



## NOVEL METHACRYLATED GELLAN GUM HYDROGELS WITH TUNABLE MECHANICAL PROPERTIES

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### KEYWORDS

Methacrylated polysaccharide, Physical and chemical crosslinking, Mechanical properties, Tissue engineering

### ABSTRACT

Gellan Gum (GG) has been recently proposed for tissue engineering applications. GG hydrogels are produced by physical crosslinking methods induced by temperature variation or by the presence of divalent cations. However, physical crosslinking methods may yield hydrogels that become weaker in physiological conditions due to the exchange of divalent by monovalent cations.

This work presents a new class of methacrylated gellan gum hydrogels (MeGG) with highly tunable properties, crosslinkable by both physical and chemical mechanisms. We herein demonstrate that the chemical incorporation of methacrylate groups yields hydrogels with mechanical properties that can be easily tuned by varying the parameters of the crosslinking mechanisms used.

### INTRODUCTION

*In situ* forming hydrogels are attractive as vehicles for drug delivery and as cell carriers due to the minimally invasive implantation. GG is a FDA-approved anionic microbial polysaccharide that forms gels in response to the temperature decrease or by ionic bonding in the presence of cations. Recently, it has shown promising results in cartilage tissue engineering (Oliveira *et al*, 2010). However, the major drawback of physically crosslinked hydrogels, like GG, is the limited stability *in vivo* due to the exchange of divalent with monovalent cations that are present in higher concentrations in the physiological environment. Another critically important attribute of hydrogels intended especially for tissue engineering are the

mechanical properties. Different tissues have different tissue regeneration rates and different mechanical properties (Engler *et al*, 2006). Thus, biomaterial scaffolds should provide mechanical properties that are compatible with the required application. Although hydrogel scaffolds mimic the extracellular matrix three dimensional environment, their major drawback is the low mechanical stability. *In situ* forming chemical hydrogels offer advantage over physical hydrogel in terms of their mechanical strength, integrity and swelling properties fine tuned by the degree of methacrylation. Thus, here we describe the functionalization of GG with double bonds, leading to the formation of both physically and chemically crosslinkable GG hydrogels. We herein show that those hydrogels have tunable mechanical properties in the range of 0.15 and 148 kPa. This range of mechanical properties is relevant for different tissue engineering applications going from soft tissues (as the skin) to stiff tissues (bone) (Engler *et al*, 2006).

### MATERIALS AND METHODS

Synthesis of MeGG: MeGG was synthesized by reacting GG with methacrylic anhydride (MA) at 90 °C (6 hours). Either 2 or 8 mL of MA was added at 50 °C, in order to synthesize MeGG with low (Low-MeGG) or high (High-MeGG) degrees of methacrylation, respectively. pH (8.0) was adjusted periodically. The modified MeGG solution was dialyzed and lyophilized. Characterization of MeGG. The chemical modification to GG was assessed by proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy and Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR).

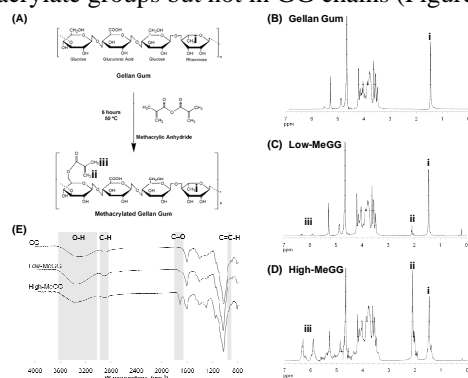
Preparation of hydrogels: Low-MeGG, High-MeGG and GG were dissolved at 0.5, 1 and 2% (w/v) in dH<sub>2</sub>O at 50 °C. Then, calcium chloride (CaCl<sub>2</sub>, 0.08 mg/mL, unless mentioned otherwise) and/or 0.5% (w/v) Irigacure 2959 were added to the dissolved materials to

fabricate hydrogels with different crosslinking mechanisms. GG and physically crosslinked MeGG hydrogels were produced by casting the solution into molds and allowing the temperature to cool to room temperature. Chemically crosslinked hydrogels were obtained by exposing to UV light for 60 sec.

