Development of a Photocrosslinkable Methacrylated Methylcellulose and Gelatin bioink for Cartilage Tissue Regeneration

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***Abstract*—Articular cartilage disease can cause pain, mobility issues, and disability. Clinical treatment includes microfracture, subchondral drilling, graft transplantation, and eventually total joint replacement implant. However, these approaches can present specific problems and limitations. Three-dimensional (3D) bioprinted scaffolds utilising hydrogels can provide a suitable 3D biochemical and biophysical environment, thus is a promising strategy for cartilage tissue therapy and regeneration. This study aims to develop a new hydrogel bioink with improved printability, mechanical, and biological properties for cartilage regeneration. A photocrosslinkable methacrylated methylcellulose (MCMA) and gelatin (GelMA) hybrid bioink is evaluated in this preliminary investigation. The results showed that methylcellulose and gelatin were successfully functionalised, which enabled photocrosslinking of the bioinks. The compressive modulus and shear-thinning behaviour of bioinks increases with higher content of MCMA, all bioinks displayed printability, and scaffolds were successfully fabricated.**

I. INTRODUCTION

Articular cartilage disease is primarily caused by trauma or degenerative pathologies, approximately 450 million people in the world over the age of 30 suffer from such afflictions [1]. Joint diseases such as osteoarthritis cause pain and stiffness, and the cartilage has limited self-healing ability due to a lack of vasculature. Current non-surgical treatments like pain management only relieve the symptoms, and the main drawbacks of surgical interventions such as graft transplantation are abnormal wear, implant irritation and high economic cost, furthermore, the graft can be difficult to match the native defect shape [2-3].

To overcome the major limitations of conventional therapies, scaffold-based cartilage tissue engineering using bioprinting technologies have been developed. Bioprinting combines additive manufacturing techniques with a hydrogel-based bioink containing cells and growth factors. A hydrogel is a 3D network composed of polymer chains with tuneable chemical and physical characteristics [4]. Hydrogels mimic the native extracellular matrix (ECM) due to their structure and hydrophilicity. Subsequently, a bioprinted hydrogel-based scaffold can provide a suitable 3D biophysical and biochemical environment for cell migration, attachment, and proliferation [5-6].

In this study methylcellulose (MC), a type of cellulose derivative, and gelatin, a hydrolysed form of collagen, were explored. MC is biocompatible, biodegradable, and can provide improved printability and structure fidelity. Gelatin provides appropriate mechanical properties, enhanced biocompatibility due to the presence of cell binding motifs and is biodegradable enzymatically. These hydrogels can be crosslinked by both physical and chemical methods; chemical crosslinks typically provide a more stable hydrogel network through covalent bonding between polymer chains. Photopolymerisation provides an efficient crosslinking method, which allows rapid curing under mild conditions. A common approach is the modification of the polymer with functional groups such as methacrylate or acrylate. The photocurable polymer solution with the addition of photoinitiators undergo polymerisation under light irradiation, ultraviolet (UV) or visible light, free radicals generated from the photoinitiators initiate the chain polymerisation of carbon-carbon double bonds in the modified polymer solutions to form a crosslinked network [7].

This preliminary study explored the development of a photocrosslinkable methacrylated methylcellulose (MCMA) and gelatin (GelMA) bioink for extrusion-based bioprinting. This technique was selected due to the ability to print with high cell densities and a range of bioink viscosities, the print head can also be equipped with a light source for photocrosslinking [8-10]. The hydrogel bioinks were characterised for their mechanical, rheological, and degradation properties. 3D scaffolds were printed and photocrosslinked showing potential suitability for cartilage tissue engineering applications.

II. MATERIALS AND METHODS

A. Hydrogel Design and Fabrication

Methylcellulose (MC, M7140, Mw≈14000 g/mol, Sigma-Aldrich, UK) and gelatin (type B, bovine, Mw=20000-25000 g/mol, Sigma-Aldrich, UK) were modified by methacrylic anhydride (MA) (94%, Sigma-Aldrich, UK), Fig. 1 shows the schematic of the synthesis of both MCMA and GelMA. Briefly, 6 g MC powder was fully dispersed and hydrated in 100 mL prewarmed Dulbecco's Phosphate Buffered Saline (PBS) (pH 7.4, with $MgCl_2$ and $CaCl_2$, Sigma-Aldrich, UK) solution at 90°C under magnet stirring, then 200 mL of PBS at 4°C was added into the solution to complete the solubilisation process, the solution was stirred for a further 30 min, and then stored at

