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Nanofibrous architecture of silk fibroin scaffolds prepared with a mild self-assembly process

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Abstract

Besides excellent biocompatibility and biodegradability, a useful tissue engineering scaffold should provide suitable macropores and nanofibrous structure, similar to extracellular matrix (ECM), to induce desired cellular activities and to guide tissue regeneration. In the present study, a mild process to prepare porous and nanofibrous silk based scaffolds from aqueous solution is described. Using collagen to control the self-assembly of silk, nanofibrous silk scaffolds were firstly achieved through lyophilization. Water annealing was used to generate insolubility in the silk based scaffolds, thereby avoiding the use of organic solvents. The nano-fibrils formed in the silk-collagen scaffolds had diameters of 20-100 nanometers, similar with native collagen in ECM. The silk-collagen scaffolds dissolved slowly in PBS solution, with about a 28% mass lost after 4 weeks. Following the dissolution or degradation, the nanofibrous structure inside the macropore walls emerged and interacted with cells directly. During in vitro cell culture, the nanofibrous silk-collagen scaffolds containing 7.4% collagen demonstrated significantly improved cell-compatibility when compared with salt-leached silk scaffolds and silk-collagen scaffolds containing 20% collagen that emerged less nano-fibrils. Therefore, this new process provides useful scaffolds for tissue engineering applications. Furthermore, the process involves all-aqueous, room temperature and pressure processing without the use of toxic chemicals or solvents, offering new green chemistry approaches, as well as options to load bioactive drugs or growth factors into process.

Keywords

Biomaterials; Scaffold; Silk; Nanofiber; Tissue engineering

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1. Introduction

In tissue engineering, scaffolds play a pivotal role by defining the three-dimensional templates and synthetic ECM environments for tissue regeneration [1]. A qualified scaffold needs to satisfy basic structural parameters such as mechanical strength, biocompatibility, biodegradability, ease of fabrication, and interconnected pores with >100 micron diameter. The ECM is an important part of the natural signaling environment to regulate cell growth and tissue regeneration in vivo [2]. The ECM provides a framework for scaffold design and fabrication in which an important common feature is the nanoscaled dimensions of the physical structures [3]. Collagen is the major component of ECM and is organized in fibrillar bundles with a diameter range of 50-500 nanometers. This nanoscaled fibrillar structure is critical to cellular activities [4]. Mimicking the nanofibrous structure of collagen, synthetic polymer scaffold has been prepared and profoundly affect biological activity of cells [5]. Therefore, nano-sized architectures of scaffolds are critical to cell function and tissue regeneration.

Spatiotemporal control of growth factors delivery to mimic physiological patterns also considered critical for tissue regeneration. A qualified scaffold should be able to imbibe or/and release various growth factors with controlled rate to facilitate the formation of specific tissues [6-10]. However, the challenge of such scaffolds lies in the preservation of growth factor integrity and function during the preparation of the scaffolds. In order to overcome the challenge, generating porous scaffolds through a green process, avoiding organic solvents and harsh conditions, would address this need [11].

In recent years, silk fibroin has been widely explored in many biomedical applications due to its impressive biocompatibility, biodegradability, minimal inflammatory reactions and excellent mechanical properties, such as for cell-support matrices for osteoblasts, hepatocytes and fibroblasts, scaffolds for ligament, bone and blood vessel tissue engineering, and controlled release systems for various drugs and growth factors [12-24]. Although salt leaching, freeze-drying, freeze-thaw, gas foaming, and electro-spinning have been successfully used to prepare silk-based scaffolds [25-29], a challenge remains to prepare nanofibrous silk fibroin scaffolds that are similar to the nano-fibrillar structure of collagen in vivo, and avoid organic solvents such as methanol or hexafluoroisopropanol (HFIP), concentrated salts or harsh processing conditions.

Silk fibroin self-assembles to form nano-fibrillar structure, a feature that can be exploited towards the goals of this study. In the present research, a new strategy was sought for silk scaffold processing to control the self-assembly process of silk fibroin to form nanofibrous architectures. Avoidance of organic solvents and harsh chemicals was favored into this process. Silk-based nanofibrous scaffolds were prepared under an all-aqueous process and under ambient conditions, beneficial to the future use of these material systems in cell and tissue studies with delivery of growth factors.

