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Research Paper

Degradation regulated bioactive hydrogel as the bioink with desirable moldability for microfluidic biofabrication

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ABSTRACT

Bioink development is vital in biofabriacation for generating three-dimensional (3D) tissue-like constructs. As potential candidates of bioinks, hydrogels need to meet the requirements of good moldability, initially strong mechanical properties and prominent bioactivity to guarantee cell vitality and further assembly. Enzyme-induced dynamic degradation is an efficient and biocompatible approach to improve the bioactivity of hydrogels through releasing space continuously for cell proliferation and promoting the functional establishing of engineered tissue. Here a novel bioink was designed by introducing alginate lyase into composite Alginate-GelMA hydrogels. Results showed that bioink with proper lyase content exhibited desirable modability and cytocompatibility. Then cell-laden osteon-like microfibers were engineered with the microfluidic device and diverse complex 3D constructs were also successfully assembled. This degradation-regulated bioink showed great promise in a variety of applications in tissue engineering and biomedical investigation.

1. Introduction

Biofabrication is emerging as an enabling tool to construct three-dimensional (3D) tissue-like structures composed of biomaterials and cells for generating the injured tissue or failing organs via a single manufacturing procedure (Fukuda et al., 2006; Mironov et al., 2009; Yanagawa, Sugiura, & Kanamori, 2016). Different from traditional top-down engineering methods, these bionic 3D constructs can be engineered with precise configuration and composition (Nakamura, Iwanaga, Henmi, Arai, & Nishiyama, 2010). In the last few decades great progress in biofabrication has been made, but challenges still exist to obstruct its development. The first is that fabrication process may be detrimental for cells. The second is the lack of desirable bioinks that not only support the handling during the fabrication but also promote cell growth and function expression from the cell compatibility point of view (Chung et al., 2013; Ferris et al., 2013; Malda et al., 2013).

The joint application of microfluidic technology and hydrogel is a possible way to address the two problems. Compared with other fabrication approaches, microfluidic technology shows distinct advantage of versatility, high throughput and cytocompatibility (Cheng et al., 2016; Domachuk et al., 2010; Haeberle & Zengerle, 2007). A vast number of biomaterials like multifunctional microfibers and microparticles have been successfully fabricated using microfluidic technology. On the other hand, hydrogels possess high-water content, unique means of cell encapsulation, diverse gelation means like ionic and ultraviolet crosslinking, as well as characters that resemble the native extracellular matrix, thus hold a great promise as potential candidate of bioinks (Annabi et al., 2014; Dan et al., 2012; Lewandowska-Łańcucka et al., 2016). Whereas, there are still severe challenges in hydrogel-based bioinks.

To meet the molding demand in fabrication, hydrogel firstly needs to be rapidly deposited or gelled with suitable mechanical strength (Chang, Boland, Williams, & Hoying, 2011). Besides, as a substrate for cell growth, hydrogel materials should exhibit good biological activity to promote cell survival, proliferation and differentiation (Ferris et al., 2013; Phuong Nguyen, Phuong Doan, Dang, Khoa Nguyen, & Quyen Tran, 2014). Nevertheless, most hydrogels cannot meet the two requirements simultaneously. For example, collagen or gelatin hydrogels are well-known for their cytocompatibility due to that they contain Arg-Gly-Asp (RGD), the cell-binding domains of extracellular matrix proteins that promote cells to adhere and survive. However, their in-situ gelation speed cannot satisfy the needs in fabrication process, and sometimes the initial mechanical strength are usually too soft to sustain their original structure (Baker et al., 2015; Brinkman et al., 2003; Yue et al., 2015). On the other hand, materials with superior mechanical properties such as alginate and polyethylene glycol are usually bioinert

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due to the lack of adhesive domains, which may restrain cells from survival (Fonseca et al., 2013; Sun et al., 2012). To overcome this dilemma, composite hydrogels are proposed to introduce biocompatible ingredient with adhesive sites into bioinert hydrogel materials that are mechanically strong (Zuo et al., 2016). To some extent, composite hydrogels with double or multiple networks could further strengthen its mechanical properties (Naficy, Kawakami, Sadegholvaad, Wakisaka, & Spinks, 2013).

However, it should be worth noting that cells cannot always exhibit ideal vitality even in the composite hydrogel with components that contain adhesive domains. This is because that cells encapsulated in 3D microenvironment require relatively soft matrix and continuously released space to facilitate cell migration and proliferation. Under the premise of sufficient initial strength for biofabrication, hydrogel matrix with sustainable degradation following the cell growth might produce space for cell migration and proliferation (Kloxin, Kasko, & Salinas, 2009). Hence dynamic degradation design of the bioink is indispensable. In fact, the cell itself encapsulated in the hydrogel could somewhat induce the degradation of the matrix slightly through its metabolism, but the speed might be too slow to supply enough space for normal cell proliferation (Lutolf et al., 2003). To realize the efficient and dynamic degradation of hydrogels, various strategies were adopted. April et al. grafted o-nitrobenzylether-based photodegradable monomer onto the PEG-bis-amine to form the degradable hydrogel under cytocompatible wavelengths irradiation (Kloxin, Tibbitt, & Anseth, 2010). While this chemical modification may leave harmful monomers for cells and be irrealizable to many natural derived polymers. Recently, it was verified that the application of specific enzymes was able to induce matrix degradation with little harm for cells. For example, collagenase was used to degrade collagen microspheres in alginate hydrogel to produce space for cell migration and spreading, suggesting enzyme-mediated degradation is a valid approach to dynamically control the biological activity of 3D matrix (Jun, Yuwono, Paramonov, Hartgerink, & Hartgerink, 2010; Zhong, Sun, Wei, Zhu, & Guo, 2014).