4°C. 10% v/v of MA was subsequently added into the MC solution drop by drop at 4°C, while a 5% w/v sodium hydroxide solution (NaOH) was used to adjust the pH value to 8.0. Next, to achieve complete reaction between MC and methacrylic anhydride, the MCMA solution was purified by dialysis process for 72 h, until the extra MA was fully removed. The MCMA solution was frozen at -80°C overnight and lyophilised for 3 days to obtain the methacrylate functionalised MC. 8 g lyophilised MCMA polymer was dissolved in 100 mL PBS to obtain 8% w/v MCMA. Preparation of GelMA polymer is similar to the process of MCMA polymer. 3.6 g gelatin powder was added and fully dissolved in 100 mL PBS for 4 h at 45°C under magnet stirring. After functionalisation and lyophilisation, 10% w/v GelMA was obtained by dissolving 10 g GelMA polymer in 100 mL PBS.

In this research, different contents of both materials were investigated, as shown in Table 1. 10 mW/cm^2 UV irradiation for 10 min and 1% w/v water-soluble photoinitiator [2-methyl-N-(2-hydroxyethy) propionamide] (VA-086, Wako, USA) were used for photopolymerisation of the composite hydrogels. Figure 2 shows five groups of blended hydrogel bioinks before and after photopolymerisation.

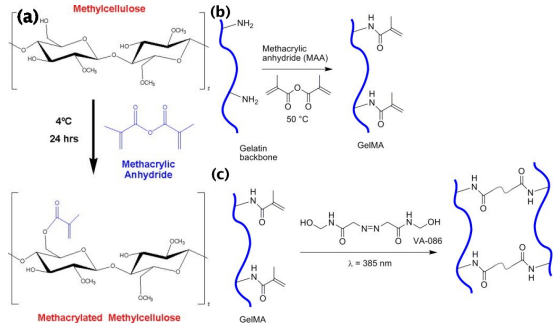

Figure 1. Schematic of the synthesis of MCMA and GelMA. (a) MC functionalisation. b) gelatin functionalisation. c) gelatin photocrosslinking (Adapted from [11-12]).

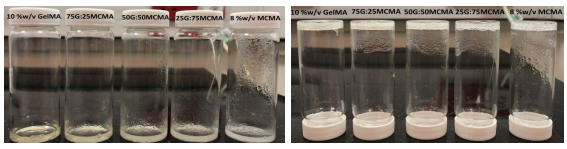

Figure 2. GelMA/MCMA blended hydrogel bioinks before (left) and after (right) pohotopolymerization process.

TABLE I. CONTENT OF BLENDED HYDROGELS

Samples	*Ratio (GelMA: MCMA)*	*GelMA Concentration (%w/v)*	*MCMA Concentration (%w/v)*
1	/	10	/
2	75:25	7.5	2
3	50:50	5	4
4	25:75	2.5	6
5	/	/	8

B. Mechanical Evaluation

Cylindrical photocrosslinked hydrogel samples (diameter: 10 mm and thickness: 10 mm) were prepared and subsequently soaked in PBS solution overnight at 4°C before testing. The mechanical evaluation was achieved by compression tests using the INSTRON 3344 system (Instron®, USA) equipped with a 10 N load cell, the compression tests were performed with the strain ranging from 0 to 20% with a compression strain ramp speed of 0.5 mm/min. Compressive strain and stress were recorded and the compressive modulus was calculated by stress-strain curves.

C. Rheology Characterization

To investigate shear-thinning behaviour of the bioinks, a flow sweep was performed using a Hybrid Rheometer HR-2 (TA Instruments, USA) equipped with a cone plate system (60 mm, 2°, and 50 µm geometry gap) at 25°C. The shear rate ramp was from 0.1 to 1000 s^{-1}, 5 points were obtained per decade for both viscosity and stress.

D. Swelling and Degradation

Five groups of crosslinked cylindrical hydrogels (diameter: 6 mm and thickness: 5 mm) were prepared and lyophilised before being incubated at 37°C in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, UK) for 12 days, the lyophilised dry weight (W_i) of each sample was measured before incubation, then the swollen weight (W_s) was measured every two days. The swelling ratio (Q) was calculated by the given formula:

$$Q = W_s / W_i \quad (1)$$

The degradation of hydrogels was performed by the mass loss percentage, which was calculated based on the formula:

$$\% \text{ mass loss} = (W_i - W_d)/W_i * 100 \quad (2)$$

where W_d is the weight of the samples after incubation and subsequent lyophilisation at each time point after. The DMEM was changed every two days for swelling and degradation experiments.

