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Solvent and temperature effects on folding of electrospun collagen nanofibers

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ABSTRACT

Electrospinning is a well-known method to obtain nanofibers and collagen is a very attractive material for biological applications due to its bioactive nature. However, the preservation of the natural structure of collagen at the end products due to the different production parameters is an open question. In this study the importance of the preparation temperature for CD measurements and the solvent effect that employed in the electrospinning process was discussed. The decrease of the preparation temperature by 10° increased the measured PP-II (folded) fraction ratio from 37% to 52.5% and the nanofibers that were obtained from acidic solvent scored 59% of PP-II fraction ratio.

1. Introduction

Collagen type I makes up 70–90% of the collagen in human body. It is present in the form of elongated fibers in various tissues. These building blocks are rod-like triple helices that are stabilized by intramolecular hydrogen bonds between Gly and Hyp in adjacent chains [1]. This 3D folded structure makes collagen a bioactive material due to its special topography and certain order of aminoacids in the 3D structure by sending the necessary signals for the cell activities. Electrospinning is a suitable way to produce fibers with diameters smaller than 1 μm and has a number of advantages [2]. In order to regenerate materials in forms of nanofibers, it is necessary to dissolve them in suitable solvents and many other production parameters could affect the final structures. Extracted type I collagen is favored for biomedical applications; under appropriate conditions it will spontaneously self-assemble to form biodegradable and biocompatible insoluble fibrils [3]. Architecture, topography, biochemical and mechanical features of the nanofibrous scaffolds have been shown to significantly improve cellular events and in vivo cellular regenerations [4]. Liu et al. [5] and Yang et al. [6] investigated the mechanical properties of single electrospun collagen type I fibers. The bending moduli of the cross-linked collagen fibers range from 1.3 to 7.8 GPa. The electrospun fibers showed anisotropic mechanical properties with two orders of magnitude lower shear modulus compared to the bending modulus. Dos Santos et al. [7] improved the electrospinning technique to impart the same morphological and mechanical properties to each point of the produced structure. Cai et al. [8] correlated the process parameters as well as relative humidity and evaporation rate to fiber diameter. Whited et al. [9] showed the correlation of fiber orientation and cell alignment. Oh et al. [10] enhanced the stability of stem cells by the nanofibers via an electrospinning technique.

We demonstrated that the electrospun collagen nanofibers have been mostly unfolded in our previous work and speculated that it is mostly due to the acting high shear forces during the electrospinning process [11,12]. Collagen has better preserved its native structure in acidic solvents; however there has been no real success to produce electrospun nanofibers from acidic solutions till now. In this work, we were able to produce electrospun nanofibers from acidic solutions, by increasing the collagen concentration to 40% and the results were compared with the electrospun nanofibers that were obtained from fluorinated solutions.

2. Experimental

The collagen-type I used in these experiments was a water-insoluble lyophilized foam powder consisting of tropocollagen extracted from bovine dermis (generously donated by the Kensey Nash Corporation, USA) and was used without further purification. Acetic acid (HAc, 99%) was purchased from Merck KGaA, Darmstadt. 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, ≥99.8%) was purchased from Sigma-Aldrich and used as received. Two different samples were prepared by dissolving collagen in different solvent systems:

