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A strategy for strong interface bonding by 3D bioprinting of oppositely charged κ-carrageenan and gelatin hydrogels

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ABSTRACT

A promising approach for improving the interfacial bonding of a three-dimensionally (3D) printed multilayered structure has been investigated by taking advantage of the electrostatic interactions between two hydrogels with oppositely charges. Here, two hydrogels namely gelatin and κ-carrageenan, which are the cationic and anionic hydrogels respectively, are used. It is found that the interfacial bonding strength between these two oppositely charged hydrogels is significantly higher than that of a bilayered gelatin or a bilayered κ-carrageenan. The bioprinted multilayered κ-carrageenan-gelatin hydrogel construct demonstrates a very good biocompatibility and a good structure integrity at 37 °C. Our strategy also overcomes the limitation of using gelatin for bio-fabrication at 37 °C, without further post crosslinking.

1. Introduction

Three-dimensional (3D) bioprinting technologies show great potentials in fabricating complex biological tissues with controlled geometries. For 3D bioprinting, cells are usually directly loaded into a hydrogel for printing (Mannoor et al., 2013; Ng, Yeong, & Naing, 2016; Ng, Qi, Yeong, & Naing, 2018; Tan, Tan, Yeong, & Tor, 2016; Zhuang, Sun, An, Chua, & Chew, 2018).

The peptide arginine-glycine-aspartate (RGD) promotes cell adhesion, and proliferation (Sakai, Hirose, Taguchi, Ogushi, & Kawakami, 2009). Some natural hydrogels contain RGD, such as gelatin. Therefore, for bio-fabrication, gelatin should be a good candidate of biomaterials. But gelatin hydrogel has a limitation: it cannot maintain the shape of its printed 3D construct due to its poor mechanical strength and unsatisfactory printability (Hospodiuk, Dey, Sosnoski, & Ozbolat, 2017; Smith et al., 2004). Gelatin is a water-soluble protein that derive from collagen (Gomez-Guillen et al., 2002; Jungst, Smolan, Schacht, Scheibel, & Groll, 2016). Moreover, gelatin inherits the superior performance of collagen to promote cell adhesion (Shi et al., 2017), but it dissolves in cell culture media as a colloidal sol at a cell culture temperature of 37 °C. Thus, gelatin itself is seldom being considered as a candidate for 3D bioprinting prior to chemical and physical treatments (e.g. cross-linking) (Zhang, Ouyang, Lim, Ramakrishna, & Huang, 2005). For example, gelatin methacrylate (GelMA) is commonly used in 3D bioprinting, in which 3D structures are formed after exposing the hydrogel to UV light for covalent crosslinking of the GelMA chains (Zhang et al., 2005). However, it has been reported that UV curing might be unfavorable to cells (Atabak Ghanizadeh, Miguel, Nicholas, & Wenmiao, 2015; Malda et al., 2013). Hence, it is of our great interest in developing a strategy to use gelatin for bioprinting, which can overcome the limitation of using gelatin for bioprinting, and also create 3D constructs with good cell viability for cell adhesion, and high structural stability at 37 °C without further post crosslinking.

Hydrogels are found to be cationic (e.g. gelatin), anionic (e.g. κ-carrageenan) (Samal et al., 2012; Souguir, Picton, & Le Cerf, 2012), as well as neutral (e.g. dextran). κ-carrageenan is known as a strong, but brittle hydrogel, which has also been used as an injectable medium for delivering cells and macromolecules (Mihaila et al., 2013), and for 3D printing structure with ultrastretchable and self-healing properties after UV curing (Liu & Li, 2017). Utilizing two oppositely charged hydrogels in bioprinting maybe a way to overcome the drawbacks of printing gelatin independently. Moreover, layer defects may exist in a layer-by-layer 3D printing process (Gleghorn, Lee, Cabodi, Stroock, & Bonassar, 2008; Lee et al., 2007; Li, Tan, Leong, & Li, 2017). It is interesting to study the interfacial bonding between a cationic hydrogel and an anionic hydrogel in 3D printing, which has not been reported in the literature.

Here, we investigate an approach to 3D bioprint a multilayered structure with strong interfacial bonding by exploiting the electrostatic interaction betwixt a cationic hydrogel, and an anionic hydrogel. We make use of the thixotropic gelatin and κ-carrageenan as the cationic and anionic hydrogels, respectively. The κ-carrageenan and gelatin hydrogels were printed alternately using a 3D bioprinter, which resulted in a 3D construct named κ-carrageenan-gelatin. Rheological

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properties of κ-carrageenan and gelatin hydrogels were studied, which helps to simulate their rheological behaviors before, during, and after extrusion-based printing condition. Thereafter, the interfacial bonding between a κ-carrageenan hydrogel layer and a gelatin hydrogel layer was also investigated. Moreover, the effectiveness of κ-carrageenan-gelatin for bioprinting was examined, such as structural integrity and biocompatibility.

2. Experimental

2.1. Hydrogels preparation

κ-carrageenan (molecular weight ~3.0×10^5g/mol, Sigma-Aldrich, Singapore) hydrogel was prepared by gradually adding the κ-carrageenan into a hot Dulbecco's phosphate buffered saline (DPBS, Sigma-Aldrich, Singapore) solution at about 80 °C while stirring. The prepared κ-carrageenan hydrogels were named κ-carrageenan1, κ-carrageenan1.5, κ-carrageenan2, and κ-carrageenan2.5, corresponding to the κ-carrageenan concentrations of 1%, 1.5%, 2% and 2.5% (wt/wt) in the DPBS solutions, respectively.