2. Materials and Methods

2.1 Preparation of silk solutions

B. mori silk fibroin solutions were prepared according to our previously published procedures [25]. Cocoons were boiled for 20 min in an aqueous solution of 0.02M Na₂CO₃, and then rinsed thoroughly with water to extract sericin proteins. The extracted silk was dissolved in 9.3M LiBr solution at 60°C for 4h, yielding a 20wt% solution. This solution was dialyzed in water using Slide-a-Lyzer dialysis cassettes (Pierce, MWCO 3500) for 72h. The final concentration of aqueous silk solution was ~7.5wt%, determined by weighting the remaining solid after drying.

2.2 Preparation of nano-fibrous silk-collagen scaffolds

Collagen solution (rat tail tendon collagen type I) from the biomaterials lab of Tsinghua University was prepared as a 0.3wt% solution by 10mM hydrochloric acid at 4°C. The collagen solution was blended with silk solution at 4°C using different contents of collagen and silk by changing the volume ratio. In order to achieve scaffolds with various pore sizes, water was added into the blend solutions to adjust silk and collagen concentrations. For the structural studies the silk concentrations were kept at 1% while the collagen concentrations were 0, 0.08, 0.11 and 0.25%, resulting in ratios of silk and collagen in the dry state of 100:0, 92.6: 7.4, 90:10, and 80: 20, respectively. For the cell growth, silk-collagen solutions with silk/collagen concentrations of 1/0.08% and 1/0.25% were prepared in order to achieve silk-collagen scaffolds having similar pore sizes to our previous silk scaffold prepared by salt-leaching. The blend of aqueous silk-collagen solutions with different concentrations and ratios of silk and collagen were directly placed at -20°C for about 12 h to freeze them and then lyophilized for about 48h. After lyophilization, the scaffolds were placed on a removable platform under which water was filled in a desiccator with a 25 in. Hg vacuum for 6h to produce water-insoluble scaffold [30]. As controls, silk scaffolds were also prepared by salt-leaching [25].

2.3 Structural Analysis

The structure of the various scaffolds was analyzed by FTIR on a JASCO FTIR 6200 spectrometer (JASCO, Tokyo, Japan) equipped with a MIRacle™ attenuated total reflection (ATR) Ge crystal cell in reflection mode. For each measurement, 32 scans were coded with resolution 4 cm⁻¹, with the wave number ranging from 400-4000 cm⁻¹. Fourier self-deconvolution (FSD) of the infrared spectra covering the amide I region (1595-1705cm⁻¹) was performed by Opus 5.0 software. Deconvolution was performed using Lorentzian line shape with a half-bandwidth of 25cm⁻¹ and a noise reduction factor of 0.3. FSD spectra were curve-fitted to measure the relative areas of the amide I region components [31].

X-ray diffraction was also performed on samples with a Rigaku-Ultima-RINT 2000 diffractometer (Japan) with CuK radiation at 40kV and 30 mA and scanning rate of 0.6°/min. Before examination, the dried scaffolds were pressed into sheets with a hydraulic compressor.

2.4 Scanning Electron Microscopy (SEM)

The surface and cross-section images of the silk-based scaffolds were examined by SEM (Supra 55 VP, Zeiss, Oberkochen, Germany) at 3kV to avoid the destruction of silk structure. Before SEM examination, the dried silk-based scaffolds were cut with a razor blade in liquid nitrogen and then coated with platinum.

2.5 Swelling properties

Silk-collagen scaffolds were immersed in distilled water at room temperature for 4h. After excess water was removed, the wet weight of the scaffold (Ws) was determined. Samples were then dried in an oven at 60°C overnight to equilibrium and the dry weight of scaffolds (Wd) was determined. The swelling ratio of the scaffold and the water content in the scaffold were calculated as follows:

$$\text{Swelling ratio} = (W_s - W_d) / W_d$$

$$\text{Water uptake (\%)} = [(W_s - W_d) / W_s] \times 100.$$

2.6 Silk dissolution

Water-insoluble scaffolds were incubated at 37°C in 40 ml of phosphate saline (PBS). Each solution contained an approximately equivalent mass (40 ± 5 mg) of silk-based scaffold. Solutions were replenished with fresh PBS daily. At designated time points the samples were rinsed with distilled water and prepared for mass balance assessment. The morphologies after degradation or dissolution were also examined using a Zeiss Supra 55 VP SEM (Zeiss, Oberkochen, Germany).

