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Fabrication and assembly of porous micropatterned scaffolds for modular tissue engineering

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ABSTRACT

In this paper, we present an efficient and simple approach to fabricate a novel microscaffold with high pattern fidelity and an open porous structure for modular tissue engineering. We characterized the microstructure, explored the formation mechanism, and evaluated the growth of cells in the microscaffold. Moreover, microscaffold blocks were assembled by surface tension-driven assembly to construct engineered tissue with controlled cell distribution and cell interactions. This microscaffold could be potentially applied as a building block to create 3D biomimetic engineered tissue.

1. Introduction

Despite the significant advances made in tissue engineering, traditional bulk scaffolds are characterized by drawbacks such as low initial cell seeding density and non-homogeneous cell distribution. Furthermore, precise control of the temporal and spatial distribution of cells to construct more sophisticated tissue is very difficult. Modular tissue engineering has recently shown promise, as it could address these challenges by using microscale design to construct modular tissues that can be used as building blocks to create larger tissues [1,2]. However, the studies reported thus far have primarily focused on microgels. Two major problems that plague the application of mirogels are the heavy physical constraint on cells and the limited diffusivity of bioactive molecules [3]. It is generally accepted that 3D porous scaffolds are more beneficial for bioactive molecule diffusion, cellular transportation, and ultimately tissue formation [4]. Therefore, developing porous microscaffolds is crucial for modular tissue engineering.

Methacrylated gelatin (GelMA), which can be easily patterned into microgels of different geometries through photolithography, has been extensively used in tissue engineering [5]. As a simple method, freeze-drying has been widely applied to generate porous scaffolds from hydrogels without porogens or organic solvents [6]. Nevertheless, it is difficult to obtain microscaffolds with high pattern accuracy directly by freeze-drying due to the swellability of hydrogels. Moreover, because the gelatin matrix is too soft to resist the interfacial tension caused by solvent evaporation, surface skin forms on the hydrogel after freeze-drying [7]. Therefore, these problems can be addressed by increasing the stiffness and decreasing the swellability of the matrix.

Herein, we report a simple approach for the fabrication of porous microscaffolds with high pattern fidelity. In this study, we introduced silk fibroin (SF) into the GelMA prepolymer to regulate the matrix stiffness and swelling property of the microgels. Patterned microscaffolds were then fabricated by lyophilization of microgels prepared by photolithography. Microscaffold assembly complexes with controlled cell distribution were ultimately achieved by surface tension-driven assembly.

2. Experimental

The GelMA prepolymer was synthesized as described previously [8], and the SF solution was extracted from silk fiber by lithium bromide. The entire manufacturing process of the microscaffolds is shown in Fig. 1. The fabrication process, characterization details and statistical analysis can be found in the Supporting Information (SI). For the cell experiments, 5 ml of human osteosarcoma (MG63) cell suspension containing 1×10^5 cells was seeded on the scaffold. After 1, 3 and 5 days of culture, the

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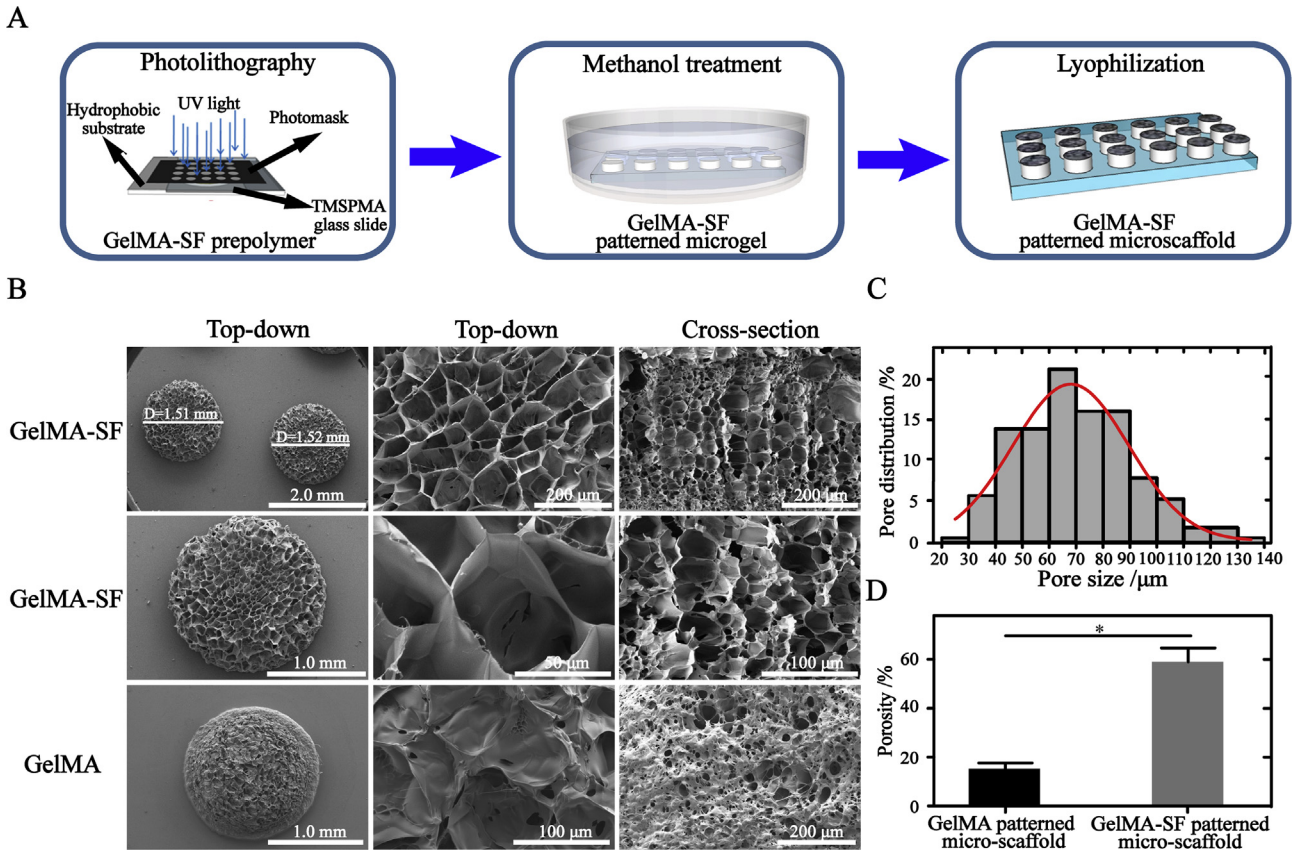