E. Printability

A 3D-bioplotter (EnvisionTEC, Germany) with a pneumatic extrusion-based system was used to examine the printability of the bioinks, a 50GelMA/50MC bioink was assessed initially. The bioinks were loaded into a syringe and extruded through a 22GA (640 µm) nozzle with a printing speed of 26 mm/s at 37°C. The printed constructs were designed in SolidWorks (Dassault Systemes, France) and the file was imported into VisualMachines (3D Sprint, USA). Hydrogel scaffolds with a 0°/90° deposition pattern and 30 mm x 30 mm square shape were printed using different extrusion pressures 1.00, 1.20 and 1.40 bar. The printed scaffolds were imaged by VHX-500F Digital Microscope (KEYENCE, UK),

the filament size was measured using Image J software. The porosity of scaffolds was calculated by the equations:

$$Porosity(\%) = \left(1 - \frac{V_{scaffold}}{V_{total}}\right) \times 100 \quad (3)$$

$$V_{scaffold} = \pi\left(\frac{Diameter_{filament}}{2}\right)^2 \times length_{filament} \times number_{filament} \quad (4)$$

F. Data Analysis

All experiments are repeated using three samples (n=3) and data represents mean ± standard deviation. OriginPro® software (OriginLab, USA) was used to analyse obtained data for mechanical evaluation, rheology characterisation and swelling and degradation.

III. RESULTS AND DISCUSSION

A. Mechanical Evaluation

Tissue specific mechanical properties are desirable for hydrogels in tissue engineering applications. A non-linear relationship between compressive strain and stress was displayed for all hydrogel groups (Fig. 3a). The compressive stress increased when the strain gradually increased to 20%, and GelMA hydrogels showed the largest compressive stress which was 4.5±0.029 kPa at 20% strain. For blended hydrogels, 25GelMA/75MCMA displayed the largest stress when the strain reached 20%.

The values of compressive modulus are shown in Figure 3b. GelMA hydrogels had the highest compressive modulus and 25GelMA/75MCMA showed the highest compressive modulus from all blended hydrogels. As the concentration of MCMA increased, the modulus of blended hydrogels increased as well, but they were still smaller than pure GelMA (0.369±0.020 kPa) and MCMA (0.161±0.033 kPa). The hydrogels lack an appropriate compressive modulus to support the mechanical properties of articular cartilage, optimisation such as increasing the concentrations of both materials is needed to match the native properties.

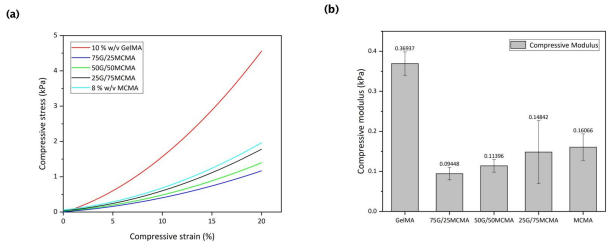

Figure 3. Mechanical evaluation of the hydrogels. (a) Compressive strain-stress curve. (b) Compressive modulus.

B. Rheology Characterisation

The viscosity and stress vs. shear rate of the bioinks is presented in Figure 4. Shear-thinning, a non-Newtonian behaviour, is a crucial factor to ensure printability of bioinks, as a lower viscosity is observed at high shear rates during the extrusion process [13]. Shear-thinning behaviour was observed in all samples, and the most prominent shear-thinning behaviour was found in pure MCMA bioink. The 25GelMA/75MCMA bioink showed the highest viscosity at a lower shear rate in all blended samples, which demonstrated that it could provide better shape fidelity. It is because after deposition, the extruded bioinks become viscous and can maintain shape fidelity before photocrosslinking.

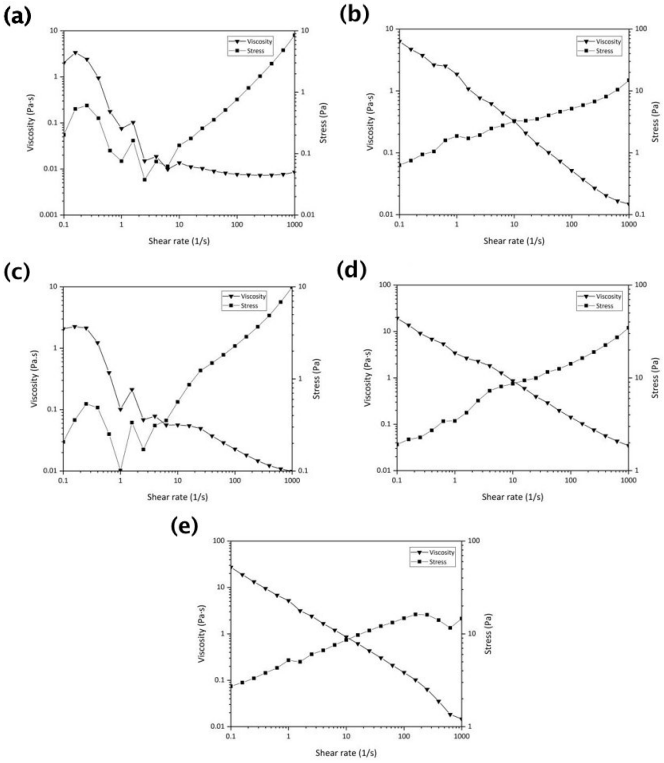

Figure 4. Characterisation of rheological behaviour (a) GelMA, (b) 75GelMA/25MCMA, (c) 50GelMA/50MCMA, (d) 25GelMA/75MCMA, and (e) MCMA hydrogels.

