultured in chondrocyte basal medium (Lonza) supplemented with 5% fetal bovine serum (Lonza), chondrocyte growth factors (Lonza, 1 ml R3-IGF-1, 2.5 ml bFGF, 1 ml insulin, 0.5 ml GA-1000 and 0.5 ml transferrin per 500 ml) and a 1% antibiotic antimycotic solution (including 10,000 units penicillin, 10 mg streptomycin and 25g amphotericin B per ml, Sigmã Aldrich Co., St. Louis, MO) at 37°C and 5% CO_2 in a humid environment. In order to examine the effects of plasma treatment on the cellular behaviors of nHAC-kn, the cells were seeded onto SF/WK scaffold treated without or with plasma and incubated for 4 hours (with an initial cell density of 2.0×10^5 cells per a scaffold for attachment assay) and 1, 3 and 7 days (with an initial cell density of 1.0×10^4 cells per a scaffold for proliferation assay).

7. Cell attachment and proliferation assays

MTT assay [reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan product] was used to determine the cell attachment and proliferation onto SF/WK scaffold. Neonatal human knee articular chondrocytes cultured onto 3-D porous SF/WK scaffold treated without or with plasma were

incubated with 0.5 mg/ml of MTT in the last 4 hours of the culture period tested at 37°C in the dark. The media were decanted and then washed twice with phosphate-buffered saline. The produced formazan salts were dissolved with dimethylsulphoxide, and the absorbance was determined at 570 nm by an ELISA reader (SpectraMax 340, Molecular Device, Sunnyvale, CA). Additionally, the morphologies of cells grown onto the non-treated or plasma-treated SF/WK scaffolds were observed after 7 days of incubation by SEM. In brief, the scaffold was washed with 0.1 M cacodylate buffer (pH 7.4) to remove unattached cells. The cells were fixed with 2.5% glutaraldehyde solution overnight at 4°C, dehydrated with a series of increasing concentration of ethanol solution and then vacuum-dried. The scaffold was mounted and sputter-coated with gold/platinum using an ion coater and then observed with a scanning electron microscope (Hitachi S-800) at an accelerating voltage of 20 kV.

8. Glycosaminoglycan (GAG) assay

GAG content was determined by a dimethylmethylene blue (DMMB) dye binding assay (Blyscan kit, Biocolor Ltd., Newtownabbey, Northern Ireland) according to the manufacturer's instructions. Chondroitin sulfate, provided with the kit, was used as the GAG standard. Briefly, the NSF scaffolds cultured with nHAC-kn were washed

with PBS and digested overnight at 37^{o}C in papain solution (1 mg/ml, Sigma Aldrich) for 24 hours. The digested solution was mixed with DMMB periodically at 25°C for 30 minutes. Solutions were then centrifuged at 10,000*g* for 10 min, and the supernatant (unbound dye) discarded. The remaining pellet (insoluble GAG-dye complex) was suspended in the provided dissociation reagent, and absorbance measurements were taken at 656 nm.

9. Statistical analysis

All variables were tested in triplicate for each experiment, which was repeated twice (n=6). Results are expressed as means ± standard deviation. A p-value of less than 0.05 was considered statistically significant. Comparing to the control group, the effects of argon plasma on increasing attachment, cell proliferation and GAG concentrations were analyzed by Student's t-test.

III. RESULTS

1. Surface structure and morphology of 3-D porous SF/WK scaffold

As shown in Fig. 2 and 3 the surface morphologies of the non-treated and plasma-treated SF/WK scaffold were observed by Scanning electron microscopy (SEM). It was revealed that the scaffold prepared in this study had uniformly distributed pores, relatively uniform pore size (390~550μm.) and high porosity (about 91%), but low pore interconnectivity. Plasma treatment did not adversely affect the surface morphology of the scaffold. The plasma-treated scaffold (Fig. 3) was shown to be significantly different compared to that of the non-treated scaffold (Fig. 2). In detail, the roughness and pore size of the scaffold was largely increased after the plasma treatment. Also, the degree of pore interconnectivity and the surface area of scaffold were markedly increased after the plasma treatment. Before the plasma treatment, the pore shape was inverted conical, but after the plasma treatment the pore had cylindrical shape.