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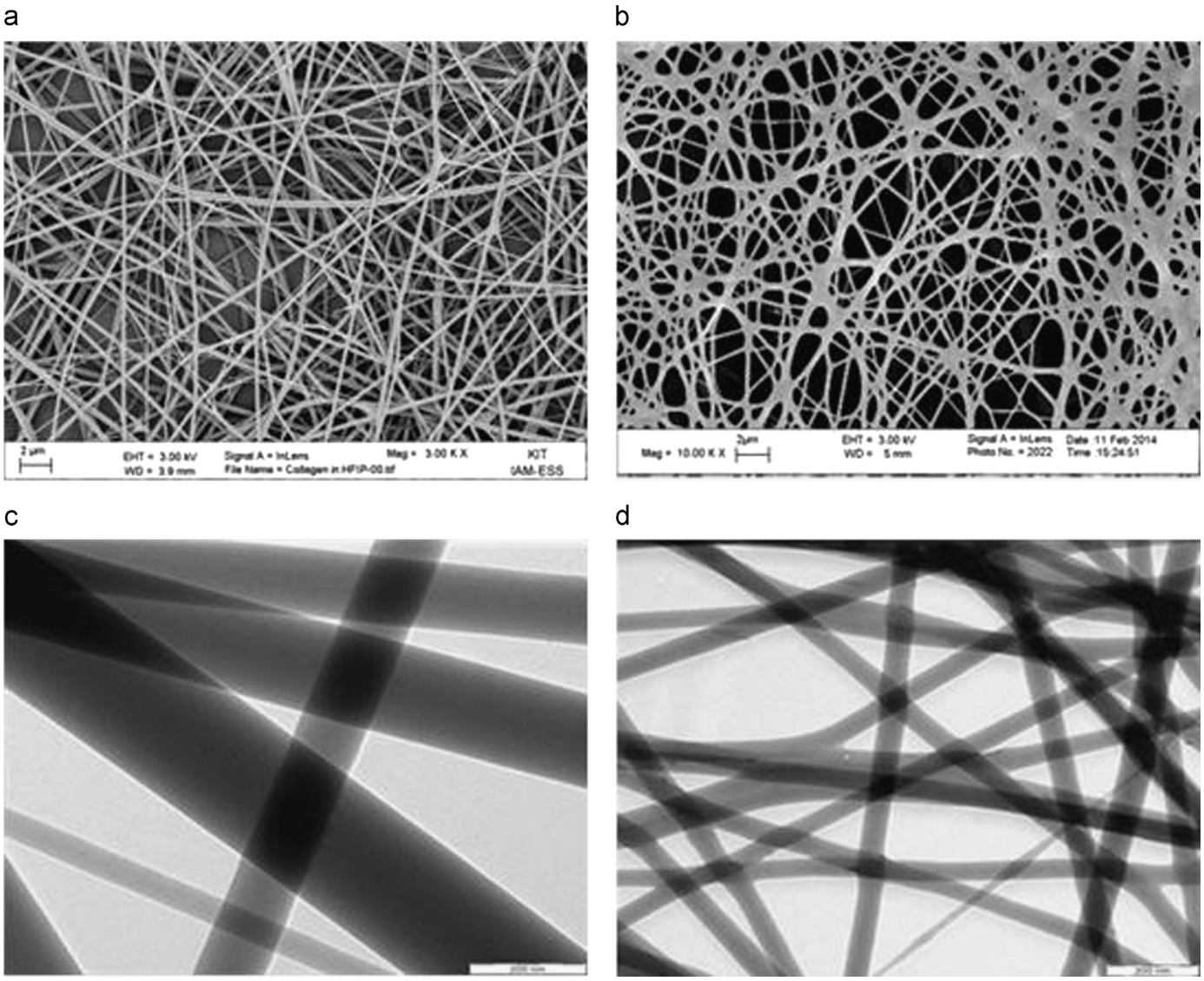

Fig. 1. SEM images of electrospun nanofibers from (a) HFP and (b) 40% HAc, and TEM images of electrospun nanofibers from (c) HFP and (d) 40% HAc.

(1) 10% w/v collagen solution was prepared by dissolving collagen in HFP; (2) 40% w/v collagen solution was prepared by dissolving collagen in 40% v/v HAc. The prepared electrospinning solutions were loaded into a 2 mL syringe (Omnifix, B. Braun, Melsungen, Germany) with a blunt end nozzle, controlled by a syringe pump (Pump 33 Harvard Apparatus, Holliston, USA). The solution was pushed through a capillary blunt steel needle (21 gauge, 0.7 mm i.d. $\times$ 50 mm length) at a constant speed (0.5 μL/min). The steel needle was coupled to a high voltage source (Spellman Bertan Series 205B, NY, USA). The electric potential was needed to start the spinning process and thus form a jet. The applied DC voltage was held at 16 kV. A Cu collector was placed 15 cm from the needle tip to collect the electrospun collagen nanofibers. The nanofiber meshes were collected on cover-glasses placed onto the collector.

3. Results and discussion

Fig. 1 (a) and (b) shows the SEM images, and (c) and (d) shows the TEM images of the electrospun nanofibers obtained from two different solvents. Electrospinning yielded randomly oriented and

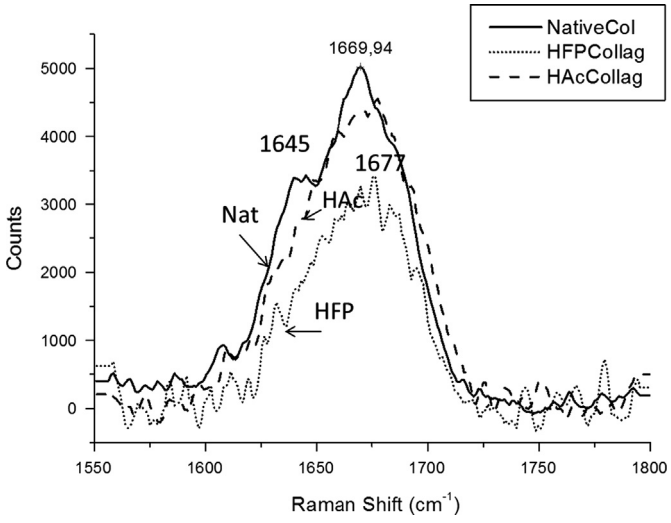

Fig. 2. Raman shift of (a) native collagen, (b) electrospun collagen nanofibers obtained from HAc, and (c) electrospun collagen nanofibers obtained from HFP.