2.7 Cultivation of human primary fibroblasts on silk-collagen scaffolds

To evaluate the cytocompatibility of silk-collagen scaffold, human primary fibroblasts (ScienCellTM, Carlsbad, CA) were employed, which were cultured in low glucose-Dulbecco's Modified Eagle Medium (L-DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% UI/ml Streptomycin-Penicillin (all from Invitrogen, Carlsbad, CA). Silk - collagen scaffolds were punched into small discs with a diameter of 5 mm and a thickness of 2 mm and sterilized by 70% ethanol immersion for 30 min. After pre-conditioned with the culture medium overnight, the scaffolds were transferred into a non-treated 24-well cell culture plate (BD, Biosciences, San Jose, CA) and seeded with the human fibroblasts at the density of 1×10^4 cell.cm⁻². The salt-leaching silk scaffolds with a same cell-seeding density served as control. The cultures were cultivated in a humidified incubator at 37°C and 5% CO₂, and the medium was refreshed every other day.

2.8 DNA Content

To study cell proliferation on the scaffolds, samples were harvested at indicated time points (from Day 1 to day 11), and the DNA contents were measured by using PicoGreen DNA Assay following the protocols of the manufacturer (Molecular Probes, Eugene, OR). Samples (n=3 per group) were measured at excitation wavelength of 480 nm and emission wavelength of 530 nm using a Fluoroskan Ascent FL spectrofluorometer (Thermo Life Science, Basingstoke, UK). The amount of DNA was calculated by interpolation from a standard curve prepared using lambda DNA in 10 mM Tris-HCl (pH=7.4), 5×10^{-3} M NaCl, 0.1×10^{-3} M EDTA over a range of concentrations.

2.9 Cell viability and morphology

The viability of the fibroblasts on the scaffolds was examined by live/dead assay (Molecular Probes, Eugene, OR). Briefly, scaffolds seeded with cells were washed with PBS, and incubated in 2×10^{-3} M calcein AM (staining live cells) and 4×10^{-3} M EthD-1 (Staining dead cells) in PBS for 30 min at 37°C. The cells were washed again with PBS and then imaged using a Leica DMIRE2 confocal microscope with a TCS SP2 scanner (Leica Microsystems, Mannheim/Wetzlar, Germany) at excitation of 495 nm and emission of 515 nm. The cell morphology on the scaffolds was investigated by SEM. Following harvest, the seeded silk scaffolds were washed with PBS 3 times and fixed in 10% neutral buffered formalin solution (Sigma-Aldrich, St. Louis, Mo) at 4°C. The samples were further fixed with OsO₄ (2%, Electron Microscopy Science, Hatfield, PA) for 2h at room temperature after the samples were washed two times with PBS. Fixed samples were dehydrated through exposure to a gradient of alcohol (50%, 70%, 85%, 95%, 100%) followed by air drying in a fume hood. Specimens were examined using a Zeiss Supra 55 VP SEM (Zeiss, Oberkochen, Germany).

3 Results and Discussion

3.1 Morphology of silk-collagen scaffolds

Figure 1 shows the influence of collagen content on scaffold structure. All scaffolds were prepared from silk-collagen blend solutions in which the silk concentration was 1% while the collagen concentrations were 0, 0.08, 0.11 and 0.25%, resulting in ratios of silk and collagen in the dry state of 100:0, 92.6: 7.4, 90: 10, and 80: 20, respectively. The scaffolds from pure silk solution formed separate layers or lamellar structures rather than porous structures. By adding the collagen in the silk solution, porous structures were formed through lyophilizing the frozen silk-collagen blends. The results indicated that adding collagen to silk solutions resulted in loss of lamellar formation. Besides the improvement of porous structure, the adding of collagen also facilitated the formation of nanofilaments. Although no specific nanostructure was found on the surface of silk-collagen scaffolds in which the content of collagen was 7.4% and 10%, respectively, the regular nanofibrous structures with diameters of 20-100 nanometers were formed in the macropore walls of the scaffolds, which could be found from the cross-section morphology of the walls (Figure 2). When the content of collagen was increased to 20%, the nanoparticles that might be part of nanofilaments inside the walls appeared on the surface. The results indicated that collagen that easily formed nano-filament in aqueous solution induced silk to self-assemble to nanofilament. The nanofilament further restrained the formation of lamellar structures in the lyophilizing process. In order to confirm the assumption, silk solution in which silk was composed of nanofilaments was prepared through slowly concentrating method [32]. Then silk scaffold with excellent porous structures was directly achieved through lyophilizing the frozen silk solution (Figure 3), indicating the effect of nanofilament on the formation of porous structure.