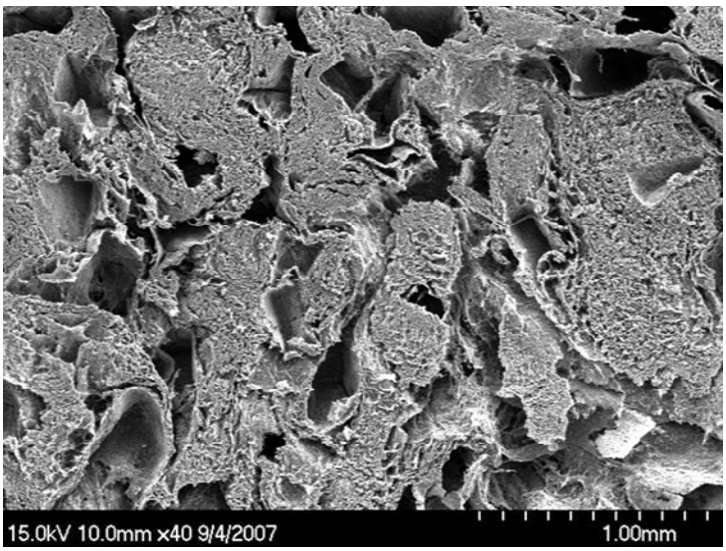

Figure 2. Non-treated silk fibroin/wool keratose (50/50) scaffold.

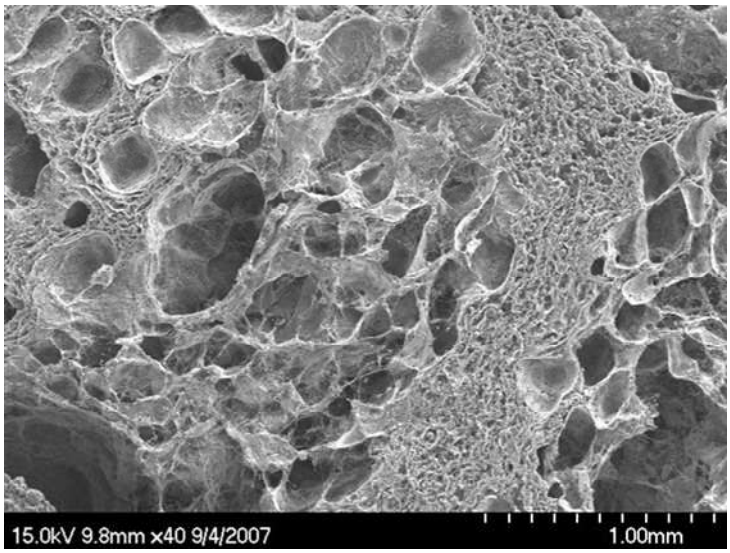

Figure 3. Argon plasma-treated silk fibroin/wool keratose (50/50) scaffold. The roughness and pore size of the scaffold was largely increased after the plasma treatment.

2. Hydrophilicity of SF/WK (50/50) scaffold treated with plasma

To determine the effects of plasma treatment on the hydrophilicity of nHAC-kn onto SF/WK scaffold, water contact angle was investigated (Fig. 4a, b). It was found that the hydrophilicity of the scaffolds was significantly ($p < 0.05$) increased by plasma treatment (Fig. 4b). The value after the treatment was approximately 6 times lower than before the treatment (Table 2).

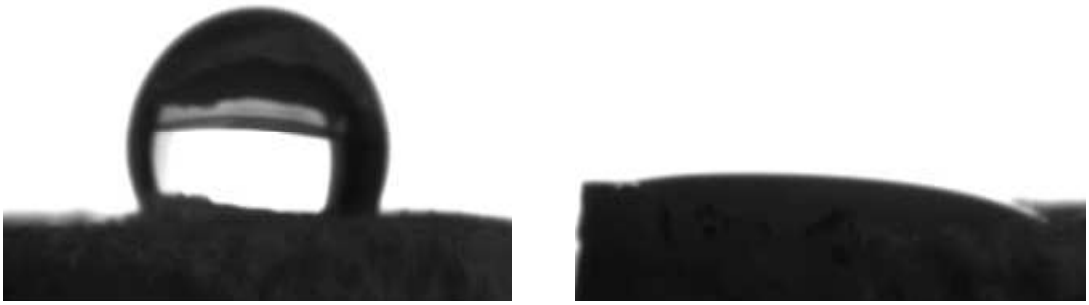

Figure 4. Water contact angle of non-treated and plasma-treated SF/WK scaffold. It was found that the hydrophilicity of the scaffold was significantly increased by plasma treatment.

