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Microfluidic-based generation of functional microfibers for biomimetic complex tissue construction

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ABSTRACT

Microfluidic-based fiber system displays great potential in reconstructing naturally complex tissues. In these systems, fabrication of the basic fiber is a significant factor in ensuring a functional construction. The fiber should possess the strong mechanical rigidity for assembly, predefined microenvironment for cell spatial distribution and high biocompatibility for cell functional expression. Herein we presented a composite material by the combination of methacrylated gelatin (GelMA) and alginate for fiber engineering with capillary microfluidic device. Being regulated by GelMA incorporation, the composite hydrogels exhibited higher mechanical moduli, better stretching performance, and lower swelling compared to pure alginate one. On the basis of the composite material and capillary microfluidic device, we constructed the double-layer hollow microfibers to simulate complex tissues. The microfibers could be precisely controlled in size and multi-layered structure by varying flow rates and outlet diameter, and it showed satisfied application in woven-structure assembly. As an example to mimic a functional tissue, a biomimetic osteon-like structure was fabricated by encapsulating human umbilical vascular endothelial cells (HUVECs) in middle layer to imitate vascular vessel and human osteoblast-like cells (MG63) in the outer layer to act role of bone. During the incubation period, both MG63 and HUVECs exhibited not only a robust growth, but also up-regulated gene expression. These results demonstrated this microfluidic-based composite microfibers system is a promising alternative in complex tissue regeneration.

Statement of Significance

Cell-laden microfibers based on microfluidic device is attracting interest for reconstructing naturally complex tissues. One shortage is the lack of suitable materials which satisfy microfluidic fabrication and cell biofunctional survival. This study reports the first combination of alginate-GelMA composite and capillary-based microfluidic technology. The composite materials possess high mechanical properties for fabrication and assembly, and tunable environment for cell spatial encapsulation. Significantly, the engineered double-layer hollow microfiber with osteon-like structure showed enhanced cellular bioactivity and realized initially functional establishment. This microfluidic-based composite microfiber not only explores a competitive candidate in complex tissues reconstruction, but also expands the biological application of microfluidic technology. This developing interdisciplinary area should be widely interested to the readers of biofabrication, biomaterials and tissue engineering.

1. Introduction

Most natural tissues within human body are composed of complex building blocks, such as lobule in liver, muscular fibril in muscle, and osteon in cortical bone [1]. To date, many biomimetic scaffolds have been made to reconstruct these complex tissues by various bottom-up approaches, mostly including soft lithography, photolithography, three dimensional (3D) printing, and microfluidic technology [2–5].

Among these approaches, microfluidic-based hydrogel fiber has been remarkably developed and showed potential for complex

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tissue regeneration [6]. On one hand, hydrogels hold high content of physiological fluids and biologically mimic the natural extracellular matrix (ECM) [7]. On the other hand, owing to the laminar effect, the microfluidic device enables the formation of coaxial flow among multiple fluids without turbulence, and microfibers can be quickly produced by solidifying the prepolymer fluid using photopolymerization or ion crosslinking treatment. According to variable design of the microfluidic devices, this route has shown enormous potential to synthesize continuously polymeric fibers at microscale, provide fine control over fiber shape, size, chemical anisotropy, and biological activity, and realize 3D cell culture with well functional distribution [8]. For instance, Yao Cheng et al. presented a multiple laminar-flow microfluidic device for the scalable formation of microfibers with tunable, morphological, chemical, and biological features [9]. Moreover, these microfibers can be further utilized in textile fabrication to form high-order woven structures [5].

Although reasonable progresses have been achieved through the microfluidic technology, it is still a big challenge to prepare the fundamental material which has been recognized as the key factor to enable the functional construction [10]. Ideally, the microfibers should be prepared with the following attributes: fast gelation, proper mechanical properties for mechanical assembly, high biocompatibility for cell growth, and tunable microenvironment for specific cell partition to resemble cell distribution in native tissue.

As a prepolymer candidate, alginate is probably the most widely used one in creating microfibers and 3D construct because of its rapid ionic gelation (e.g., by the addition of calcium chloride, $CaCl_2$) as well as the high mechanical strength of its hydrogel [11,12]. However, the poor biocompatibility associated with alginate limits its biological application. Diverse cells, such as hepatocyte, fibroblast, endothelial cell, and islet cell, have been encapsulated in alginate fibers, and they could just maintain a low viability during the whole incubation [6,13,14]. To overcome this disadvantage, attempts to avoid the use of alginate for cell encapsulation are arising [5,15]. Typically, Onoe et al. fabricated a core-shell hydrogel microfibers with alginate as shell and ECM proteins containing cells as core to induce cell specific differentiation, and these microfibers could be further assembled into woven structure [5]. Nevertheless, with removal of the shell alginate, the remaining cell-ECM mixture core lacked sufficient mechanical strength to be further manipulated. As a photocrosslinkable material, methacrylated gelatin (GelMA) can quickly gel after a short time of ultraviolet exposure, and it is famous for its excellent biocompatibility as well as cost-efficiency. However, the weak mechanical rigidity of GelMA hydrogel greatly hinders its applications as an independent material for microfibers assembly [16,17]. Based on above descriptions, a proper combination of alginate and GelMA should be potentially exploited as the material for microfibers fabrication with both mechanical rigidity and cellular bioactivity.

