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Differences in cytocompatibility between collagen, gelatin and keratin

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ABSTRACT

Keratins are cysteine-rich intermediate filament proteins found in the cytoskeleton of the epithelial cells and in the matrix of hair, feathers, wool, nails and horns. The natural abundance of cell adhesion sequences, RGD (Arg-Gly-Asp) and LDV (Leu-Asp-Val), makes them suitable for tissue engineering applications. The purpose of our study is to evaluate their cytocompatibility as compared to well-known collagen and gelatin proteins. Herein, collagen, gelatin and keratin were blended with poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) and electrospun to afford nanofibrous mats, respectively. These PHBV/protein composite mats were characterized by field emission scanning electron microscopy (FE-SEM), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), and dynamic mechanical analysis (DMA). The cytocompatibility was evaluated with cell adhesion, cell viability and cell proliferation. The data from MTT and BrDU revealed that collagen had significantly superior cytocompatibility as compared to gelatin and keratin. Gelatin showed a better cytocompatibility than keratin without statistical significance difference. Finally, we gave the reasons to account for the above conclusions.

1. Introduction

Tissue engineering (TE) is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function [1]. Scaffolds for TE play a pivotal role because they guide cells to grow, synthesize extracellular matrix and other biological molecules, and facilitate the formation of functional tissues and organs. There are several requirements in the design of scaffolds for tissue engineering. Many of these requirements are complex and not yet fully understood. In addition to being biocompatible both in bulk and degraded form, these scaffolds should possess appropriate mechanical properties to provide the correct stress environment for the neotissues. Also, the scaffolds should be porous and permeable to permit the ingress of cells and nutrients, and should exhibit the appropriate surface structure and chemistry for cell attachment. From those aspects, they should positively interact with cells, including enhanced cell adhesion, growth, migration, and differentiated function. Natural polymers, especially for proteins, have been used for tissue engineering applications. Proteins are advantageous in that they contain information (for example, particular amino acid sequences) that facilitates cell attachment or maintenance of differentiated function. Among them, collagen is one of the key structural proteins found in the extracellular matrices of many connective tissues in mammals, making up about 25% to 35% of the whole-body protein content [2]. Gelatin, the denatured form of collagen, has almost identical composition and biological properties as those of the parent collagen. Much attention has been focused on the use of gelatin as a tissue engineering material due to its low cost [3,4].

Keratins are cysteine-rich intermediate filament proteins found in the cytoskeleton of the epithelial cells and in the matrix of hair, feathers, wool, nails and horns. The natural abundance of cell adhesion sequences, RGD (Arg-Gly-Asp) and LDV (Leu-Asp-Val), makes them suitable as a biomaterial for tissue engineering applications [5]. Keratins can be utilized as wound dressing [6], scaffold for tissue engineering [7], coating for implantable devices [8,9] and cell encapsulant [10]. However, keratins are extremely insoluble due to their extensive cross-linking and high hydrophobic residue content. Various attempts have been made to extract keratin based on oxidization [11], reduction [12–16], and sulfitolysis [17].

Electrospinning has been recognized as an efficient technique for the fabrication of polymer nanofiber mats [18]. The key features of these mats are: high porosity which makes them oxygen permeable, a high surface to volume ratio and most important, a similar morphology to the natural extracellular matrix (ECM) of skin, which promotes cell adhesion, migration and proliferation.

In our previous work, keratins were coelectrospun with PHBV [19,20] and PLA [21], respectively. These mats could accelerate cell adhesion and proliferation. The purpose of our study was to assess their cytocompatibility as compared to well-known collagen and gelatin proteins. Herein, proteins of collagen, gelatin and keratin were co-electrospun with PHBV at the ratio of 7 and 3 to produce PHBV/protein composite nanofibrous scaffolds, respectively. The characteristics of nanofibrous scaffolds were examined using field emission scanning electron microscopy (FE-SEM), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS),

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Table 1
Electrospinning conditions for different polymer solutions.

	Concentration (wt.%)	Voltage (kV)	Distance[a] (cm)	Flow rate (ml/h)
PHBV	6	7	15	1.5
PHBV-Col(7/3)	6	12	22	1
PHBV-Gel(7/3)	6	7	12	2
PHBV-Ker(7/3)	6	10	15	1

[a] The distance is between the spinneret and collector.

and dynamic mechanical analysis (DMA). The cytocompatibility of PHBV/protein mats was measured using MTT and BrDU assay.

2. Materials and methods

2.1. Materials

Keratins were extracted from raw materials (MP Biomedical Company, Germany) and modified as reported [21]. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate). (PHBV, PHV content: 5%), gelatin (bovine skin, type B powder), collagen (calf skin, type I powder), 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) and dialysis bags (MWCO 12,000–14,000) were purchased from the Sigma-Aldrich Chemical Co (MO, USA).

