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Role of scaffold mean pore size in meniscus regeneration

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ABSTRACT

Recently, meniscus tissue engineering offers a promising management for meniscus regeneration. Although rarely reported, the microarchitectures of scaffolds can deeply influence the behaviors of endogenous or exogenous stem/progenitor cells and subsequent tissue formation in meniscus tissue engineering. Herein, a series of three-dimensional (3D) poly(ε-caprolactone) (PCL) scaffolds with three distinct mean pore sizes (*i.e.*, 215, 320, and 515 μm) were fabricated *via* fused deposition modeling. The scaffold with the mean pore size of 215 μm significantly improved both the proliferation and extracellular matrix (ECM) production/deposition of mesenchymal stem cells compared to all other groups *in vitro*. Moreover, scaffolds with mean pore size of 215 μm exhibited the greatest tensile and compressive moduli in all the acellular and cellular studies. In addition, the relatively better results of fibrocartilaginous tissue formation and chondroprotection were observed in the 215 μm scaffold group after substituting the rabbit medial meniscectomy for 12 weeks. Overall, the mean pore size of 3D-printed PCL scaffold could affect cell behavior, ECM production, biomechanics, and repair effect significantly. The PCL scaffold with mean pore size of 215 μm presented superior results both *in vitro* and *in vivo*, which could be an alternative for meniscus tissue engineering.

Statement of Significance

Meniscus tissue engineering provides a promising strategy for meniscus regeneration. In this regard, the microarchitectures (*e.g.*, mean pore size) of scaffolds remarkably impact the behaviors of cells and subsequent tissue formation, which has been rarely reported. Herein, three three-dimensional poly(ε-caprolactone) scaffolds with different mean pore sizes (*i.e.*, 215, 320, and 515 μm) were fabricated *via* fused deposition modeling. The results suggested that the mean pore size significantly affected the behaviors of endogenous or exogenous stem/progenitor cells and subsequent tissue formation. This study furthers our understanding of the cell-scaffold interaction in meniscus tissue engineering, which provides unique insight into the design of meniscus scaffolds for future clinical application.

1. Introduction

Meniscus plays a crucial role in load absorption, lubrication, and maintaining stability of the knee joint. Meniscal tear is one of the most frequently recorded sports medicine injuries with mean incidence of 66/100,000 in the United States [1]. As a usual surgical option, either partial or total meniscectomy would increase the stress on articular cartilage, even leading to knee damage and osteoarthritis [2]. Various surgical attempts to repair the torn meniscus have been used but are only effective in the vascular zone and often associated with a re-rupture rate of 30% [3]. Meniscus tissue engineering, which aims to regenerate the damaged tissue, offers a potential solution strategy to facilitate traditional repair or meniscectomy [4]. However, there still remains an unmet need for optimal biomaterials with both appropriate framework and sufficient mechanical strength to support the behaviors of endogenous or exogenous stem/progenitor cells for regeneration of meniscus.

The microarchitectures of biomaterials have been shown to significantly affect the behaviors of cells both *in vitro* and *in vivo* [5,6].

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In particular, the mean pore size of scaffold can modulate cell-matrix interaction effectively [7,8]. It has been demonstrated that larger pores of scaffolds facilitate cell diffusion and migration, while smaller ones provide a higher surface area of scaffold for cell adhesion [9]. The subsequent cell proliferation, differentiation, and matrix deposition may contribute to the biochemical and mechanical properties of regenerated constructs [6,7]. Therefore, the understanding of mechanisms, by which scaffold architecture (*e.g.*, mean pore size) affects cell behavior and subsequent tissue formation, is a prerequisite for successful meniscus tissue engineering.

So far, few studies have investigated the optimal mean pore sizes of scaffolds for resembling tissue engineered meniscus [10,11]. As a typical example, Mandal et al. designed a multilayered silk scaffold composed of three individual layers with different pore sizes (*i.e.*, 350–400, 500–600, and 60–80 μm) and orientations. Although the implanted seed cells could migrate though the large pores and the smaller pores improved the deposition of extracellular matrix (ECM), the whole platform exhibited inferior mechanical property compared to native meniscus [11]. When generating the tissue engineered meniscus as a weight-bearing construct, attention should also be paid to the mechanical properties of the biomaterial itself, which interact with the microarchitecture [4,12].

In this context, we utilized fused deposition modeling (FDM) to fabricate a series of three-dimensional (3D) poly(ε-caprolactone) (PCL) scaffolds with three distinct mean pore sizes (*i.e.*, 215, 320, and 515 μm). The above mean pore sizes were selected based on the previously reported results that the selected ones have positive effects on tissue formation [7,12–14]. The purpose of the present research was to investigate the influences of various mean pore sizes of scaffold on (1) *in vitro* proliferation, differentiation, and matrix production and deposition of mesenchymal stem cells (MSCs), which has been widely used as the seed cells for meniscus tissue engineering; (2) *in vivo* tissue regeneration (*i.e.*, cellular infiltration, vascularization, and fibrocartilaginous tissue formation) and the chondroprotection in a rabbit model (Fig. 1). The results of present study might verify the effect of mean pore size on outcome of the 3D-printed PCL scaffold used for meniscus tissue engineering and further offer unique insight into meniscus regeneration.