The results also revealed the effect of water annealing on nano-fibrous structures (Figure 4). Previous study has shown that silk fibroin films $<70\mu\text{m}$ thick rapidly solubilized in water under water-annealing process[30]. For pure silk scaffolds, the majority of the scaffold was lost in the water-annealing treatment (Data not shown). Unlike pure silk scaffolds, silk-collagen scaffolds maintained integrity under the same process and result in the transformation of soluble scaffolds into water insoluble states. The macropore wall of the scaffolds containing 7.4% and 10% of collagen in the dry state was still composed of nanofibrous structure, while the nano-fibrils were reduced and macroscale fibrils increased in the scaffolds containing 20% of collagen. Compared with other silk based scaffolds prepared by salt-leaching, freeze-drying, freeze-thaw, gas foaming, and electro-spinning, the scaffolds in the present study formed better hierarchical porous structures with morphologies similar to that of natural collagen fibers.

3.2 Structural Analysis

Structural changes in the silk-collagen scaffolds were determined by FTIR (Figure 5A). The spectra of collagen scaffolds showed amide I bands at 1640 and 1668 cm^{-1} and an amide II band at 1550 cm^{-1} , while the main peaks of pure silk appeared at 1650 cm^{-1} and 1535 cm^{-1} . The results indicated that pure collagen and silk scaffolds were mainly composed of random and α -helix structures. Peaks at 1625 cm^{-1} and 1511 cm^{-1} appeared in the spectra of the silk-collagen scaffolds, suggesting an increase of β -sheet content [31, 33]. After annealed with water for 6h, the more crystal structures, silk I and silk II, were formed with an increase of the peak at 1650 cm^{-1} .

The secondary structure of the scaffolds was further characterized by deconvolution of the IR spectra. The relative ratios of different secondary conformations were calculated from the amide I region (Table 1). The β -sheet content of silk-collagen scaffolds was 18.7, 18.9 and

19.1%, when the collagen content was 7.5, 10 and 20%, respectively, higher than silk scaffolds without collagen prepared with the same lyophilization process (17.1%). At the same time, turns also increased from 29.4% to 39.4, 40.1 and 46.7%, while random conformation content decreased from 24.1 to 21.1, 19.8, 20.9%, following the increase of collagen content from 0 to 7.4, 10 and 20%, respectively. In our recent study, silk hydrogel mainly composed of intermediate structures rather than β -sheet was prepared under electric fields, confirming that some intermediate structures of silk also have water-stability [34]. Based on our previous and present studies, we believed that the increase of β -sheet and intermediate structures improved the stability of silk-collagen scaffolds, making the scaffolds intact in water-annealing process. Once water-annealed, the intermediate conformation (turns) transformed into crystal structure (silk I and silk II), resulting in water stability of the silk-collagen scaffolds. Similar to water-annealed silk films in our previous study [30], the main amide I bands of the silk-collagen scaffolds were still at 1651 and 1625 cm^{-1} , the typical silk I and silk II peaks, after the water-annealing process. The structural changes of silk fibroin were confirmed by XRD results (Figure 5B). The soluble silk and silk-collagen scaffolds showed an amorphous state, characterized by the presence of a broad peak in the 2θ scattering angle range from 10.5° to 25° . After water-annealing treatment, the silk-collagen scaffolds were characterized by diffraction peaks at 2θ values of 12.2° (I), 19.5° (I), 20.2° (II), 24.6° (I) and 27.2° (I), indicating the formation of silk I and silk II structure, especially the formation of silk I structure [35]. Many studies have shown that silk I is a larger and more hydrated crystal structure than the β -sheet [30]. Compared with salt-leaching silk scaffolds with high β -sheet content, the increase of silk I content in silk scaffolds indicated that more hydrophilic features were achieved. These results indicated that the interaction between silk and collagen accelerated the self-assembly of silk, inducing the more stable intermediate and crystal structures in terms of water insolubility, which relate to the formation of nanofibrous structures during the lyophilization process.

