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Characterisation of hyaluronic acid methylcellulose hydrogels for 3D bioprinting

Nicholas Law[a,b,1], Brandon Doney[a,b,1], Hayley Glover[a,b], Yahua Qin[b], Zachary M. Aman[b], Timothy B. Sercombe[b], Lawrence J. Liew[c], Rodney J. Dilley[c,d], Barry J. Doyle[a,b,e,*]

[a] Vascular Engineering Laboratory, Harry Perkins Institute of Medical Research, Nedlands, and Centre for Medical Research, The University of Western Australia, Crawley, Perth, Australia
[b] School of Mechanical and Chemical Engineering, The University of Western Australia, Perth, Australia
[c] Ear Science Institute Australia, Perth, Australia
[d] Ear Sciences Centre and Centre for Cell Therapy and Regenerative Medicine, School of Medicine, The University of Western Australia, Perth, Australia
[e] BHF Centre for Cardiovascular Science, The University of Edinburgh, UK

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ABSTRACT

Hydrogels containing hyaluronic acid (HA) and methylcellulose (MC) have shown promising results for three dimensional (3D) bioprinting applications. However, several parameters influence the applicability bioprinting and there is scarce data in the literature characterising HAMC. We assessed eight concentrations of HAMC for printability, swelling and stability over time, rheological and structural behaviour, and viability of mesenchymal stem cells.

We show that HAMC blends behave as viscous solutions at 4 °C and have faster gelation times at higher temperatures, typically gelling upon reaching 37 °C. We found the storage, loss and compressive moduli to be dependent on HAMC concentration and incubation time at 37 °C, and show the compressive modulus to be strain-rate dependent. Swelling and stability was influenced by time, more so than pH environment. We demonstrated that mesenchymal stem cell viability was above 75% in bioprinted structures and cells remain viable for at least one week after 3D bioprinting.

The mechanical properties of HAMC are highly tuneable and we show that higher concentrations of HAMC are particularly suited to cell-encapsulated 3D bioprinting applications that require scaffold structure and delivery of cells.

1. Introduction

Hydrogels have been used across a wide range of biomedical applications and are showing great potential in the field of tissue engineering. Hydrogels are three-dimensional (3D) cross-linked scaffolds of water-soluble polymers, which form a macromolecular network capable of retaining high water content. Due to their often poor mechanical integrity, hydrogels are classified as soft gels with structural similarity to some human soft tissues (Bajaj et al., 2014). However, their hydrophilic polymer networks enable the diffusion of glucose and other nutrients, thus supporting the growth of cells. Additionally, by altering the concentration of hydrogel components, mechanical properties can often be tailored.

Recently, hydrogels have been used with 3D bioprinting. To be effective as a 3D bioink, hydrogels must exhibit desirable characteristics, such as good printability at low air pressure (< 200 kPa) (Murphy et al., 2013), minimal swelling and contraction (Murphy et al., 2013; Sun et al., 2011), and excellent biocompatibility (Bajaj et al., 2014). Depending on the application, good structural integrity (Irvine et al., 2015), the ability to maintain physiological pH values and fast gelation times (Mayol et al., 2014) are also highly desirable.

Although there are several hydrogels showing promise, blends of hyaluronic acid (HA) and methylcellulose (MC) have been used for applications including dermal wound repair (Murphy et al., 2013; Mayol et al., 2014), retinal repair (Ballios et al., 2010, 2015), stroke (Cooke et al., 2011; Caicco et al., 2013a; Tuladhar et al., 2015) and spinal cord repair (Gupta et al., 2006; Baumann et al., 2010, 2009; Wang et al., 2009; Caicco et al., 2013b). Most studies have focused on low concentration HAMC blends (Ballios et al., 2010, 2015; Cooke et al., 2011; Caicco et al., 2013a; Tuladhar et al., 2015) for delivery of therapies to specific sites. However, these lower concentrations of HAMC do not have the mechanical properties required to print 3D

* Corresponding author at: Harry Perkins Institute of Medical Research, 6 Verdun Street, Nedlands, Perth, WA 6009, Australia.
[1] Both authors contributed equally.

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scaffolds for applications requiring structural shape and controlled release of therapies.

Therefore, here we aim to characterise HAMC parameters that enable its use as a viable bioink for tissue engineering applications requiring cell delivery via either a soluble delivery vehicle or a 3D scaffold. We have investigated a wide range of HAMC blends from viscous gels to structurally stable blends capable of bioprinting 3D scaffolds. We have characterised these biomaterials using a suite of experimental tests to determine printability, swelling and stability, rheological properties, compressive modulus, and performance of mesenchymal stem cells within the biomaterial. Overall, we have shown HAMC to have highly tuneable properties capable of supporting cell viability and that, depending on the HAMC blend, could be useful across a range of biomedical and 3D bioprinting applications.