2.2. Fabrication of nanofibrous mats by electrospinning

The transparent polymer solution for electrospinning was obtained by dissolving protein and PHBV in HFIP with sufficient stirring at room temperature. The blended solution was delivered to a metal needle (18 G) connected to a high voltage power supply. In this study, the typical parameters of electrospinning were listed in Table 1. To maintain good mechanical property, the ratio of the mass of PHBV to that of keratin was fixed at the rate of 7 to 3.

2.3. Surface characterization

The morphology of the electrospun fibers was observed by a field-emission scanning electron microscope (FE-SEM, Hitachi S-4300, Japan). ATR-FTIR measurements were performed on a Nicolet 170sx Fourier transform infrared spectrometer (USA), coupled with ATR accessory. XPS spectra were obtained on an ESCALab MK II (V. G. Scientific Co. Ltd., UK) spectrometer using Al K_α radiation. The binding energy was referenced by setting the C_{1s} hydrocarbon peak to 285 eV.

2.4. Dynamic mechanical analysis

The mechanical properties of nanofiber mats were tested using a TA Instruments dynamic mechanical analyzer (DMA) Q800. For DMA test, the mats were cut along the nanofiber winding direction with a typical size of 20 mm (length) × 6 mm (width). The DMA multistress mode and strain sweep were used. From the stress–strain curve, the slope was calculated as elastic modulus. The test temperature was 20 °C for all the experiments.

2.5. Cell attachment and spreading [22,23]

In order to examine the interaction of nanofibers with cells, circular nanofibrous sheets were fitted in a 24-well culture plate and subsequently immersed in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco) and 1% penicillin G-streptomycin. To seed the cells, 300 μl of NIH 3T3 cell solution (5×10^4 cells/cm^{-2}) was added and incubated in a humidified atmosphere of 5% CO_2 at 37 °C. After incubation for 4 h, the medium solution was removed. These mats were rinsed twice with PBS, and fixed by 2.5% glutaraldehyde aqueous solution for 20 min. The sample sheets were then dehydrated in a graded concentration of ethanol (25, 50, 75, 90 and 100%) for 10 min each. Finally, the sample sheets were air dried in

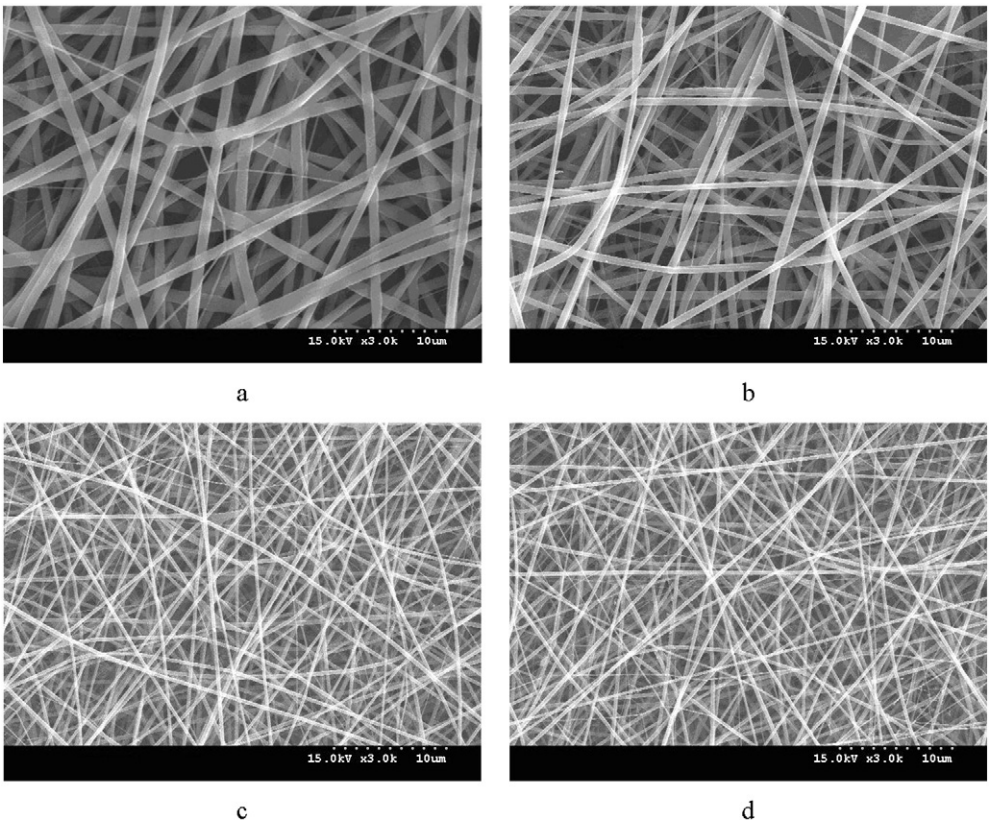

Fig. 1. SEM micrographs of electrospun fibers of PHBV (a), PHBV/keratin = 7/3 (b), PHBV/gelatin = 7/3 (c), and PHBV/collagen = 7/3 (d).