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Wet-spun silk fibroin scaffold with hierarchical structure for ligament tissue engineering

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ABSTRACT

A regenerated silk fiber scaffold for ligament tissue engineering is prepared. The scaffold showed a designed hierarchical structure involving nanofibril, microfiber, and fiber bundles. This scaffolds after 2 times drawing demonstrated the matching mechanical performance to human anterior cruciate ligament (ACL). Additionally, the biodegradation test shows that the regenerated silk scaffold losses 8% weight after incubation in PBS for about 60 days and 62% weight in actinomycetes protease solution for about 48 h. Thus, this novel regenerated SF scaffolds, combining with their hierarchical structure, mechanical properties, and biodegradability, could be applied with promise in ligament tissue engineering.

1. Introduction

The ACL is the major intra-articular ligament of the knee and is critical to normal kinematics and stability. Annually, more than 200,000 patients are diagnosed with ACL disruptions [1,2]. Due to inherently poor healing potential, ACL ruptures do not heal and surgical replacement is often required [3]. Tissue engineering is a superior strategy to replace, repair and regenerate the damaged ACL. An ideal scaffold for ACL tissue engineering should be biodegradable, porous, and exhibit sufficient mechanical properties and hierarchical structure similar to extra cellular matrix (ECM), which has the potential to provide appropriate mechanical support and biochemical stimulation to allow formation of neoligament tissue [4].

Silk has drawn increased attention as a choice for tissue engineering because of its biocompatibility, biodegradability, and remarkable performance [5,6]. Several groups have explored silk-based scaffolds for ligament tissue engineering by twisting or braiding silk in its natural fiber forms. These scaffolds showed excellent cell biocompability and induction the formation of ligament-like ECM [7], three-dimensionally braided scaffolds for ACL repair [8–10], the braid-twist scaffolds for ACL repair [11].

The goal of this study was to develop a scaffold which features a structure mimicking hierarchical collagen structure (such as molecules, fibrils, fibril bundles) of ligaments, high performance and biodegradability. The hierarchical structure, mechanical properties and biodegradability of regenerated silk fibroin ligament-like scaffolds were characterized.

2. Experimental

Raw silk fibers were degummed twice with 0.05% (w/v) Na_2CO_3 solution at 100 °C for 60 min and then rinsed thoroughly with deionized water to remove glue-like sericin proteins. The degummed silk fibroin (SF) was directly dissolved in $CaCl_2$–FA solvent with $CaCl_2$ concentration of 4% (w/v) for 3 h at normal atmosphere temperature to prepare 15% (w/v) SF solution.

The above SF–$CaCl_2$–FA solution was spun into water coagulation bath using a syringe and a pump with a 22 gauge needle and 5 mL/h flow rate at room temperature, and the filaments were collected by take-up roller with 100 cm/min (Fig. 1). 600 filaments were collected together to form a fiber bundle (Fig. 1D). 10 groups of fiber bundles were fixed together to form artificial ACL scaffold (Fig. 1F). After wet-spinning, SF filaments were soaked in deionized water overnight for removing salt ions, and then were drafted with draw-ratios of 0.5, 1, and 2 times for use. The ends of 10 groups of fiber bundles were fixed by immersion in 10 wt% SF–NaCl–FA solution with a weight ratios of NaCl to silk 10:1. Then the filament scaffold was desalted and frozen at −20 °C for 12 h,

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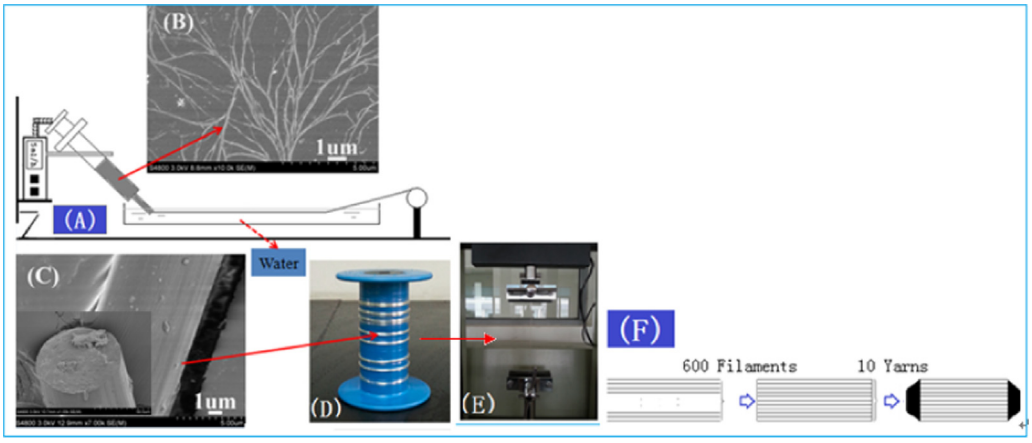

Fig. 1. (A) Schematic of custom-made wet-spinning device, (B) SEM image of SF nanofibrils in spinning solution, (C) cross-section and longitudinal views of SF filaments, (D) filaments, (E) stretching, and (F) procedure of scaffold by filaments.

