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The effect of elastic biodegradable polyurethane electrospun nanofibers on the differentiation of mesenchymal stem cells

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ABSTRACT

Biodegradable polyurethane (PU) was synthesized based on using poly(ε-caprolactone) (PCL) as the soft segment. Fibers in different diameters (200–400 nm, 600–800 nm, and 1.4–1.6 μm) were then made by electrospinning PU solution in N,N-dimethylacetamide and 2,2,2-trifluoroethanol. Human bone marrow derived mesenchymal stem cells (hMSCs) in the form of single dispersed cells or aggregates were seeded on the electrospun meshes for evaluation of cell behavior. Differentiation experiments showed that hMSC aggregates on electrospun fibers had greater differentiation capacities than single cells. Besides, nanofibers of 200–400 nm diameters significantly promoted the osteogenic and chondrogenic differentiation of hMSCs than fibers of the other diameters. The effect of substrate elasticity was further elucidated by comparing cell behaviors on the nanofibers of PCL-based PU and those of pure PCL. The more elastic PU nanofibers demonstrated more osteogenic and chondrogenic induction potential than PCL electrospun fibers. We suggested that the elastic nanofibers seeded with hMSC aggregates may be advantageous for cartilage and bone tissue engineering.

1. Introduction

Mesenchymal stem cells (MSCs) are a promising cell source for tissue engineering applications mainly because of its multipotency to differentiate into various cell types such as osteoblasts [1], adipocytes [2], chondrocytes [3], myocytes [4] and neural cells [5]. Their fate is greatly influenced by both intrinsic and extrinsic signals, including cell–cell and cell–substrate interactions, the gradients of oxygen, nutrient, and protein concentrations, as well as the mechanical properties of the substrate [6]. Biological responses of stem cells could be quite different in two-dimensional (2D) and three-dimensional (3D) environments [7]. The traditional 2D culture method tends to separate single cells from their neighbors, which does not resemble the real in vivo situation. The 3D cell culture involves the generation of cellular aggregates or the growth of cells on 3D substrates ("scaffolds"), which may improve the cell–cell communication and differentiation potential of stem cells [8].

Electrospinning, a fast and simple polymer processing technique, is not only capable of producing a wide variety of polymeric nanofibers, but also able to control the sizes, shapes, and porosity to achieve desired products [9]. Due to the above advantages, electrospinning is widely used in fabricating 3D scaffolds for regeneration of tissues such as blood vessel [10], bone [11], nerve [12], cartilage [13], skin [14], heart [15], and ligament [16]. Recent studies have shown that the adhesion, migration, proliferation, and differentiation of stem cells could be influenced by the topography [17], porosity [18], and mechanical properties of the substrate materials [19]. In particular, nanofibers that simulate the fibrillar structure of natural native extracellular matrix (ECM) can improve the differentiation efficiency of stem cells [20]. Electrospun substrates with high porosities or large pore sizes may have better proliferation capacities because cells can grow into the substrates [21]. Moreover, the osteogenesis of stem cells could be achieved by increasing the hardness of the matrix, while chondrogenesis could be induced by the softness of the substrate [22]. An ideal scaffold should mimic both the biomechanical properties and architecture of a natural tissue. Therefore, making nanofibrous scaffolds from elastic polymers such as polyurethane (PU) could be a potential approach for tissue engineering [23–25].

Cellular aggregates of MSCs have received much attention recently for their possible applications in tissue engineering

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[26–29]. The behavior of MSC aggregates on electrospun 3D scaffolds remains unclear. There is no direct evidence that the differentiation capacity of aggregates after spreading on a fibrous matrix can still maintain their advantages over single (dispersed) cells on the same substrate. In this study, we first synthesized biodegradable polyurethane (PU) elastomers. Elastic fibers in nano- to micro-scale were then fabricated by electrospinning. The influence of topography and mechanical properties of different fibrous scaffolds on cell adhesion, proliferation, and differentiation potentials of MSC aggregates vs. single cells was investigated. Specifically, we sought to optimize the combination of electrospun fibers and MSC aggregates for cartilage and bone tissue engineering.

2. Materials and methods

2.1. PU synthesis

The biodegradable PU used in this study was synthesized by a green process. Isophorone diisocyanate (IPDI, Evonik Degussa GmbH) was reacted with stirred (180 rpm) poly(ε-caprolactone) diol (PCL diol, molecular weight ~2 kDa, Sigma) under nitrogen for 3 h at 75 °C in the presence of 0.03% stannous octoate ($Sn(Oct)_2$, Alfa Aesar) as the catalyst. After prepolymerization, 2,2-bis(hydroxymethyl) propionic acid (DMPA, Sigma) and an appropriate amount (13 mL) of methyl ethyl ketone (MEK, J.T. Baker) were added to the reactor. After stirring for 1 h (180 rpm), the reaction temperature was then dropped to 50 °C. Neutralization was completed by adding triethylamine (TEA, R.D.H) into the reactor. The neutralized prepolymer was then dispersed in deionized water (110 mL) and stirred vigorously at 1100 rpm before ethylenediamine (EDA, Tedia) in 110 mL of deionized water was further added with continuing stirring to complete the reaction. The stoichiometric ratio used for synthesis was optimized as IPDI/PCL diol/DMPA/EDA/TEA = 3.52:1:1:1.52:1 [30]. The solvent was removed to obtain the solid form of biodegradable PU. The molecular weight defined by gel permeation chromatography was ~160 kDa. The weight percent of PCL segment in the final polymer was ~65%. On the other hand, the pure PCL polymer was purchased (molecular weight 70–90 kDa, Sigma) and used as received.