Hence, we herein attempted to demonstrate the promising preparation of Alginate-GelMA composite for microfluidic application. In this composite material, gelation of alginate was supposed to generate a mainly supportive network, in the meantime, incorporation of GelMA aimed to offer the hydrogel an additional network. We expected that the incorporation of GelMA could not only complement the mechanical property of microfibers to fulfill the assembly, but also strengthen its biological activity to induce cell growth. Furthermore, by the design of the microfluidic device, we proposed to fabricate the microfibers with a double-layer hollow structure to mimic the simplified osteon which possessed a unique concentric double-ring conformation [18]. In addition, the feasibility of the engineered microfibers in constructing woven structures was investigated.

2. Materials and methods

2.1. Materials

Gelatin (Porcine Type A, Cat Nos. G2500), methacrylic anhydride, 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (I_{2959}), fluorescein diacetate (FDA), and propidium iodide (PI) were obtained from Sigma–Aldrich (USA). Sodium alginate was supplied by Wako (Osaka, Japan). Cylindrical glass capillary was purchased from Beijing Zhongcheng Quartz Glass Products Co. Ltd. Square glass capillary was obtained from Casix Inc. 5 min epoxy (glue) was provided by DEVCON (USA). Molecular probes (CM-DIL, CM-FDA), fluorescence microballoon were purchased from Invitrogen (USA). Unless otherwise stated, all other reagents were obtained from Chengdu Kelong Chem Co.

2.2. Alginate-GelMA composite prepolymer solution preparation

2.2.1. GelMA Synthesis

GelMA was synthesized as given in previous report [3]. In brief, 10 g of gelatin powder was first dissolved in 100 mL Dulbecco's Phosphate Buffered Saline (DPBS) under 50 °C, and then 10 mL of methacrylic anhydride was slowly added. The solution was stirred under 50 °C. After 3 h, 500 mL DPBS was added as a dilution, and the mixture was stirred under 50 °C for 15 min. Lastly the solution was dialyzed in a 12–14 kDa dialysis tube against distilled water for 1 week at 37 °C. The dialysis water was changed twice a day. The final solution was freeze-dried to get GelMA solid which was stored at room temperature.

2.2.2. Preparation of the composite solution

Firstly, alginate, I_{2959} and GelMA solid were together added into DPBS, and then the mixture was stirred under 60 °C until all the solid phases were fully dissolved. In this prepolymer solution, the concentration of alginate and I_{2959} was set at a constant value of 1.0 w/v% and 0.5 w/v%, respectively, and the concentration value for GelMA was selected at 5 w/v% and 10 w/v%. I_{2959} was used as a photoinitiator to induce the gelation of GelMA. As a control group, the prepolymer solution with 1/1.5 w/v% alginate alone was used. Here we named the four groups as A1.5G0, A1G0, A1G5, and A1G10 (Supplementary Table 1).

2.3. Fabrication and characterization of double-layer hollow Alginate-GelMA composite microfibers

2.3.1. Fabrication of microfibers in use of microfluidic device

As shown in Supplementary Fig. 1, the double coaxial laminar flow microfluidic device was comprised of glass capillaries and pinheads. Cylindrical glass capillaries (outer diameter: 1 mm; inner diameter: 550 μm) was pulled with a puller (PC-10, Narishige) to form thin tips (~120 μm in inner diameter for D_1; ~160 μm for D_2; 550–200 μm for D_3). Square glass capillaries (outer diameter: 1.4 mm; inner diameter: 1 mm) were used to connect two adjacent cylindrical capillaries. These glass capillary tubes were assembled with pinheads, and fixed with 5 min epoxy. All pinheads were connected to syringes with Teflon tubes, and the syringes were set to syringe pumps (Lange Baoding Co. Ltd, Hebei, China) to precisely control the flow rates.

To fabricate double-layer hollow microfibers, 2 w/v% hyaluronic acid (HA) was injected from 1st inlet while the alginate-based composite solution was pumped into 2nd and 3rd inlets. The outlet of the microfluidic device was immersed in 100 mM $CaCl_2$ solution. Due to the laminar effect, these three fluids formed coaxial flows in the glass capillary without turbulence, and the alginate-based fluids in 2nd and 3rd was quickly gelled when they reached the out-