3.3 Swelling

The water-binding ability of scaffolds is an important feature to evaluate biomaterial properties for tissue engineering. Compared with salt-leached silk scaffolds, the swelling ratio as well as water uptake increased significantly with an increase of collagen content in the scaffolds (Table 2). This finding is likely due to the presence of hydrophilic collagen in the silk-collagen scaffolds.

3.4 Dissolution

In our recent study, a water-insoluble silk films were prepared through controlling the drying rate [32]. The silk films dissolved slowly, with about a 30% mass lost after 2 weeks in PBS solution. Interestingly, silk-collagen scaffolds prepared in our present study had similar secondary structure composition with the above silk films. The silk-collagen scaffolds also dissolved slowly in PBS solution, losing 20% and 21% mass after 2 weeks, and then 28% and 26% mass after 4 weeks when collagen contents were 7.4% and 20%, respectively (Figure 6A). The morphology changes following dissolution were also investigated (Figure 6B). Following the dissolution, the compact nanofibrous structure was found on the surface of the macropore walls. At the same time, many nanofilaments with diameter of 50-500nm appeared in the silk-collagen scaffold containing 7.4% of collagen while filaments with diameter of several microns were found in the silk-collagen scaffold containing 20% of collagen. The results indicated that the nanofibrous structure emerged following the dissolution or degradation, which might promote cell adhesion, growth and migration in vitro or in vivo.

3.5 Cell compatibility

To assess cell compatibility of the nanofibrous porous sponges, primary fibroblast cells were cultured in the silk-collagen scaffolds. In order to clarify the influence of nanofibrous structure and collagen content on cytocompatibility, scaffolds containing 7.4 and 20% collagen were used to compare with salt-leached silk scaffolds. The growth behavior of fibroblasts on silk and silk-collagen scaffolds was characterized by Pico Green™ DNA content assay at days 1, 3, 5, 7, 9 and 11d (Figure 7). For the silk scaffolds, a significant increase in DNA content was observed and reached maximum at day 9, and slightly decreased at day 11. However, a continuous increase in DNA content was observed for the cells cultured on the silk-collagen scaffolds over the 11-day culture period. Moreover, the DNA content at day 11 was significantly higher than those from silk scaffolds. The results indicated that silk-collagen scaffolds provided a suitable and perhaps improved environment for cell growth. Although collagen has better cyto-compatibility than silk fibroin, and has been used to improve cell growth and proliferation on silk-based films [36], the growth of cells on silk-collagen scaffolds with different collagen contents had no significant difference before day 7, and then significantly better on silk-collagen scaffolds with lower collagen content (7.4%) than that with a higher collagen content (20%) after day 7, which indicated that the improvement of cell compatibility was not only due to the adding of collagen. Extracellular matrix (ECM) molecules are good framework for synthetic scaffold design and fabrication. It has been recognized that nanoscaled fibrillar structure of ECM are critical to cell function and tissue regeneration [3]. Different from the nanofibrous architecture of PLLA/PLGA scaffolds prepared by other groups [1], the nanofibrous structure of silk-collagen scaffolds containing 7.4% of collagen was masked by the smooth surface of the wall at the beginning of cell culture. Following the slow dissolution and degradation in aqueous solution, the nanofibrous structure would emerge. Considering that the silk-collagen scaffolds with higher and lower collagen contents had similar secondary structure and hydrophilic properties, more emerged nanofibrous structure presumably provided a more suitable environment for the proliferation of cells on the silk-collagen scaffolds containing less collagen, resulting in the improvement of cell growth after day 7.