Table 2. Water contact angle of non-treated and plasma-treated silk fibroin/wool keratose (50/50) scaffold.

	Water contact angle, Θw
(a) Plasma non-treated	70.4
(b) Plasma treated	12.2

3. Cellular behavior of neonatal human knee articular chondrocytes (nHAC-kn) SF/WK scaffold treated with plasma

In order to determine the effects of plasma treatment on the cellular behaviors of nHAC-kn onto SF/WK scaffold, cell attachment and proliferation were investigated. It was found that the attachment of the cells onto the scaffold was increased by plasma treatment even though it was not statistically significant (Table 3). The value after the plasma treatment was approximately 1.3 times higher than before the treatment (Fig. 5). The proliferation of nHAC-kn onto SF/WK scaffold was investigated on 1, 3, 7 days of incubation. It was found that the proliferation of the cells onto the scaffold was not significantly ($p < 0.05$) increased by plasma treatment. But in the plasma-treatment group, the rate of cell growth was markedly increased

with the prolonging incubation period (3, 7 days). That was not seen in non- plasma treatment group (Fig. 6). These results were then confirmed by SEM micrographs showing the cellular morphology of nHAC-kn cultured for 1, 3, 7 days nto SF/WK scaffold treated without or with plasma. The cells on the non-treated scaffold party covered and locally attached to the surface. In contrast, the cells on the plasma-treated scaffold formed an almost single layer with maintaining the natural original morphology of articular chondrocytes. Also the cells on the plasma-treated scaffold had highly organized pattern and shape (Fig. 7).

Table 3. Attachment of normal human articular chondrocytes on non-treated and plasma-treated silk fibroin/wool keratose (50/50) scaffold (analyzed by a Student's *t*-test, n= 6).

	Plasma non-treated	**Plasma-treated**
Average (% of control)	100	136
standard deviation	23.6	16.27
p value	0.1517	

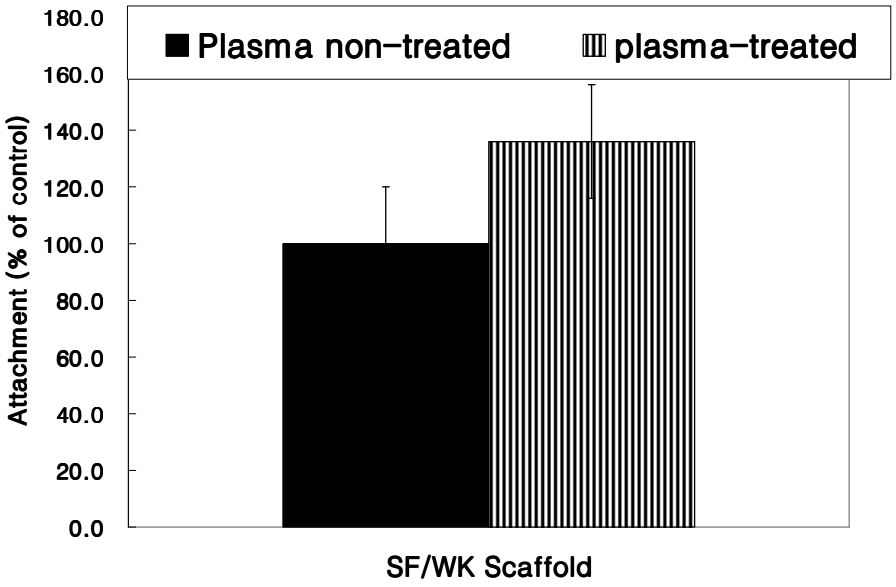

Figure 5. Attachment of normal human articular chondrocytes on non-treated and argon plasma-treated silk fibroin/wool keratose (50/50) scaffold.