2.2. Electrospinning process

The setup of the electrospinning apparatus was constructed from a high-voltage power supplier (You-Shang Technical Corp., Taiwan), a syringe pump (KDS-100, KD Scientific, USA), a syringe with a stainless steel blunt-ended needle (20G, Terumo, Japan), and a plate type collector (Fig. 1(a)). The electrospinning was conducted in a custom-designed transparent acrylic chamber equipped with a hygrometer and a thermometer. The local humidity (40%) was controlled by a desiccant (silica gel). The chamber was placed in a humidity/temperature controlled room. In order to narrow down the distribution of fiber diameters, the mixed solvent system of a low-boiling point solvent 2,2,2-trifluoroethanol (TFE, Tedia; bp. 78 °C) and a high-boiling point solvent N,N-dimethylacetamide (DMAc, Tedia; bp. 165 °C) was used. PU was dissolved at the concentration of 6 wt% in the mixture of TFE and DMAc with different mixing ratios and stirred for overnight. The polymer solution was then poured into the syringe and drawn to the collecting plate by applying a high voltage. The flow rate, the applied voltage, and the distance between the nozzle and the collector could be adjusted. The temperature and the relative humidity were controlled at 21.5 ± 0.5 °C and 40 ± 4%.

After optimization, PU (5, 8, and 11 wt%) in a mixed solvent (wt% 60:40) was used to obtain electrospun membranes comprising of nanofibers ("ES1" groups), sub-microfibers ("ES2" groups), and microfibers ("ES3" groups). The relative humidity and temperature were controlled as mentioned. Other electrospinning parameters

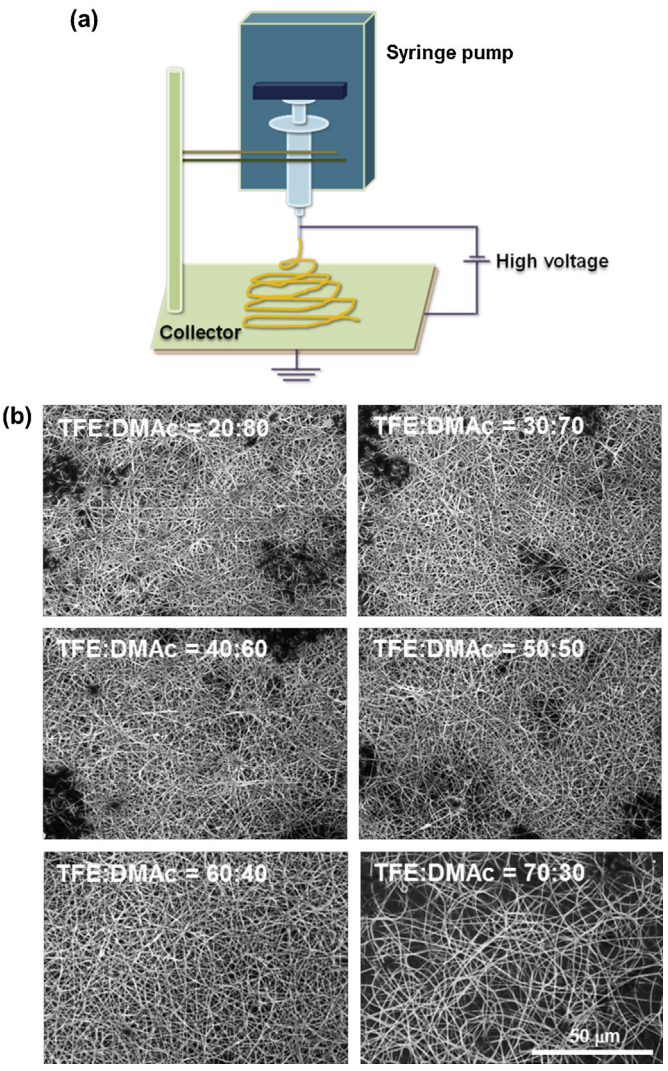

Fig. 1. (a) The electrospinning apparatus. (b) The effect of mixed solvent (TFE/DMAc) ratio on the morphology of electrospun fibers.

such as the flow rate, the applied voltage, and the distance between nozzle and collector were varied in the ranges of 0.3–0.6 mL/h, 10–15 kV, and 15–32 cm, respectively. Electrospun meshes for cell studies were spun onto the PU-coated coverslip glass, while those for physico-chemical characterization were spun onto glass slides and then removed by immersion into ice water. The PU-coated coverslip glass was prepared by spin coating 3% solution in TFE onto 15 mm diameter coverslip glass (Assistant, Glaswarenfabrik Karl Hecht KG, Germany) at 2750 rpm for 20 s and dried in vacuum for 1 day, which also served as the control ("PU film" groups). PCL electrospun meshes ("PCL-ES" groups) were prepared for comparison. For the purpose, the solution of 10 wt% PCL in TFE was spun onto PCL-coated coverslip glass at a voltage of 20 kV, a flow rate of 0.3 mL/h, and a distance of 32 cm. The relative humidity and temperature were controlled as mentioned. The PCL-coated coverslip glass was prepared similarly to that of PU by 5% solution of PCL in TFE. All electrospun meshes were dried in vacuum for 2 days to remove residual solvent.

2.3. Characterization of electrospun fibers

The electrospun meshes were examined by a scanning electron microscope (SEM, Hitachi S-4800) at an accelerating voltage