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3D multi-layered fibrous cellulose structure using an electrohydrodynamic process for tissue engineering

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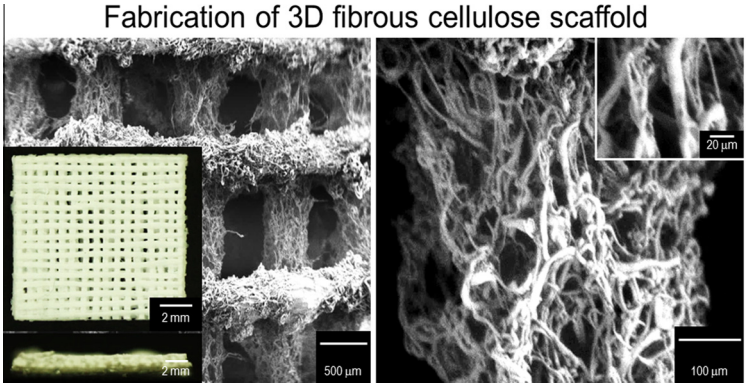
GRAPHICAL ABSTRACT



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ABSTRACT

Micro/nanofibrous structures have been applied widely in various tissue-engineering applications because the topological structures are similar to the extracellular matrix (ECM), which encourages a high degree of cell adhesion and growth. However, it has been difficult to produce a three-dimensional (3D) fibrous structure using controllable macro-pores. Recently, cellulose has been considered a high-potential natural-origin biomaterial, but its use in 3D biomedical structures has been limited due to its narrow processing window. Here, we suggest a new 3D cellulose scaffold consisting of multi-layered struts made of submicron-sized entangled fibers that were fabricated using an electrohydrodynamic direct jet (EHDJ) process that is spin-printing. By optimizing processing conditions (electric field strength, cellulose feeding rate, and distance between nozzle and target), we can achieve a multi-layered cellulose structure consisting of the cylindrically entangled cellulose fibers. To compare the properties of the fabricated 3D cellulose structure, we used a PCL fibrous scaffold, which has a similar fibrous morphology and pore geometry, as a control. The physical and *in vitro* biocompatibilities of both fibrous scaffolds were assessed using human dermal fibroblasts, and the cellulose structure showed higher cell adhesion and metabolic activities compared with the control. These results suggest the EHDJ process to be an effective fabricating tool for tissue engineering and the cellulose scaffold has high potential as a tissue regenerative material.

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1. Introduction

Scaffold designs for regenerative medicine have been changed noticeably by the development of three-dimensional (3D) manufacturing technology [1]. Recently, 3D printing techniques have enabled the fabrication of accurate and highly reproducible 3D biomedical scaffolds having controllable inner micro-pore structures (pore size, porosity, tortuosity) [2–5]. However, some biomaterials, including natural biopolymers, have limitations in achieving 3D controllable pore structures due to their poor processability. In addition, although 3D printing techniques for designing macroscale structures may be near perfect, the controllability of the range from nano- to microscale structures remains limited. However, recently, the design of biomedical scaffolds has expanded the controllability of internal structures into the nano/micro-scale region. According to Bari et al., promising scaffold structures to regulate behaviors, such as proliferation, cell fate, differentiation, and apoptosis, of stem cells can be a combinational structure consisting of micro- and nanoscale features [6]. Also, a new innovative structure has been suggested by Kumar et al. [7]. They recommended a new ideal model of a biomedical scaffold for tissue regeneration, which consisted of multi-layered struts, but the microsized struts could be made up of bundled nanofibers, which can induce considerably greater cell adhesion and proliferation.

Recently, various electrospinning processes have been studied. Edirisinghe et al. used a gyration process to develop nanosized fibers [8] and also Wang et al. produced beads using self-assembly-driven electrospinning method [9]. Jayasinghe et al. developed EHD printing process in order to obtain three dimensional scaffolds [10]. Moreover, Hashimdeen et al. was able to fabricate 3D structures using EHD process connected to a 3D robot system [11]. However, although these EHD-based processes have introduced new 2D and 3D fibrous structures, those cannot produce realistic 3D fibrous macropore structures. To achieve an 'ideal' 3D scaffold, we previously established a new system configured with an electrohydrodynamic direct jetting (EHDJ) process and an ethanol target bath [12,13]. Generally, EHDJ process uses a fine jet obtained from the apex of a liquid cone and this process has various printing modes including cone-jet mode, multi-jet mode, and micro-dripping mode [14–16]. Each mode has certain levels of feeding rate of solution and applied electric field within which it happens [16,17]. With the cone-jet mode of the process, we investigated various processing parameters (the effect of target media, electric field strength, flow rate of feeding solution, weight fraction of poly(ε-caprolactone) (PCL)) to fabricate a PCL scaffold consisting of multilayered fibrous bundles and macro-scale pores. Using the optimized processing parameters, we achieved a structure close to the ideal scaffold suggested by Kumar et al. [7]. The PCL scaffold showed significantly higher cellular activities with MC3T3-E1 cells (~threefold greater adhesion and ~twofold greater proliferation) compared with a scaffold fabricated using a 'pure' 3D printing process. In this study, we expand the technology to a natural biopolymer, which cannot be fabricated readily using a 3D printing process.

