Alignment of 3D nano-/micro-structured silk fibroin-chitoson scaffolds using DEP for tissue engineering

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Statement of Purpose: Silk fibroin-chitosan (SFCS) scaffolds, with physiological compatibility and mechanical pliability, have been successfully applied to abdominal wall reconstruction, skin wound healing and bone regeneration[1]. However, SFCS scaffolds used in previous studies formed stacked sheets structure, which did not capture the multiscale nature of the extracellular matrix (ECM) architecture of nano-/micro-fibrous structures. Studies have shown that use of nano-/micro-combined scaffolds are capable of promoting physiologically more relevant microtissue formation in vitro[2]. Aligned fibrous scaffolds present promising potential for guiding cell integration and promote cell differentiation[3]. Dielectrophoresis (DEP), through which protein molecules could be patterned in the AC electric fields[4], has been applied to align SFCS scaffolds in two dimension (2D) (monolayer formation) for endothelial and stem cell interaction study[4]. In this work, we assessed alignment of nano-/micro-fibrous SFCS scaffolds in three dimension (3D) multilayer formation fabricated using DEP with simultaneous contributing effects from freezing temperature and AC frequency.

Methods:

SFCS preparation

The blend of 75:25 SFCS solution was prepared as previously described[1]. Briefly, raw silk was degummed in 0.25% (w/v) sodium carbonate and 0.25% (w/v) sodium dodecylfulfate at 100 ^{o}C for 1 h. The degummed silk was then dissolved in calcium nitrate tetrahydrate and methonal solution; chitosan was dissolved in 2% acetic acid solution at the same concentration as silk fibroin solution and was mixed with silk fibroin solution in a ratio of 1:3.

SFCS alignment using DEP

SFCS scaffolds were aligned using DEP as previously described[5]. To form multilayer scaffolds, 1.0 mL SFCS solution was pipetted onto electrodes (triangular castellation arrays) which were fabricated on top of glass microscope slides. The electrodes were connected to an AC power supply. A 10 V_{pp} sine wave (100 kHz or 1 MHz) was applied to the sample for 45 min at room temperature; then the sample was transferred to -20 ^{o}C freezer or liquid nitrogen container (-190 ^{o}C). Frozen samples were lyophilized overnight and crystallized (in 50:50 (v/v) methanol:sodium hydroxide (1 N) solution for 15 min) before examination.

Scaffolds evaluation using light microscopy Scaffolds were imaged using light microscopy before and after crystallization. Polarized light microscopy was also used to assess the alignment and orientation of the fibrous structures within the scaffolds.

Results: Nano-/micro-fibrous structures were observed in SFCS samples treated by DEP and post-DEP freezing (Figure 1). Patterned alignment was seen in samples prepared at -190 ^{o}C (Figure 1(a-c)). The alignment was along the electric field lines, and Figure 1(c) presents the aligned SFCS fibers in a monolayer. Less aligned fibrous structures were formed in samples frozen at -20 ^{o}C after DEP (Figure 1(d-f)), and Figure 1 (f) presents the SFCS fibers formed in a monolayer. Similar fibrous structures were observed when changing AC frequency from 100 kHz (Figure 1(a) and (d)) to 1 MHz (Figure 1(b) and (e)).

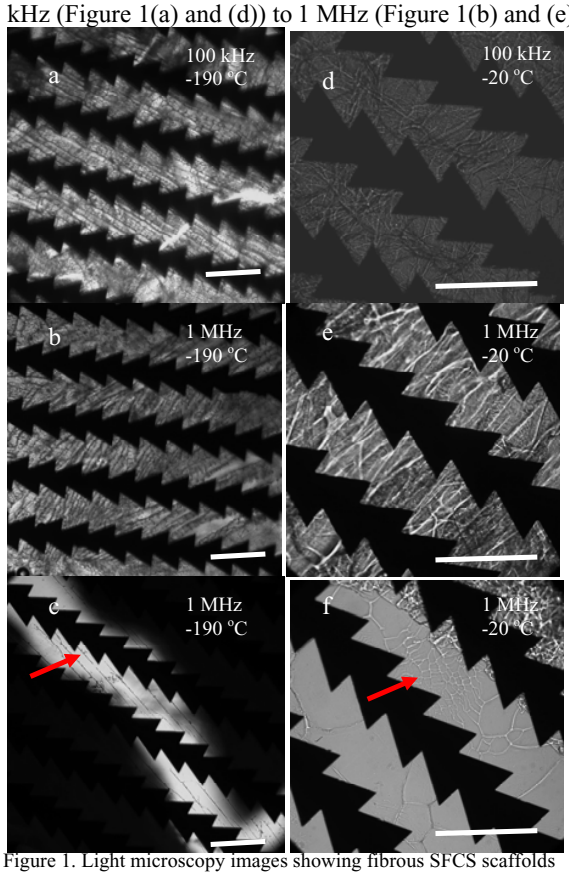

Figure 1. Light microscopy images showing fibrous SFCS scaffolds fabricated by DEP (V_{pp} = 10 V) at different frequencies (100 kHz for (a) and (d) and 1 MHz for (b), (c), (e), (f)) and post-DEP freezing at different temperatures (-190 ^{o}C f o r (a-c), -20 ^{o}C for (d-f)). Scale bar =50 µm.

Conclusions: The results supported the idea of fabricating nano-/micro-fibrous SFCS scaffolds using DEP. DEP frequency and post-DEP freezing temperature are important parameters in 3D fibrous SFCS scaffold alignment. The effect from DEP frequency was not obvious in the results. Post-DEP freezing temperature (-20 ^{o}C or -190 ^{o}C) may affect water freezing process, which may indirectly affect SFCS alignment. Future work will be focused on DEP alignment parameter optimization, scaffold mechanical property characterization, and effect of aligned scaffolds on adipose-derived stem cells differentiation *in vitro*.

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