The viability and morphology of fibroblast cells was examined by SEM and confocal microscopy (Figure 8 and 9). By day 11, the macroporous structure still could be observed for the silk scaffolds in which fewer cells spread. Cell-seeded silk-collagen scaffolds containing 20% collagen were covered by a cell sheet with possible ECM formation. Although only containing 7.4% collagen, the nanofibrous silk-collagen scaffolds further improved cytocompatibility. The nanofibrous scaffolds seeded with fibroblasts were fully covered with what appeared to be a thick multilayer cell sheet and ECM concealed the scaffolds. This was confirmed by confocal. The nanofibrous structure in the silk-collagen scaffolds containing 7.4% collagen supported the proliferation of fibroblasts further. Cell mainly formed confluent cell sheets and most cells were not spherical. Fibroblast shape was dependent on ECM or scaffold microstructure. Upon seeding within 3D nano-fibrous collagen ECMs, fibroblast generally adapted from an initial rounded shape to a bipolar spindle shape [37]. In present study, fibroblast maintained rounded shape on the salt-leaching silk scaffolds while transformed to spindle shape on the silk-collagen scaffolds (Figure 9). The results confirmed that fibrous microstructure appeared on the silk-collagen scaffolds, which changed the fibroblast shape and further facilitated the proliferation and growth of fibroblast.

Although the biocompatibility of the salt-leaching silk scaffolds has been confirmed in previous studies [25], it is still critical to further increase biocompatibility of silk scaffolds to satisfy the requirements of different cell and tissue regeneration needs in vivo and/or in vitro. In the present research, a fibrous architecture was achieved to mimic the natural ECM by controlling the self-assembly of silk in an aqueous and green process, resulting in the

further improvement of cell interactions. Since in our recent studies we found that silk fibroin is also a good carrier for protein drugs such as the growth factors and enzymes [38,39], besides improving cell interactions, new ways to load different growth factors without the loss of activity can be considered in this new approach.

4. Conclusion

Three-dimensional macroporous silk scaffolds with nanofibrous architectures were prepared from silk-collagen aqueous solutions using freeze-drying. By changing the collagen content to control the self-assembly of silk, the nanostructure of the macroporous wall transformed from irregular to nanofibrous structures and then to macroscaled fibrils. The nanofibrous architecture of silk-collagen scaffolds, similar to natural ECM, provided fibroblasts with a favorable microenvironment to enhance growth and proliferation.

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Figure 1.

SEM images of silk-collagen scaffolds derived from 1% silk solution with different contents of collagen: (a) collagen concentration 0%, the ratio in dry state of scaffolds, 0%; (b) collagen concentration 0.08%, the ratio in dry state, 7.4%; (c) collagen concentration 0.11%, the ratio in dry state 10%; and (d) collagen concentration 0.25%, the ratio in dry state 20%.

Figure 2.

Surface and cross-section images of macropore wall of silk-collagen scaffolds derived from 1% silk solution with different contents of collagen: (a) collagen concentration 0%, the ratio in dry state of scaffolds, 0%; (b) collagen concentration 0.08%, the ratio in dry state, 7.4%; (c) collagen concentration 0.11%, the ratio in dry state 10%; and (d) collagen concentration 0.25%, the ratio in dry state 20%. (Left, surface images; Right, cross-section images)

Figure 3.

The relationship between nanofibrous structure of silk in solution and macropore structure of scaffolds: (a) AFM images of silk in solution. The solution was treated by slowly concentrating process, and then diluted to below 0.01% to get single layer on mica surface; (b) SEM image of silk scaffold derived from the treated solution through lyophilizing process. Before lyophilization, the treated solution was diluted to 2% to achieve suitable pore structure.

Figure 4.

SEM images of water annealed silk-collagen scaffolds derived from 1% silk solution with different contents of collagen: (a) collagen concentration 0.08%, the ratio in dry state, 7.4%; (b) collagen concentration 0.11%, the ratio in dry state 10%; and (c) collagen concentration 0.25%, the ratio in dry state 20%.

Figure 5.

(A). FTIR spectra of silk-collagen scaffolds before and after water annealing: (a) collagen prepared using the same process with silk-collagen scaffolds, without water annealing; (b) pure silk prepared using the same process with silk-collagen scaffolds, without water annealing; (c) collagen content 7.4%, without water annealing; (d) collagen content 10%, without water annealing; (e) collagen content 20%, without water annealing; (f) collagen content 7.5%, water annealing; (g) collagen content 10%, water annealing; and (h) collagen content 20%, water annealing; (B) XRD data from silk-collagen scaffolds before and after water annealing: (a) pure silk prepared using the same process with silk-collagen scaffolds, without water annealing; (b) collagen content 7.4%, without water annealing; (c) collagen content 10%, without water annealing; (d) collagen content 20%, without water annealing;

(e) collagen content 7.4%, water annealing; (f) collagen content 10%, water annealing; and (g) collagen content 20%, water annealing.