Therefore, through the introduction of enzyme-mediated degradation, herein we designed a desirable bioink with excellent mechanical strength and prominent bioactivity by compositing alginate and methacrylic anhydride modified gelatin (GelMA) together. Alginate was used due to its fast-ionic gelation and remarkable mechanical strength, showing highly match for microfluidic fabrication as bioinks. Gelatin could provide bioactive domains for supporting cells to adhere and proliferate. Alginate lysase was chosen to specifically digest the bioinert alginate through selectively cleaving the glucosidic bond (Wong, Preston, & Schiller, 2000), just as Fig. 1A illustrates. During the continuous degradation process, the photocrosslinked GelMA network can maintain the architecture of the hydrogel, and inner space can be produced due to the digestion of alginate network, thus facilitating cell migration, proliferation and function expression. The degradation, swelling ratio and compressive modulus of the composite materials with different concentration of alginate lyase were characterized. Biological activity was also studied via 3D cell encapsulation. Then this composite material system was applied to generate double-layer hollow microfibers that simulate the native osteon with the double coaxial laminar flow microfluidic device. Cell viability, morphology and relative gene expression are detected as well. At last, higher-ordered, larger constructs were assembled with the microfibers by varied means to prove the applicability of this approach in biofabrication.

2. Materials and methods

2.1. Materials

Alginate lyase, sodium alginate (229 kDa), gelatin (Porcine Type A, Cat Nos. G2500), methacrylic anhydride, photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2 methyl-1-propanone (Irgacure 2959), propidium iodide (PI), and fluorescein diacetate (FDA) were all purchased from Sigma–Aldrich (USA). Molecular probes (CM-DIL, CM − FDA), fluorescence microballoon were obtained from Invitrogen (USA). Unless specifically stated, other chemicals were acquired from Chengdu Kelong Chem Co. 5-min epoxy (glue) was obtained from DEVCON (USA). Cylindrical/square glass capillaries was supplied by Beijing Casix Inc. Syringe pumps were purchased from Lange Baoding Co. Ltd. (Hebei, China) and he UV-light source (OmnicureS1500) was purchased from EXFO Photonic Solutions Inc. (Ontario, Canada).

2.2. Preparation of the lyase-laden alginate-GelMA solution

2.2.1. GelMA synthesis

GelMA was synthesized based on previously described protocols (Xuan et al., 2016; Yue et al., 2015; Zhou et al., 2017). Briefly, 10 g of gelatin powder was first dissolved in 100 mL Dulbecco's Phosphate Buffered Saline (DPBS) under 50 °C, and 10 mL of methacrylic anhydride was added dropwise to react with the gelatin solution under magnetic stirring for 3 h at 50 °C. Then, the mixture was 5-fold diluted with DPBS and stirred for another 15 min. Salts and unreacted methacrylic anhydride were removed from the mixture by dialysis in a 12–14 kDa dialysis tube against distilled water for 1 week at 37 °C which was refreshed twice a day. White porous GelMA foam was obtained by lyophilization of the solution and kept for following use.

2.2.2. Preparation of the lyase-laden composite prepolymer

GelMA foam, sodium alginate, I2959 (the photoinnitiator to induce the gelatin of GelMA) were added into DIW and dissolved under 60 °C. Here the concentration of GelMA, alginate and I2959 was fixed at a constant value of 10%, 1% and 0.5% respectively. After all the solid were dissolved, the temperature of the mixture was cooled around 20 °C and different ratio of alginate lyase was added. To mix alginate lyase fully with Alginate-GelMA composite, firstly we dissolved it in DIW to obtain solution with high concentration. Then the lyase solution was proportionally added into the precursor after precise calculation with gentle stir. Just as Table 1 shows, we designed five concentration gradient of alginate lyase at 0.2%, 0.1%, 0.05%, 0.01% and 0 (w/v), and the corresponding groups were named as AL0.2, AL0.1, AL0.05, AL0.01 and AL0. Here AL0 is the control.

2.3. Fabrication and characterization of lyase-laden composite hydrogel

2.3.1. Fabrication of composite hydrogels

Both hydrogel microfibers and blocks were made for different characterization. 100 μL of prepolymer solution in a 1 mL syringe was injected into the 100 mM $CaCl_2$aq through a 23G (D0 = 330 μm) nozzle and quickly gelled to form fibrous hydrogel, subsequently 15 s of UV irradiation was exposed to crosslink the GelMA in the composite and form the interpenetrating dual-network hydrogel. During the whole process the temperature of the prepolymer was maintained at 20 °C and the solid fibrous hydrogel were obtained for further characterization. For mechanical testing, hydrogel blocks were made. Generally, the prepolymer solution was pipetted into a circular Teflon mold with the size of Φ 8 mm × 2.4 mm. Then 100 mM $CaCl_2$aq was added dropwise to the mold to crosslink the alginate. Subsequently 15 s UV was perpendicularly exposed to the composite disc to crosslink GelMA. The round hydrogel disc was fabricated after removing from the mold. Compression tests were conducted both on samples just made and that incubated in DIW at 37 °C for 96 h.

2.3.2. Degradation and morphology maintaining study

The degradation rate of the composite hydrogel was detected with the solid fibrous hydrogel. Samples of five groups were immersed in the DIW at 37 °C which was refreshed every day. At predetermined time points, the samples were picked out, washed with DIW, dried and weighed. Digital photos of the samples after degradation for 24 h were taken to observe the morphology of the fibers. The degradation rate of