C. Swelling and Degradation

The swelling ratio and the mass loss over 12 days for hydrogels demonstrate that the swelling ratio of pure GelMA was significantly smaller than that of MCMA and all blended hydrogels (Fig. 5). Demonstrating that the GelMA hydrogel had a denser porous structure. The degradation rate of hydrogels affects cell behaviour such as cell migration, proliferation, and differentiation, as well as the production of native ECM, and the degradation rate should match the rate of new tissue formation. The pure MCMA hydrogel displayed the fastest degradation with a mass loss of approximately 55% after 12 days incubation. As the concentration of GelMA increased, the degradation rate increased for the blended hydrogels.

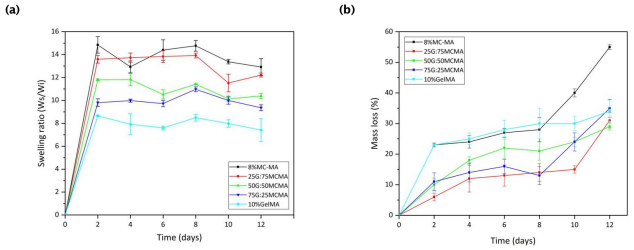

Figure 5. Swelling ratios (a) and mass loss (b) of the hydrogels over 12 days.

D. *Printability*

Optimised printing parameters is a crucial factor to ensure the fidelity and viability of a bioprinted printed construct. Low extrusion pressure or high deposition speed will cause insufficient extruded bioinks as well as the collapse of complex structures, and excess bioink will change the internal structure of scaffolds, which cannot satisfy biology properties. Two-layer scaffolds, as an initial scaffolds design, were successfully printed using 50GelMA/50MC as a preliminary investigative bioink using different extrusion pressures (Fig. 6). Table 2 shows the measured filament diameters and calculated porosities. The scaffold printed with 1.00 bar extrusion pressure showed a defined morphology and the highest shape fidelity. It can be observed that the filament diameter increased as extrusion pressure increased, the scaffold using 1.00 bar pressure had 0.73±0.06 mm filament diameter and 73.87% porosity, which were closest to the designed filament diameter (nozzle diameter) of 0.64 mm and a porosity of 79.8%.

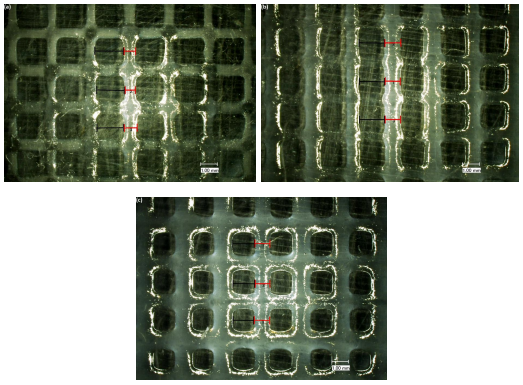

Figure 6. Microscopy images of printed 50GelMA/50MC hydrogel scaffolds in different extrusion pressure. (a) 1.00 bar. (b) 1.20 bar. (c) 1.40 bar.

TABLE II. FILAMENT DIAMETER AND POROSITY OF 50GELMA/50MC BIOINK AT DIFFERENT EXTRUSION PRESSURES

Extrusion pressure (bar)	*Filament diameter (mm)*	*Porosity (%)*
1.00	0.73±0.06	73.87
1.20	0.92±0.01	58.51
1.40	1.06±0.15	57.64

IV. CONCLUSIONS

Extrusion-based bioprinting was used to explore the fabrication of scaffolds for cartilage tissue engineering applications. GelMA/MCMA hydrogel bioinks were successfully functionalised and photocrosslinked. The bioinks showed shear-thinning behaviour and the inclusion of MCMA contributed more to the mechanical properties. Optimisation of material concentration will be explored to improve the mechanical properties and thickness of the printed scaffolds in the future. Additionally, bioprinted cell-laden hydrogel scaffolds will be investigated *in vitro* using chondrocytes and mesenchymal stem cells to assess the biological performance of the hybrid hydrogels.

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