2. Methods

2.1. Biomaterial preparation

Hyaluronic acid methylcellulose (HAMC) blends were prepared similar to previous reports (Gupta et al., 2006). Methylcellulose (MC; viscosity 15 cP, Sigma-Aldrich) was dissolved in deionized water at 90 °C with a stir bar for 4 h to wet the polymer and to produce solutions containing 0.5, 1.0, 2.0, 3.0, 5.0, 6.0, 7.0, 9.0 MC wt%. We used a heat plate and oil bath to uniformly heat the solution. Phosphate buffer saline (PBS) was added to the solution in equal quantity to water, and subsequently cooled to 0 °C using an ice bath for an additional 30 min, after which it was allowed to equilibrate for 12 h at 4 °C. Hyaluronic acid (HA; 1000–1500 kDa, Lotioncrafter) was then added to the MC solution at a range of concentrations (0.25, 0.5, 1.0, 1.0, 2.0, 2.0, 2.0, 2.0 HA wt%) and allowed to dissolve for 12 h at 4 °C. Throughout the process we used a magnetic stirrer to maintain uniformity within the material. We produced the following HAMC wt% blends; 0.25/0.5, 0.5/1.0, 1.0/2.0, 1.0/3.0, 2.0/5.0, 2.0/6.0, 2.0/7.0 and 2.0/9.0.

2.2. Printability

Solidworks (v23, Dassault Systèmes, France) was used to design a 5 × 5 mm grid pattern for printability experiments. Each HAMC blend was loaded into a syringe at 4 °C for use with the BioBot bioprinter (Beta model, BioBots, USA) and a heat plate at 37 °C was positioned below the print head. The printing process was controlled using the freeware software platform Repetier-Host (Hot-World GmbH & Co. KG, Germany) and the deposition needle speed was held constant at 3 mm/s for all tests. The initial layer height was 0.1 mm with each subsequent layer height 0.35 mm and the deposition needle size ranged from 0.1 to 0.51 mm inside diameter, with the needle diameter increasing with increasing viscosity to ensure that printing air pressure did not exceed 200 kPa (Fredriksson et al., 2008; Harkin et al., 2006). The grid pattern was printed three times for each HAMC blend and the print accuracy of the matrix was calculated using the following formula (Eq. (1)):

$$Printing\ Accuracy = \frac{|A_i - A|}{A} \times 100\% \quad (1)$$

Where A_i is the area of print (mm^2) measured using a digital calipers and A is the design area (mm^2) (Duan et al., 2013). Although we used a grid pattern, only the outer dimensions of the grids were used to calculate print area. An example printed grid is shown in Fig. S1 in the Supplementary data section.

2.3. Swelling and stability

A potential application of HAMC is the treatment of burns, where burns often exhibit different pH environments during healing. Therefore the swelling and stability of the HAMC blends was examined by placing the samples into different saline buffers with varying pH levels from 5.5 to 8.5, in 1.0 increments.

2.3.1. Buffer preparation

Four 50 mmol buffers were prepared with 150 mmol sodium chloride (NaCl) in deionized water. Saline was added to more closely resemble the extracellular conditions where the gel will be deposited. Tris Buffered Saline (TBS) was used for the pH 7.5 and .85 solutions, bis-tis buffered saline (BTBS) for pH 6.5 and citrate buffered saline (CBS) for pH 5.5. The pH was adjusted to these values at 37 °C and the resulting buffers were filtered at 0.45 μm to remove particulates.

2.3.2. Swelling

Four 0.5 mL aliquots of each HAMC blend were gelled at the bottom of separate pre-weighed replicate glass vials heated to 37 °C and re-weighed. We then added 3 mL of each of the buffers to the samples, and placed the vials in an incubator. Buffers were changed and vials re-weighed at 1 h, 3 h, 6 h, 1 day, and 3 days. The swelling ratio was calculated as defined in Eq. (2):

$$Swelling\ ratio = \frac{weight\ of\ hydrogel(t)}{weight\ of\ hydrogel(0)} \quad (2)$$

Where the weight of the hydrogel was determined by subtracting the initial weight of the vial from the total weight with sample after the surface buffer was carefully removed.

2.3.3. Stability

Four 0.5 mL aliquots of each HAMC blend were gelled at the bottom of 24-well plates at 37 °C. We then added 3 mL of each buffer and placed in an incubator. Inspections and supplementation of fresh buffer took place every day for a 15 day period to determine if samples remained intact or dissolved.

2.4. Rheological characterisation

We performed all rheological experiments using a TA Instruments Discovery Hybrid DHR-3 controlled stress rheometer, equipped with 60 mm 2° acrylic cone-and-plate geometry placed atop an integrated Peltier stage for temperature control. In each experiment, we performed three repeat trials per sample, and performed each experiment in duplicate with separately prepared batches of HAMC.

2.4.1. Frequency sweep tests

Each rheological characterisation experiment consisted of frequency sweeps at 1% strain, repeated at 4 and 37 °C. Storage (G′) and loss moduli (G″) were recorded from 0.1 to 100 Hz, with 10 points taken per decade. Samples were stored at 4 °C prior to testing. For frequency sweep tests carried out at 37 °C, samples were allowed to equilibrate for 20 min prior to testing, with the Peltier stage set to 37 °C (Caicco et al., 2013b).

2.4.2. Time sweep tests

Time sweep tests were performed to quantify gelation time with storage and loss moduli for each blend measured as a function of time. The rheometer temperature was increased from 4 to 37 °C at 5 °C/min, and the moduli were recorded at an angular frequency of 1 Hz and 1% strain. The gelation time was taken as the time from when the material reached 37 °C to the crossover point of the storage and loss moduli.

2.5. Compression testing

2.5.1. Sample preparation

The compressive behaviour of the four higher HAMC blends (2.0/5.0, 2.0/6.0, 2.0/7.0, 2.0/9.0) were tested as the lower blends could not maintain a cylindrical structure. We injected the HAMC blends at 4 °C into cylindrical moulds 6 mm high and 11 mm diameter which had