Fig. 1. Schematic of the process to fabricate the micropatterned scaffolds (A). SEM images of microscaffolds based on GelMA-SF and pure GelMA microgel (B). Pore size distribution (C) and percent porosity of the microscaffolds (D).

cellular viability and morphology were investigated. More details about the characterization are given in the SI.

After 1 day of cell culture, all the cell-laden microscaffolds were immersed in mineral oil. For 2D surface assembly, mechanical agitation using the tip of a pipette was employed to aggregate the individual microscaffolds to generate the assembly complex. For 3D sequential assembly, a syringe needle was swiped uniaxially against the linear array of microscaffolds on the glass slide. The microscaffolds were then packed. Afterwards, 6% GelMA solution was dropped on the assembly complex as an adhesive agent, and secondary photocrosslinking was conducted for 20 s to fix the microscaffold assembly.

3. Results and discussion

As shown in Fig. 1(B), the porous GelMA-SF microscaffolds demonstrated high pattern fidelity, as the diameter of the circular pattern of the photomask was 1500 μm (Fig. S1). All the GelMA-SF microscaffolds demonstrated an open porous structure. Cross-sectional images revealed interconnected porous channels in the microscaffolds that could support vascularization, nutrient diffusion, and nutrient exchange for 3D cell ingrowth. In contrast, the pure GelMA microscaffolds prepared by the same procedure appeared to be covered by a surface skin, and the morphology was deformed compared with the original photomask (Fig. 1(B)). The pore size distribution confirmed that the pore size of the microscaffolds ranged from 20 to 100 μm (Fig. 1(C)), which is a very suitable range for cellular ingrowth. The porosity of the resulting microscaffold was approximately 60%, which was significantly higher than that of the GelMA microscaffold ($P < 0.05$) (Fig. 1(D)).

Furthermore, to investigate the formation mechanism of the porous microscaffolds with high pattern fidelity, a fluorescent dye was used to observe the morphological changes during the preparation process. As shown in Fig. 2(A), before MeOH treatment, the microgel was in a swollen state, and the size of the gel was obviously increased. After treatment, the size of the microgel was reduced to the original size, and no significant dimensional changes were observed in the water. Fig. 2(B) reveals that after methanol treatment, the amide I and amide II bands of the hydrogel shifted to lower wavenumbers, indicating β-sheet structure formation. First, the crystalline β-sheet structure of SF can significantly reinforce the mechanical properties of the microgel ($P < 0.05$) by providing sufficient rigidity to counteract the surface tension and thus can prevent the formation of surface skin (Fig. 2(C)). Second, during the MeOH treatment process, excess water from the hydrogel was removed under the action of methanol dehydration, as revealed by decrease in the water content of the microgel (Fig. 2(D)); simultaneously, the MeOH solution promoted SF crystallization [9]. The resultant crystallized fragments can significantly reduce the swelling properties of the microgel due to their hydrophobic nature (Fig. 2(E)) [10]. Under the synergistic effect of these factors, porous microscaffolds with improved pattern fidelity could be fabricated by freeze-drying.

Next, the morphology of the MG-63 cells was investigated by CLSM and FESEM. The cells could quickly attach to the microscaffolds after 1 day of culture, and they continued to spread and proliferate for up to five days ($P < 0.05$) (Fig. 3(A)–(D)). Moreover, there were negligible dead cells throughout the incubation period. SEM images (Fig. 3(E)–(J)) revealed that the