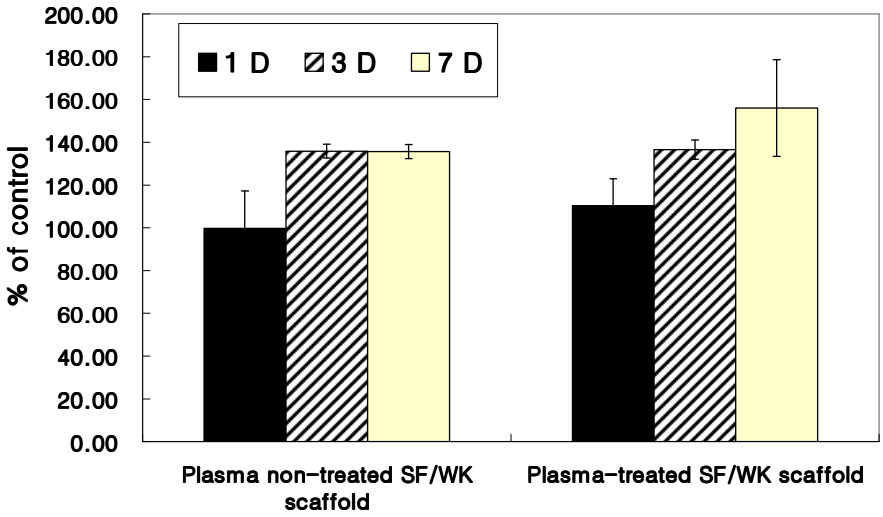

Figure 6. Proliferation of normal human articular chondrocytes on non-treated and argon plasma-treated silk fibroin/wool keratose (50/50) scaffold. In the plasma - treatment group, the rate of cell growth was markedly increased with the prolonging incubation period (3, 7 days).

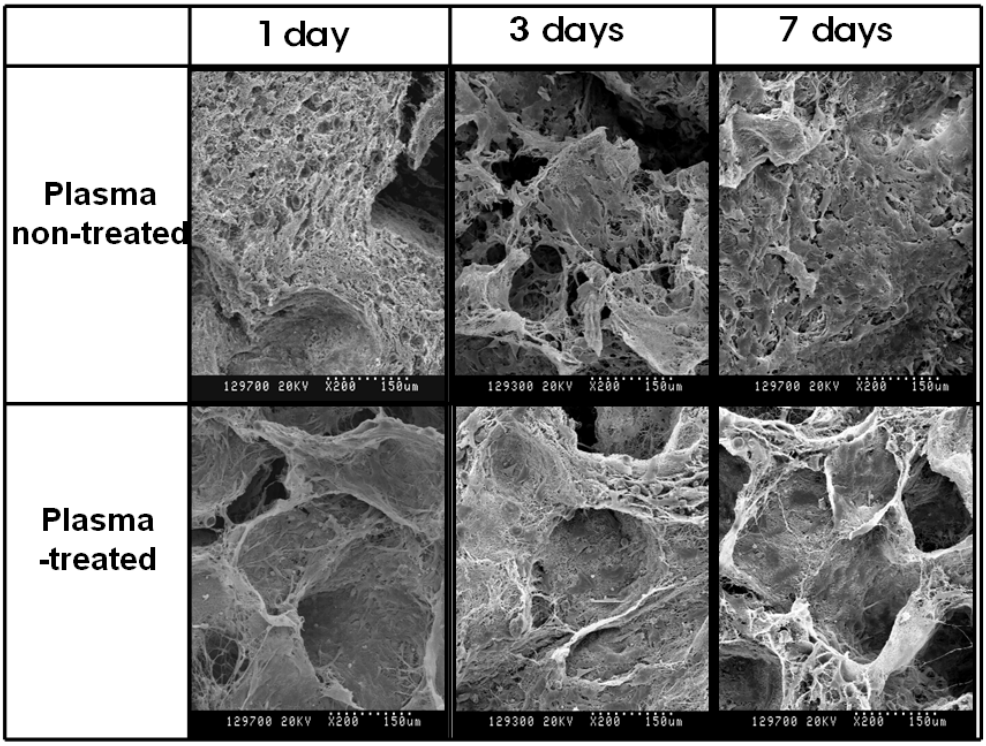

Figure 7. SEM micrographs of normal human articular chondrocytes on non-treated and treated argon-plasma silk fibroin/wool keratose (50/50) scaffold. The cells on the plasma-treated scaffold formed an almost single layer with maintaining the natural original morphology of articular chondrocytes. Also the cells on the plasma-treated scaffold had highly organized pattern and shape.