Figure 6.

Dissolution and morphology changes of water-annealed silk-collagen scaffolds in PBS solution. (A) Dissolution of water-annealed silk-collagen scaffolds, (a) collagen content 7.4%, (b) collagen content 20%. (B) SEM images of silk-collagen scaffolds incubated in PBS solution at 37°C for 2 weeks. (a)-(c), silk-collagen scaffold containing 7.4% collagen; and (d)-(e), silk-collagen scaffold containing 20% collagen.

Figure 7.

Fibroblast proliferation on silk-collagen scaffolds and silk scaffolds. Silk, silk scaffold prepared with salt-leaching process; Col-7.4, silk-collagen scaffolds containing 7.4% collagen in dry state; and Col-20, silk-collagen scaffolds containing 20% collagen in dry state. Error bars represent mean ± standard deviation with N=3 (*p<0.05, #p>0.05)

Figure 8. SEM images of fibroblasts cultured on silk scaffolds and silk-collagen scaffolds at day 11. (a) Silk scaffolds, (b) nanofibrous silk-collagen scaffold containing 7.4% collagen, and (c) non-nanofibrous silk-collagen scaffold containing 20% collagen.

Figure 9. Viability of fibroblasts cultured on silk scaffolds and silk-collagen scaffolds at day 11 using live/dead assay with confocal microscopy. (a) Silk scaffolds, (b) silk-collagen scaffold containing 7.4% collagen, and (c) silk-collagen scaffold containing 20% collagen.

Table 1

The structural conformation ratios in silk fibroin and silk fibroin-collagen scaffolds derived from deconvoluted amide I FTIR spectra

Samples	Conformation content of silk fibroin based scaffolds (%) ^{a)}			
	Random	β -sheet	Silk I (helix in collagen)	Turns
Silk	24.1 $\pm$ 1.1	17.1 $\pm$ 0.6	27.0 $\pm$ 1.1	29.4 $\pm$ 1.8
S-C7.4 ^{b)}	21.1 $\pm$ 0.8	18.7 $\pm$ 0.5	16.9 $\pm$ 0.7	39.4 $\pm$ 0.9
S-C10 ^{b)}	19.8 $\pm$ 1.0	18.9 $\pm$ 0.8	16.6 $\pm$ 0.4	40.1 $\pm$ 1.8
S-C20 ^{b)}	20.9 $\pm$ 0.5	19.1 $\pm$ 0.5	10.5 $\pm$ 1.0	46.7 $\pm$ 1.6
Wa-S-C7.4 ^{b)}	21.7 $\pm$ 0.6	23.6 $\pm$ 0.2	22.1 $\pm$ 0.4	27.8 $\pm$ 0.3
Wa-S-C10 ^{b)}	21.8 $\pm$ 1.0	23.5 $\pm$ 1.2	21.7 $\pm$ 0.8	28.3 $\pm$ 1.1
Wa-S-C20 ^{b)}	21.0 $\pm$ 0.5	24.8 $\pm$ 0.8	20.9 $\pm$ 0.6	27.8 $\pm$ 0.7

^{a)} Values are average $\pm$ standard derivation (N=4). The different data of same sample come from different FTIR spectra of different areas of same sample;

^{b)} S-C7.4, silk fibroin-collagen scaffold containing 7.4% collagen; S-C10, silk fibroin-collagen scaffold containing 10% collagen; S-C20, silk fibroin-collagen scaffold containing 20% collagen; and Wa means water-annealing treatment

Table 2

Swelling ratio and water uptake of the different scaffolds.

	Collagen content in dry state (%)			
	0 ^{a)}	7.4	10	20
Swelling ratio	0.96±0.03	1.20±0.06	1.43±0.15	1.80±0.23
Water uptake (%)	45.8±1.8	58.2±1.2	62.5±3.0	65.0±2.7

^{a)} Silk fibroin scaffolds without collagen prepared by salt-leaching rather than lyophilization.