Cellulose is the most abundant biopolymer in nature; it is a linear homo-polymer of glucose with a repeating unit ranging from 500 to 5000. Due to its low water solubility, minimal foreign body reactions, and low inflammatory responses [18], cellulose has been used widely in tissue-regeneration applications, such as bone regeneration [19], cartilage regeneration [20], and connective tissue formation [21]. Generally, the most widely used method for fabricating nanofibrous structures of cellulose is an electrospinning process, but this method does not produce 3D fibrous structures, with the exception of accumulation of a stacked fibrous mat, resulting in critically low controllability of macro-pores over 100 μm [22]. Additionally, cellulose has a high melting temperature (306 °C) that is very close to its decomposition temperature (315 °C), so it cannot be processed readily by a 3D melt-printing method to obtain a controllable macro-pore structure.

In general, 3D scaffolds are recommended in regenerative medicine and tissue engineering because the ideal tissue engineered scaffold would provide the same or similar micro-environmental function to the seeded cells as that of a native ECM [23]. As a tissue-engineered scaffold, electrospinning used to fabricate two-dimensional (2D) fibrous meshes. But the 2D fiber mats cannot generate the whole 3D cellular morphology and cell differentiation compared to 3D fibrous structures [24]. Therefore, the 3D electrospun cellulose acetate scaffold has been fabricated to provide ECM-mimicking environment in order to enhance cellular activity. It is known that the macro-pores and pore-interconnectivity within scaffolds are prerequisites in scaffold designs because they enable cells to migrate and grow efficiently and provide various pathways for nutrients and metabolic waste. For these reasons, 100% pore-interconnectivity and appropriate macro-pores (size: 100–500 μm) have been recommended in scaffolds for regenerating various tissues [25].

Here, to obtain a 3D fibrous structure of cellulose having a highly porous and controllable pore structure, we used the EHDJ process. To achieve a multi-layered cellulose structure consisting of fibrous bundles, we investigated the effects of various fabrication factors, such as feeding solution flow rate, electric field strength, and height of target solution (ethanol) in the bath, on the processing ability to evaluate the resulting cellulose structure. The cellulose fibrous scaffolds were analyzed in terms of not only their topological and physical properties—including surface morphology using scanning electron microscopy (SEM) and water uptake ability—but also their biological activities—such as protein absorption, cell-seeding efficiency, and cell metabolic activity—using human dermal fibroblasts (HDFs) compared with a PCL fibrous scaffold with a similar pore structure as a control [12], also fabricated using the EHDJ process.

2. Experimental

2.1. Materials

Cellulose acetate (lot # MKBR3194V, density = 1.3 g/cm^3, M_n = 30,000 g/mol) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). A 20-weight fraction of the cellulose in a 50:50 mixed solvent of acetone (surface tension: 24 mN/m) and dimethylformamide (DMF; surface tension: 37.1 mN/m) (Junsei Chemical Co., Tokyo, Japan) was used in the process. As a target solution, 99% ethanol (surface tension: 22.1 mN/m; Duksan, Korea) was used.

2.2. Fabrication of a 3D cellulose fibrous structure

Several electric fields (3.8–6.5 kV/cm) and flow rates (0.2–2.0 mL/h) were used to determine optimal conditions. The electric field system was connected to a three-axis robot moving system (DTR3-2210-T-SG, DASA Robot, South Korea). The speed of the nozzle connected to the robot was set to 5 mm s^{-1}. A pure ethanol solution was used as the target medium. After printing the fibrous bundles, they were washed with pure water and the bundles were freeze-dried (SFDSM06; Samwon, Busan, South Korea) at −76 °C for 2 days. In the process, the volume flow rate of the cellulose solution was manipulated using a syringe pump (KDS 230; KD Scientific, Holliston, MA, USA). Electric field strength was supplied by a power supply (SHV300RD-50K; Convertech,