4. Chondrogenic differentiation of nHAC-kn on SF/WK scaffold treated with plasma

The surface of SF/WK scaffold was modified by plasma treatment and its effect on the chondrogenic differentiation of nHAC-kn was determined by biochemical analysis. It was revealed that the cells on the plasma-treated SF/WK scaffold showed significantly ($p < 0.05$) higher *glycosaminoglycan* (GAG) synthesis than those on the non-treated SF/WK scaffold for 3 and 7 days (Table 4). However, there was no significant difference in the synthesized GAG contents between the cells grown on the non-treated and plasma-treated scaffold for initial time. (4 hours and 1 day) It was also found that the GAG synthesis on the scaffold was increased in a time-dependent manner irrespective of the plasma treatment. The rate of GAG synthesis was also markedly increased on the plasma-treated SF/WK scaffold.

Table 4. Glycosaminoglycan synthesis of normal human articular chondrocytes on non-treated and argon plasma-treated silk fibroin/wool keratose (50/50) scaffold († standard deviation, * $p < 0.05$ vs. the non-treated at the same time, analyzed by a Student's t-test, n=6). It was revealed that the cells on the plasma-treated SF/WK scaffold showed significantly ($p < 0.05$) higher *glycosaminoglycan* (GAG) synthesis than those on the non-treated SF/WK scaffold for 3 and 7 days.

		Non-treated	**Plasma-treated**	***p* value**
GAG synthesis according to time (μg/scaffold)	4 hours	0.288 (0.020)†	0.360 (0.030)†	0.20
	1 day	0.625 (0.009)†	0.567 (0.120)†	0.64
	3 days	0.744 (0.081)†	0.985 (0.071)†	0.02*****
	7 days	0.964 (0.099)†	1.174 (0.076)†	0.04*****

.IV. DISCUSSION

Plasma treatment can benefit the fabrication of scaffolds with modified surface for tissue engineering.[15] In addition, plasma treatment is a convenient method for modifying surface properties of materials such as hydrophilicity, surface energy, charge and roughness. As shown in Fig 3, plasma treatment did not adversely affect the surface morphology of the scaffold. The plasma-treated scaffold (Fig. 3) was shown to have significantly different morphology compared to that of the non-treated scaffold (Fig. 2). The roughness and pore size of the scaffolds were largely increased after the plasma treatment. Also, the degree of pore interconnectivity and the surface area of scaffold were markedly increased by plasma treatment. Before the plasma treatment, the pores had inverted conical shape, but after the plasma treatment, the pores had cylindrical shape. These properties may be more suitable for cell growth because it not only provides larger space in which the seeded cells live but also makes cell migration easier. It is well known that the pore size as well as the degree of pore interconnectivity of polymer scaffolds are important parameters of scaffold design that determine the fluid flow through the porous scaffold.[16]

The hydrophilicity of SF/WK scaffold was dramatically increased by plasma treatment. Hydrophilic scaffold has much more benefits to cell adhesion and growth than hydrophobic ones. The attachment and proliferation of the cells on the scaffold

was increased by plasma treatment, even though there was no statistical significance. ($p < 0.05$) In the plasma-treated group, the rate of cell growth was markedly increased as the incubation periods prolonged (3, 7 days). That was not observed in the non-treated group. This result suggests that the surface of the plasma-treated NSF scaffold was more favorable for the cell spreading, growth and proliferation than that of the non-treated one. Recent study has reported that with the use of plasma treatment and acrylic acid grafting, hydrophilic functional groups can be successfully introduced onto the surface of electrospun nanofibrous scaffold and this surface-modified scaffold significant improvement on cell attachment and proliferation *in vitro*.[17] Moreover, it was shown that the surface-modified nanofibrous scaffolds of polycaprolactone and hydroxyapatite had potential in mineralization of osteoblast for bone tissue engineering.[18]

On SEM micrographs, the cells on the non-treated scaffold group covered and locally attached to the surface but the cells on the plasma-treated scaffold formed almost a single layer while maintaining the natural original morphology of articular chondrocytes. These results suggest that the surface-modified scaffolds are effective for the attachment and growth of nHAC-kn although the non-treated NSF is favorable to the cells as well. SF/WK scaffold has been used for *in vitro* cartilage tissue engineering in combination with adult human chondrocytes.[19] Furthermore, the

ability of electrospun silk matrices to support the attachment, spreading and growth of bone marrow stromal cells *in vitro*, combined with a biocompatibility and biodegradable properties of the silk protein matrix, suggest potential use of these biomaterial matrices as scaffolds for tissue engineering.[20]

