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Effect of humidity and benign solvent composition on electrospinning of collagen nanofibrous sheets

Seon Young Bak [a], Gye Jin Yoon [a], Sang Woo Lee [a], Hyun Woo Kim [a,b,*]

[a] Graduate Program of Nano Science and Technology, Graduate School of Yonsei University, Seoul, Republic of Korea
[b] Department of Orthopaedic Surgery, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea

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ABSTRACT

This study investigated the effects of relative humidity (RH; 30% and 60%) and the presence of an environmentally benign solvent consisting of 20 × phosphate-buffered saline/ethanol (1:1, 1:1.3, and 1:1.5 ratios) on the fabrication of collagen nanofibrous sheets. The sheets formed nanofibers without beads in the 1:1.5 solvent at 30% RH. The wettability of the crosslinked sheets was roughness-dependent. Wettability increased with ethanol content at 30% RH but decreased at 60% RH. The stability of the collagen structure increased with higher ethanol content, and nanofibers in the 1:1.5 solvent showed full recovery of the diffraction pattern of pristine collagen after crosslinking. Isolated adipose tissue-derived stem cells (ADSCs) cultured on nanofibrous sheets showed good attachment at 1 week. However, because of nanofiber degradation, attachment was non-existent (1:1) or reduced (1:1.3) at 60% RH at 8 weeks. These results demonstrate that ethanol content influences the structural stability of collagen and that both humidity and ethanol content affect the morphological characteristics of collagen nanofibers and cell attachment to nanofibrous sheets.

1. Introduction

Nanofibers are useful tissue engineering scaffolds since they closely mimic natural extracellular matrix [1,2]. Electrospinning is a simple but versatile technique for fabricating nanofibers [3]; diverse natural and synthetic polymers have been used for this purpose [1,4,5]. As a natural polymer, collagen has several advantages such as biocompatibility, degradability, and low antigenicity [6,7]. However, the solvents used in the electrospinning process such as 1,1,1,3,3,3,-hexafluoro-2-propanol, 2,2,2,-trifluoroethanol, and acetic acid are toxic and lead to the instability of collagen, thus limiting its clinical potential as a fibrous matrix [8,9].

A recent study reported the electrospinning of collagen nanofibers with the environmentally benign solvents phosphate-buffered saline (PBS) and ethanol [10]. Using this solvent system, another study investigated the effects of factors such as collagen concentration, salt and ethanol compositions, and humidity level on the fabrication of optimal nanofibers [11]. However, studies on benign solvent systems are in the early stage, and the relationship between solvent composition and collagen structure of nanofibers and the ability of cells to attach to nanofibrous sheets under different conditions remain unclear.

We addressed these issues by examining the effect of solvent composition and humidity on the fabrication of collagen nanofibrous sheets, particularly with regards to their wettability and fiber morphology. The influence of ethanol content on the structural characteristics of the collagen nanofibers was evaluated by comparisons to isolated collagen. Finally, the combined effects of humidity level and ethanol content of the solvent on cell attachment were assessed.

2. Materials and methods

2.1. Materials

Type I collagen was isolated by pepsin-acetic acid treatment (Supporting information). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of nanofibrous sheets

Homogenous collagen solutions (8% w/v collagen in 20 × PBS/ethanol mixed at ratios of 1:1, 1:1.3, and 1:1.5) were vigorously vortexed for 3 min. After centrifugation (3000 × *g* for 3 min), the supernatant was transferred to a 10-ml syringe with a 21-gauge

* Corresponding author at: Department of Orthopaedic Surgery, Severance Children's Hospital, Yonsei University College of Medicine, 50–1 Yonsei-ro, Seodaemun-gu, Seoul 120–752, Republic of Korea.
E-mail address: pedhkim@yuhs.ac (H.W. Kim).

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blunt needle. The collagen solution was ejected from the syringe pump at a feed rate of 0.3 ml/h; a voltage of 18 kV was applied using a high-voltage power supply, and the needle-to-collector distance was fixed at 15 cm. The nanofibrous sheet fabricated on a cover glass (18×18 mm^2) was placed on a rotating stainless steel collector. The resultant was withdrawn and immersed in 50 mM EDC ethanol solution (95 vol%) for 24 h at room temperature for crosslinking. After then, nanofibrous sheet on cover glass was washed with DW to remove residual EDC. Electrospinning processes were carried out under the following conditions: 30% and 60% relative humidity (RH) at 25 °C. Humidity was controlled using a dehumidifier and air conditioner and continuously monitored using a 608-H1 thermohygrometer (Testo, Alton, UK) with an accuracy of 3% for RH and 0.5 °C for temperature.

2.3. Characterization of nanofibrous sheets

Nanofiber morphology was characterized using a field emission scanning electron microscope (JEOL-7001F; JEOL, Tokyo, Japan). Nanofiber diameter was measured from micrographs using ImageJ software (National Institutes of Health, Bethesda, MD, USA); at least 20 points were assessed. The contact angle of deposited ultrapure water droplets on the nanofibrous sheets was measured using a contact angle meter (CAM-200; KSV Instruments, Helsinki, Finland). Three measurements were made for each specimen. The structural characteristics of isolated collagen and its nanofibers were analyzed by Raman spectroscopy and X-ray diffraction (XRD). Raman spectroscopy (LabRam ARAMIS; Horiba Jobin Yvon, Edison, NJ, USA) was carried out using 532-nm laser at a power of 50 mW with an exposure time of 10 s. The spectrum was analyzed after baseline correction using Essential Fourier Transform Infrared software (Madison, WI, USA). The high-resolution XRD system (Rigaku, Tokyo, Japan) was operated at 45 kV and 200 mA using CuKα radiation ($\lambda = 0.15418$ nm).