2. Materials and methods

2.1. Fabrication of 3D PCL scaffolds

Medical grade PCL (number-average molecular weight (M_n) = 74,600 g mol^{-1} and melting point (MP) = 52.9 °C) was granted by Changchun SinoBiomaterials Co., Ltd. (Changchun, PR China). During the fabrication process, PCL was melted and extruded through a heated metal nozzle. The nozzle, which was controlled by computer-aided manufacturing software (Delta Tau Data Systems Inc., Chatsworth, CA, USA), could be moved both vertically and horizontally. As listed in Supplementary Table S1, the processing parameters were set to fabricate three distinct scaffolds with different mean pore sizes of 215, 320, and 515 μm, and a specification of 10 mm-diameter and 1.5 mm-thick. The surface morphologies of PCL scaffolds were observed under an S-4800 scanning electron microscope (SEM; Hitachi, Japan) operated at an acceleration voltage of 15.0 kV (Fig. S1A–C, Supplementary data). Mean pore sizes and road widths were measured with Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA; Supplementary Fig. S1D–I). Porosities were determined according to the previous reported approach [15]. The detail operation and equation were shown in Supplementary data.

2.2. Characterization of PCL scaffolds with various mean pore sizes

The surface wettability of all scaffolds was determined by measuring water contact angle (WCA) using the sessile drop method according to the previously reported protocol [16]. The degradation properties of scaffolds were assessed using mass loss ratio and water absorption ratio. The above values were calculated as shown in Supplementary data.

The surface area per unit volume (SA/V) was estimated using a previously described Eq. (1) [9]. It means a sufficiently high specific surface provided for a critical number of cells attached to the scaffold [7].

$$SA/V = \frac{3.65}{l} \times \sqrt{\frac{\rho^*}{\rho_s}} \quad (1)$$

In Eq. (1), l is the edge length of tetrakaidecahedron, ρ^* is the density of PCL scaffold, and ρ_s is the density of raw PCL. Zein et al. have demonstrated that FDM process did not result in a significant change of PCL molecular weight and crystallinity fraction [17], the relative density (*i.e.*, ρ^*/ρ_s) of PCL scaffold is 1. Based on the previous assumption that the pore diameter can be calculated from the edge length by $d = 2.78l$ [9], we calculated that SA/V of PCL scaffold used in this study is inversely related to the mean pore size (*i.e.*, d) by Eq. (2).

$$SA/V = \frac{10.15}{d} \quad (2)$$

2.3. Harvest, culture, and implantation of MSCs

All experimental protocols of animals were approved by the local Institutional Animal Care and Use Committee complied with the "Guide for the Care and Use of Laboratory Animals" published by the National Academy Press (NIH Publication No. 85-23, revised 1996). Bone marrow-derived MSCs were isolated from 3-month-old New Zealand White rabbits with average weight of approximately 3.0 kg. Isolation, cultivation, and tri-lineage differentiation potential assays of MSCs were performed according to a previously described protocol [18]. The tri-lineage differentiation potential results of MSCs were shown in Supplementary data. MSCs were seeded between the second and third passage using centrifugal method [19]. Briefly, the scaffold was placed at the bottom of 1.5 mL centrifugal tube, and 50.0 μL of concentrated cell solution (2.5×10^5 cells/scaffold) was added. The tube was centrifuged at 500 rpm for 1 min and then turned over as one loop. The process was repeated three consecutive times. For *in vitro* differentiation, the scaffolds were cultured under chemically defined Dulbecco's modified Eagle's medium (DMEM supplemented with 0.1 μM dexamethasone, 50.0 μg mL^{-1} ascorbate 2-phosphate, 40.0 μg mL^{-1} L-proline, 100.0 μg mL^{-1} sodium pyruvate, and its supplements (6.25 μg mL^{-1} insulin, 6.25 μg mL^{-1} transferrin, and 6.25 ng mL^{-1} selenous acid) with 10 ng mL^{-1} transforming growth factor-β3 (TGF-β3); Cyagen Biosciences Inc., Santa Clara, CA, USA).

2.4. Initial cell adhesion assessment

The initial cell adhesion in the scaffolds with different mean pore sizes was determined by initial adhesion number [16]. Briefly, MSCs seeded on different scaffolds were incubated for 1, 3, 5, or 7 h. At each time point, the cell-loaded scaffolds were washed with phosphate-buffered saline (PBS) and then incubated in 0.25% (*W*/*V*) trypsin-EDTA (EDTA, ethylenediaminetetraacetic acid; Gibco BRL Co. Ltd., Gaithersburg, MD, USA) for 10 min to detach cells from the scaffolds. Cell number was detected using an automated cell counter (Scepter™, Merck Millipore Co., USA).