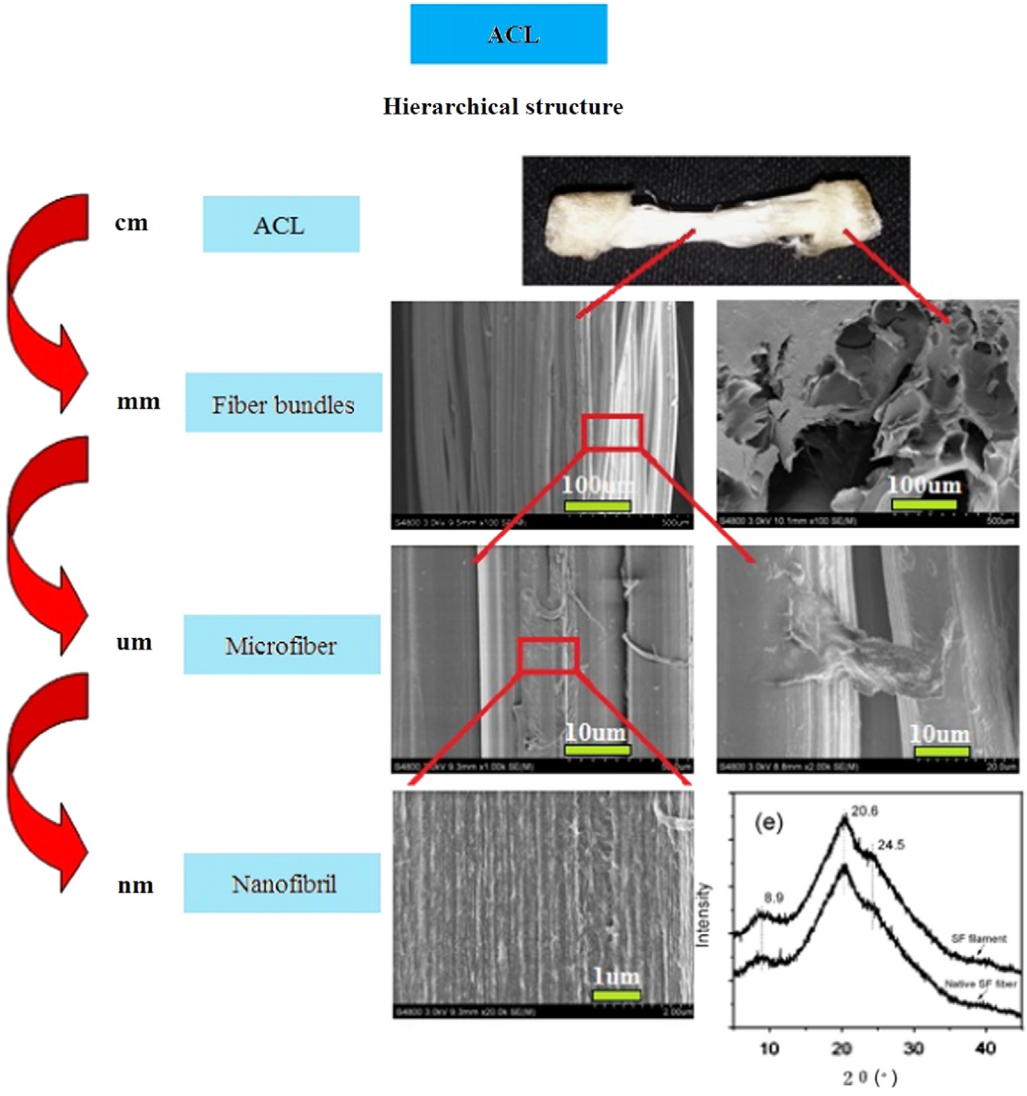

Fig. 2. SEM images (A–E) of scaffold and its XRD results (F).

freeze-dried for 24 h to form artificial ACL consisting of parallel regenerated SF fiber whose two ends were fixed in porous silk fibroin scaffold.

The morphology of the cross-section and longitudinal sections of SF filaments was observed by Hitachi S4800 scanning electron microscope (SEM, Japan). The secondary structure of SF filaments was analyzed with an X-ray diffraction instrument (X'Pert Pro MPD, PANalytical, Netherlands). Instron 3365 mechanical testing instrument (Instron, Norwood, MA) was used for artificial ACL scaffold testing (25 ± 0.5 °C; $60 \pm 5\%$ relative humidity; gauge