Gelatin hydorgel (gel strength ~ 300 g bloom, type A from porcine skin, Sigma-Aldrich, Singapore) was obtained by dissolving the gelatin powder in a hot DPBS solution at about 50 °C while thoroughly stirred the blend. Gelatin6 (6 wt% gelatin), Gelatin7 (7 wt% gelatin), Gelatin8 (8 wt% gelatin), and Gelatin9 (9 wt% gelatin) were prepared.

2.2. Printability of hydrogels with various concentrations

0/90° pattern was printed to study the printability of each hydrogel. Here, a 3D bioprinter (Biofactory bioprinter machine, RegenHu) under a working temperature at about 26 °C was utilized for 3D printing. The nozzle used for printing was 0.25 mm in inner diameter. The optimal printing pressures for extruding the hydrogels were recorded, and shown in Table 1.

2.3. Rheological study

Rheological properties of the κ-carrageenan2, and the Gelatin8 hydrogels were investigated using a rotational rheometer (DHR, TA Instruments). A 40 mm parallel plate with a measurement gap of 0.55 mm was used. Two rheological measurements were performed under the working temperature (about 26 °C) of the 3D bioprinter: steady-state flow tests in the shear rate range of 0.5–500 s^{-1}; recovery tests simulating the extrusion process during 3D printing, where an estimated shear rate was applied (see Section 2.3.1).

2.3.1. Shear rate

Based on our previous study (Li, Liu, & Li, 2016, 2017), the shear rate ($\dot{\gamma}$) exerted on a hydrogel in a nozzle (Chhabra & Richardson, 2008), could be estimated through an equation:

$$\dot{\gamma}^n = \left[\frac{VR^2}{\left(\frac{n}{3n+1}\right)\left(R^{(3n+1)/n}\right)} \right]^n . \ r \quad (1)$$

where n represents the power-law index of the hydrogel, V represents the flow rate of the hydrogel in a nozzle, R represents the inner radius of a nozzle, while r is the radial position ($0 < r < R$) of a hydrogel in a nozzle. Here, the inner diameter of the nozzle utilized was 0.25 mm. According to our previous study (Li et al., 2017), the flow rate for each hydrogel in a nozzle could be obtained.

Table 1
Optimum printing pressure for extruding each hydrogel.

Samples								
Parameters	κ-carrageenan				Gelatin			
Concentration (%)	1	1.5	2	2.5	6	7	8	9
Pressure (Bar)	0.4	0.7	0.8	1.0	0.6	0.7	0.8	0.9

2.3.2. Thixotropic property

The thixotropic properties of the hydrogels were investigated to simulate the rheological behaviors of the hydrogels during the 3D printing process (Li et al., 2016, 2017). The whole simulation process can be divided into three steps: step I represents the initial condition of a hydrogel before extrusion. A very low shear rate (0.1 s^{-1}) was applied for 60 s to imitate this stage; step II represents the condition of a hydrogel during extrusion through a fine nozzle. According to Eq. (1), an estimated shear rate (100 s^{-1}) was obtained and applied for 10 s to simulate the second step; step III represents the final state of the sheared hydrogel after extrusion. Again, a very low shear rate (0.1 s^{-1}) was applied for another 60 s to allow the hydrogel to recover its viscosity.

2.4. Interfacial bonding properties

2.4.1. Interaction between κ-carrageenan and gelatin

To observe the interaction between a cationic (e.g. gelatin) and an anionic (e.g. κ-carrageenan) hydrogel, a simple experiment was first conducted. Small pieces of the transparent κ-carrageenan2 hydrogel (not stained), and the orange Gelatin8 hydrogel (stained with an orange food-grade dye, Bake King) were sliced. After that, three pieces of the hydrogels with alternate colors, i.e. Gelatin8- κ-carrageenan2-Gelatin8, were placed next to each other. The κ-carrageenan2-κ-carrageenan2- κ-carrageenan2, and Gelatin8-Gelatin8-Gelatin8 were served as a control.

2.4.2. Lap-shear test

Aiming to quantitatively study the interfacial properties between the hydrogel sheets, lap-shear tests were conducted with an Instron machine (Instron 5569; U.K.) at ambient conditions using a 100 N load cell. Hydrogel sheets with a thickness of 2 mm were prepared by casting, each representing one layer of a 3D printed multilayered construct. Five groups of samples were fabricated, namely the bilayered κ-carrageenan2, the bilayered Gelatin8, the bilayered κ-carrageenan2-Gelatin8, the bulk κ-carrageenan2, and the bulk Gelatin8. The bilayered κ-carrageenan2-Gelatin8 was fabricated by placing a κ-carrageenan2 hydrogel sheet onto a Gelatin8 hydrogel sheet to obtain a sample with a thickness of 4 mm. The bulk κ-carrageenan2, and bulk Gelatin8 served as a control were 4 mm in thickness. The dimensions of samples used for the lap-shear test were 20 mm by 20 mm (by 4 mm thickness). The ultimate shear stress (USS), that is, the maximum shear stress that the sample able to resists failure in shear, was simply obtained from the shear stress versus time curve (Li et al., 2017).

2.5. Structure integrity and degradation of the printed κ-carrageenan2-Gelatin8 construct in DI water at 37 °C

The 3D printed κ-carrageenan2-Gelatin8 construct was immersed in deionized (DI) water in an oven (37 °C) to invesitgate its structure integrity for up to 120 h. The κ-carrageenan2 construct, and Gelatin8 construct were also printed and observed under the same condition. In order to distinguish the printed structure, a food dye was used.

Meanwhile, the dagradation of the printed κ-carrageenan2-Gelatin8 construct at 37 °C in DI water was also investigated. After recording the weight of a sample before immersing (W_0), and after immersing (W_1), respectively, the percentage of degradation (W_r) could be estimated by $W_r = (W_1/W_0) * 100\%$.