**Mechanical characterization of MeGG hydrogels:** Compression tests were performed on freshly prepared GG and MeGG hydrogels (1 mm thick, 8 mm in diameter) were compressed at a rate of 0.2 mm/min until failure of the hydrogel. The Young's modulus was defined as the slope of the linear region of the stress-strain curve in the 5-15% of the strain range.

## RESULTS

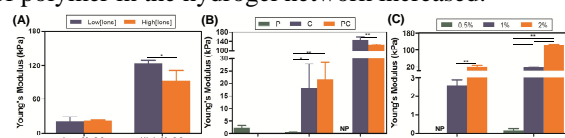
MeGG was prepared by the methacrylation of GG prepolymer (Figure 1A). <sup>1</sup>H-NMR spectroscopy confirmed the methacrylation of GG by the appearance of distinctive peaks in the double bond region ( $\delta$  5.50-7.00 ppm) and a sharp peak that corresponds to the -CH<sub>3</sub> of the methacrylate groups ( $\delta$  2.09 ppm) on the modified GG spectra. Figure 1C and Figure 1D show the <sup>1</sup>H-NMR spectra of methacrylated GG with low and high degrees of methacrylation, respectively. FTIR-ATR also showed the appearance of the carbon double bond peak at 1640 cm<sup>-1</sup>, known to be present in methacrylate groups but not in GG chains (Figure 1E).



**Figure 1.** (A) Schematic illustration of the synthesis of MeGG. <sup>1</sup>H-NMR spectra of (B) GG, (C) Low-MeGG and (D) High-MeGG recorded in D<sub>2</sub>O. (E) FTIR-ATR spectra of GG, Low-MeGG and High-MeGG.

The crosslinking mechanisms used in this study to produce each hydrogel were: by addition of Ca<sup>2+</sup> (P); by UV exposure (C); and combination of both by addition of Ca<sup>2+</sup> ions followed by UV exposure (PC). These hydrogels were then mechanically analyzed. The influence of three parameters over the mechanical properties was evaluated: the ionic concentration in the hydrogel fabrication (Figure 2A), type of crosslinking mechanism (Figure 2B) and polymer concentration (Figure 2C) while keeping the other conditions constant. A higher amount of Ca<sup>2+</sup> ions used in the fabrication of the High-MeGG hydrogels significantly decreased the Young's modulus. Moreover, the crosslinking mechanisms used to fabricate the hydrogels significantly influenced their mechanical properties. When only the physical mechanism was employed, the value of Young's modulus significantly

decreased with the increase in the degree of methacrylation of the materials. The chemical crosslinking alone led to the development of hydrogels with significantly higher Young's modulus when compared to those produced only with the physical mechanism. The combination of both crosslinking mechanisms significantly decreased the stiffness of High-MeGG hydrogels. Also, different concentrations were tested for the PC samples. The Young's modulus of the hydrogels significantly increased as the amount of polymer in the hydrogel network increased.



**Figure 2.** Influence of three parameters over the Young's modulus of GG and MeGG hydrogels: (A) concentration of ions in the hydrogel fabrication; (B) type of crosslinking mechanism; and (C) Polymer concentration. (Two-way ANOVA and Bonferroni post-hoc test showed significant differences (\*\*\*) $p < 0.0001$ , (\*\*) $p < 0.001$ , (\*) $p < 0.01$ ) between the analyzed groups. NP: not processable).

## CONCLUSIONS

MeGG hydrogels with two degrees of methacrylation were synthesized by reacting GG with different amounts of MA. The combination of physical crosslinking (temperature and the addition of cations) with chemical crosslinking (photocrosslinking) enabled the development of MeGG hydrogels with highly tunable mechanical properties.

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