Composites Science and Technology 122 (2016) 113–121

Contents lists available at ScienceDirect

Composites Science and Technology

journal homepage: http://www.elsevier.com/locate/compscitech

Direct modulus measurement of single composite nanofibers of silk fibroin/hydroxyapatite nanoparticles

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ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form
10 November 2015
Accepted 15 November 2015
Available online 1 December 2015

Keywords:
Silk fibroin
Hydroxyapatite
Composite nanofibers
Three point bending
Atomic force microscopy

ABSTRACT

When cells are populated in fibrous scaffolds, the mechanical strength of individual nanofibers has significant influence on cell behaviors such as cell attachment, proliferation, differentiation, and protein expressions. However, analysis of mechanical property of tissue scaffolds has been rather limited to macroscopic scaffolds than local cell-scale force field of scaffolds. Silk fibroin (SF) has been frequently utilized in a form of nanofibers as promising tissue scaffolding material. However, due to difficulty of processing composite nanofibers with uniformly mixed SF and nanoparticles, mechanical analysis of SF-based composite nanofibers has not been investigated yet. In this study, we fabricated "composite" nanofibers of silk fibroin (SF) with hydroxyapatite (HAp) up to 40 wt% that were uniformly dispersed in the SF nanofibers. Their mechanical moduli and dependency on the content of HAp nanoparticles were analyzed using three point bending with tipless AFM cantilever (AFM-TPB). The composite single nanofibers became stiffer with higher content of HAp nanoparticles up to 20 wt% of HAp. Further addition of HAp nanoparticles reduced the mechanical strengths of the composite single nanofibers similarly to macroscale electrospun scaffolds. DSC and XRD analysis revealed that the crystallinity of SF increased up to 20 wt% and became saturated at higher contents of HAp nanoparticles. It was also noticeable that the single composite nanofibers had two orders of magnitude higher mechanical moduli than macro scaffolds samples.

1. Introduction

Nanoscale details of tissue scaffolds have significant influences on cell behaviors such as cell attachment, proliferation, differentiation, and protein expressions. For example, it is known that nanoscale topography has direct control over cell adhesion, motility, and orientation as well as intracellular signaling pathways that are ultimately connected to gene transcription and protein expression [1]. Surface chemistry of tissue scaffolds also showed strong influence on protein adsorption that also determined cell physiology and behaviors mentioned above [2–4]. On the other hand, mechanical properties of tissue scaffolds are also known to affect cell morphology, structure and adhesion on a macro scale [5].

However, the influence of nanoscale or cellular scale mechanical properties of tissue scaffolds has not been rigorously investigated so far. Recently, microenvironmental control of mechanical property of tissue scaffolds demonstrated the feasibility of controlling cell differentiation by the mechanical modulus of tissue scaffolds [6,7]. In these works, the mechanical properties of the scaffolds were measured using macroscopic samples whose sizes ranged from a few to tens of millimeters without considering local force fields that individual cells in the scaffolds were experiencing.

Electrospinning has been frequently utilized to construct tissue scaffolds because of its architectural similarity to the extracellular matrix (ECM) of natural tissue, in terms of high porosity, wide range of pore diameter distribution and effective mechanical properties [8]. However, due to the morphological complexity of the electrospun scaffolds, it is difficult to estimate the nanoscale force fields which individual cells sense, based on the macroscale (mm ~ cm) mechanical properties [9]. For the measurement of local force fields, recent works focused on direct mechanical measurement of single nanofibers of electrospun scaffolds. Nanoscale characterization techniques such as nanoscale tensile tests [10], AFM-connected microbead tests [11], and AFM-based cantilever tests [12,13] were

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http://dx.doi.org/10.1016/j.compscitech.2015.11.019
0266-3538/

developed to measure the mechanical properties of single nanofibers.

Silk fibroin (SF) is one of the most widely-used biomaterials with its excellent biocompatibility as well as regeneration capability in various forms of tissue scaffolds including electrospun scaffolds [14,15]. In particular, for enhancement of bone regeneration and mechanical property, electrospinning of SF/hydroxyapatite (HAp) composite were investigated [16,17]. Although the macro scale mechanical measurements were performed to find out the effect of nanoparticle inclusion on the modulus of the scaffolds [18,19], the direct measurement at a single nanofiber level has not been performed. It still remains unclear whether the HAp addition in the single SF nanofibers will also increase the mechanical property of SF/HAp nanofibers with an optimal composition for maximum mechanical property in the same fashion as demonstrated in the macro scale scaffold samples [18].

In this study, nanofibers of SF/HAp composite at varying mixing ratios were electrospun over micrometer scale channels. Using tipless AFM cantilevers, the suspended SF/HAp nanofibers were gently pressed at a controlled speed to record the corresponding forces and deflections at each location (Fig. 1). Based on the force and deflection data, the bending modulus of each SF/HAp composite nanofibers at different HAp contents was calculated and compared to the modulus measured from tensile tests. For understanding the effect of HAp nanoparticles on the crystallinity and mechanical property of SF, DSC and XRD analysis were performed and the results were analyzed.