2.4. Cell studies

Each of the crosslinked nanofibrous sheets was sterilized with 70 vol% ethanol for 30 min and 40 − W ultraviolet lamps for 20 min. Each sheet was then washed with deionized water and 1 × PBS, and incubated for 1 day in 1x PBS. Before cell seeding, sheets were rinsed with conditioned medium (Supporting information). Adipose tissue-derived stem cells (ADSCs) were seeded on each sheet at a final concentration of 1×10^6 cells.

3. Results and discussion

In a 1:1 solvent (referred to as 1:1), beads were observed on the nanofibers under both RH conditions [Fig. 1(a)]. The 1:1.3 and 1:1.5 groups showed beaded fibers at 60% RH, but scarce (1:1.3) or no (1:1.5) beads at 30% RH. The average fiber diameter increased with increased ethanol content [Fig. 1(b)], indicating that average diameter and potential for bead formation are influenced by ethanol content. A decrease in ethanol content induces a repulsive force at the surface of the electrospun jet, resulting in decreased fiber diameter. On the other hand, an increased proportion of salt decreases viscosity, thereby accelerating bead formation. At high humidity, fiber diameter decreases; the slow solidification rate enables the charged jet to elongate and reduces the repulsion of charges on the jet surface, and the resultant capillary instability leads to the formation of thin fibers with beads [11,12].

Crosslinked sheets had melted fibers and surface protrusion differed between 30% and 60% RH [Fig. 1(c)]. Crosslinked nanofibrous sheets showed opposite trends for contact angle at different humidity levels (Table 1). At 30% RH, a larger fiber diameter was associated with increased roughness, with beads contributing less to roughness at this humidity. Consequently, the wettability of nanofibrous sheets increased at higher ethanol content, whereas

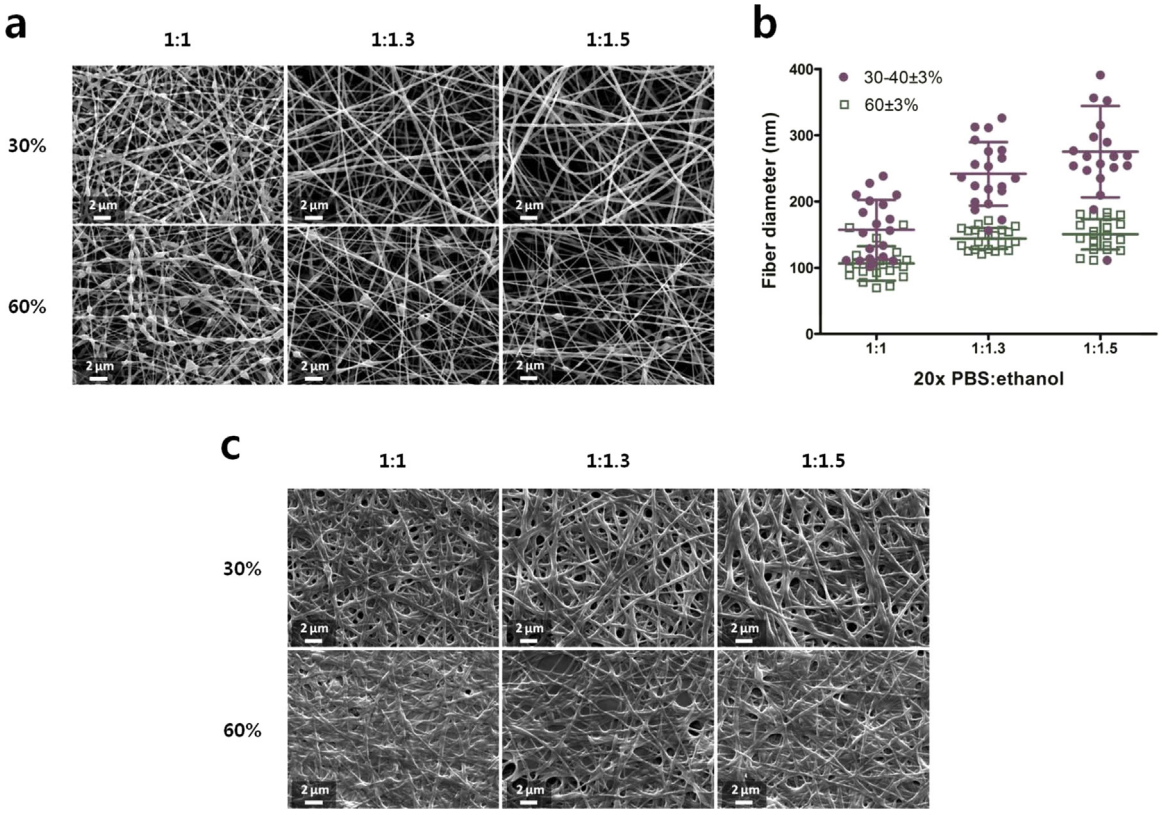

Fig. 1. (a) Morphology of non-crosslinked nanofibrous sheets. (b) Distribution of fiber diameter. (c) Morphology of crosslinked nanofibrous sheets.