Recently, it has been shown that the adhesion and re-differentiation of human chondrocytes could be regulated by controlled changes in substrate surface chemistry and composition following glow discharge gas plasma treatment.[21] Successful cartilage tissue engineering requires cells capable of undergoing chondrogenic differentiation upon treatment with appropriate biochemical factors and a three dimensional porous scaffold capable of providing a favorable environment for chondrogenic cell growth and new cartilage extracellular matrix formation. In this study, plasma-treated SF/WK scaffold shows much more GAG synthesis which suggests higher adhesion and differentiation of human chondrocytes than non-treated one. Moreover, recent report showed that cartilage regeneration using a fibroin sponge and a stirring chamber improved the potential of articular cartilage tissue engineering. [22]

Polymer surfaces are commonly modified either by chemical means, using plasma treatment or via the adsorption of monocomponent ECM proteins onto the polymer surface from solution or combination of these methods. The disadvantages of

chemical modifications are the use of organic solvent or harsh chemicals. Three dimensional aqueous-derived biomaterial scaffold was also reported, but it had decreased hydrophilicity and higher silk fibroin concentration.[23] Plasma treatments do not use such chemicals and can modify the wettability, and adhesive properties of the surface by the choice of the plasma gas or vapour and the exposure time.

V. CONCLUSION

In this study, it was shown that chondrocyte cultures on SF/WK scaffold surface-modified by microwave-induced argon plasma treatment significantly increased the hydrophilicity, cellular attachment, proliferation and GAG synthesis as well as chondrogenic differentiation Therefore, it can be suggested that the surface modification of SF/WK scaffold with plasma treatment can be effectively used for the enhancement of cartilage tissue engineering and repair.

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< ABSTRACT (IN KOREAN)>

전기 방사한 실크 피브로인 / 모직 케라토즈 (50/50) 지지체에서 아르곤 플라즈마의 효과

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천연 폴리머의 하나인 실크는 우수한 생체적합성 및 가공능력을 가지는 재료이고 자연적 분해가 가능한 섬유질 단백질로써 직물기술로 제작할 수 있어 조직공학에 적합한 3차원 구조의 지지체로 사용 할 수 있다. 전기방사로 제작된 지지체는 자연적인 나노섬유상 구조가 형성됨으로써 세포 외 기질의 교원질 섬유상 구조와 아주 유사한 구조적 특성을 갖는다. 최근 나노섬유상 구조의 지지체에 세포를 배양해, 세포의 성장이 우수하다는 많은 보고가 이어지고 있다. 아르곤 플라즈마는 약한 경계층을 없애고, 더 나은 접착을 위한 표면 거칠기를 증가 시키기 위해 표면 고분자의 교차결합, 표면의 오염제거, 표면 유래 물질의 제거와 같은 다양한 용도로 사용되어왔

다. 하지만, 그동안 아르곤 플라즈마를 이용한 스캐폴드의 표면개질에는 연구가 적었다.

본 연구는, 실크 피브로인과 이의 단점을 보완하기 위해 모직 케라토즈를 50:50으로 섞어 전기방사하여 3차원구조의 나노섬유상 지지체를 제작하였다. 표면에 아르곤 플라즈마를 처리하여 표면거칠기를 증가시킨 스캐폴드를 실험군으로 하고 아무것도 전처치 하지 않은 스캐폴드를 대조군으로 하여 표면의 물접촉각을 측정하여 비교하여 친수성의 변화를 평가하고 연골세포를 배양하여 세포의 분화와 증식 그리고 글라이코스아미노칸의 형성을 관찰 하여 다음과 같은 결과를 얻었다.

첫째, 지지체의 구조를 전자현미경으로 관찰한 결과 실험군에서 세공의 크기가 증가하고 그 형태 또한 원통형으로 바뀌었다. 둘째, 실험군은 대조군 비하여 친수성이 증가하였다. 셋째, 연골세포가 실험군에서 대조군에 비하여 더 잘 생착하고 더 잘 증식되었다. 넷째, 연골형성의 특징을 관찰할 수 있는 글라이코스아미노칸 역시 실험군에서 뚜렷한 증가를 보였다.
이런 결과들로 전기방사한 실크 피브로인/모직 케라토즈 지지체를 아르곤 플라즈마로 전처리하여 연골세포의 증식과 분화에 더 좋은 영향을 줌이 확인되었다. 따라서 추후 연골 조직 공학에 유용하게 사용될 수 있을 것이다.

핵심되는 말 : 나노섬유상 실크 피브로인, 아르곤 플라즈마, 연골세포, 연골, 지지체