2. Materials & methods

2.1. Preparation of SF/HAp mixture solution for electrospinning

For preparation of an aqueous SF solution for electrospinning, SF protein was extracted from *Bombyx mori* cocoons by a degumming process using 0.2 M of sodium carbonate and 0.01 M of sodium oleate aqueous solution. The solution was boiled for 30 min and rinsed with deionized (DI) water to remove sericin protein from raw silk worm cocoons. The degummed SF was dried for 24 h in a convection oven at a room temperature. The dried SF fibers were dissolved in 9.3 M LiBr solution at 60 °C for 30 min, then dialyzed in DI water for at least 3 days.

To prevent aggregation and achieve uniform dispersion of HAp particles, the particle surface was coated with γ-glycidoxypropyltrimethoxysilane (GPTMS). One side of the GPTMS was attached to the hydroxyl groups of HAp surfaces, while the other side (epoxide functional group) interacted with the side chains of the amino acids of silk fibroin [18,20]. The GPTMS-coated HAp nanoparticles were suspended in DI water to obtain 0%, 10%, 20%, 30% and 40% (w/w) concentration. Each suspension was sonicated for 5 min and mixed with aqueous SF solution (9%) to prepare a desired mixing ratio of HAp/SF. Aqueous poly (ethylene oxide) (PEO) (Mw = 900,000, Sigma, Product. No #189456) solution (5%, w/v) was added in HAp-SF solution (SF:PEO = 3:1) to enhance viscosity for stable electrospinning.

2.2. Electrospinning of SF/HAp nanofibers over micro channels

Silicon micro channels that were 20 μm wide and 20 μm deep were fabricated by photolithography and DRIE processes. A commercialized electrospinning system (ESR200RD, Nano NC Inc., Seoul, Rep. of Korea) was used for experiments. The SF/HAp solution for electrospinning was pumped at a feed rate of 1.2 ml/h using a 17 gauge metal needle. The needle tip was connected to a 14 kV voltage power supply with 15 cm distance to a collector, the silicon micro channels. After collection on the silicon micro channels, SF/HAp composite nanofibers were treated in 99.5% methanol for 5 min. The collected SF/HAp nanofibers on micro channels were immersed in DI water for 24 h to remove PEO from SF fibers in order to avoid any effect of PEO on the mechanical property of SF/HAp nanofibers.

2.3. Three point bending tests of suspended fiber with AFM

Single SF/HAp composite nanofibers that were aligned in a perpendicular direction to the silicon micro channels were carefully selected. Contact mode imaging was conducted using a commercial AFM system (Multimode 8, Bruker Corp., Massachusetts, USA) with a silicon nitride AFM tip (SNL-10, F, Bruker Corp., Massachusetts, USA) to confirm the fixation between fibers and silicon surface. To avoid surface damage or indentation of fiber surface, we used a "tipless" triangular silicon nitride AFM cantilever (MLCT-O10, type F, Bruker Corp., Massachusetts, USA) for three point bending test. Unlike common AFM cantilever contacts, the tipless cantilever made a "line contact" between the AFM cantilever and nanofibers.

The spring constant of the tipless cantilever was obtained using the thermal tuning mode of AFM analysis software (Nanoscope Analysis 1.5 ver., Bruker Corp., Massachusetts, USA) [21]. Cantilever deflection was calibrated on a non-deformable silicon surface (Fig. S1a). On a deformable sample like suspended fiber, fiber deflection was obtained by subtraction of cantilever deflection from piezo displacement ($\delta = \Delta Z - \Delta d$, Fig. S1b). With the spring constant of cantilever (k) and the deflection of cantilever (Δd), force applied by cantilever (F) is represented by simple Hooke's law, $F = k \times \Delta d$. In a force-deflection curve, the slope of the force-deflection curve($dF/$

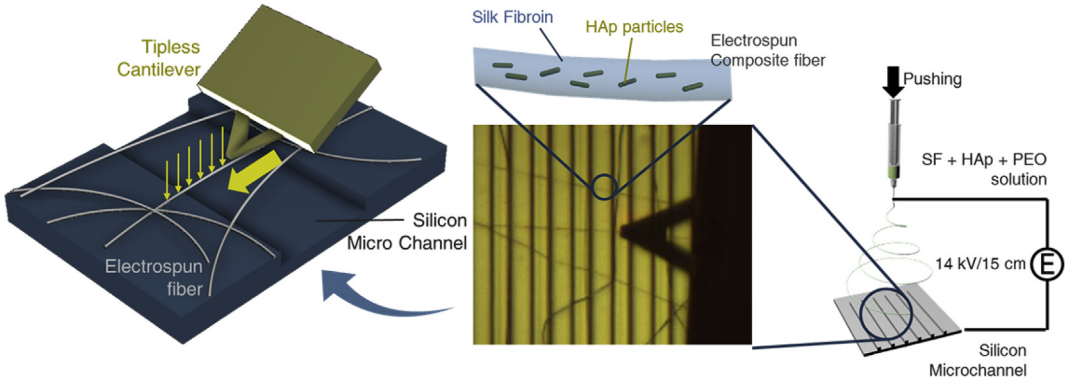

Fig. 1. Mechanical property measurement of SF/HAp composite nanofibers electrospun over micro channels using tipless AFM cantilever.