A Novel Modified Chitosan PEG Bio-ink for use in Additive Manufacturing

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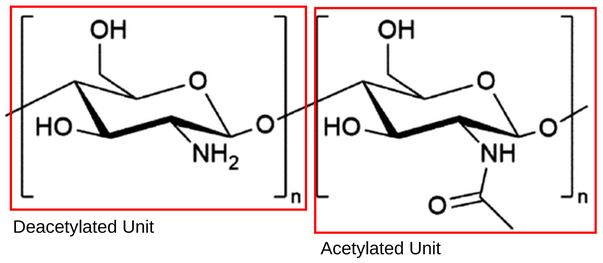
INTRODUCTION

The 3D printing of chitosan hydrogels has attracted wide interest because of their excellent biocompatibility, biodegradability, and low cost. [1] Chitosan has been shown to be able to be chemically modified with photo-polymerizable methacrylate (MA) group. However, printed chitosan scaffolds lack mechanical strength, limiting their use in tissue engineering. [1] Polyethylene glycol (PEG)-based hydrogels have proven extremely versatile for tissue engineering applications with superb mechanical strength. [2,3]

In this study, we created a methacrylate modified chitosan (N-MAC), by modifying the N-acetyl-D-glucosamine (acetylated unit) of the chitosan polymer for use in additive manufacturing, and by cross-linking chitosan with difunctional polyethylene glycol dimethacrylate (PEGDMA) we plan to create a double-network gel with improved strength characteristics over that of chitosan hydrogels. The aim is to create a composite photopolymerizable N-MAC-PEGDMA bio-ink for use in additive manufacturing.



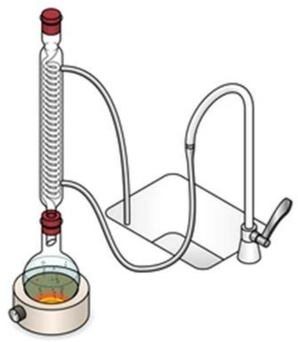
Deacetylated Unit

Acetylated Unit

METHODS

N-MAC Synthesis

A 1.25 weight % solution of 80% DDA chitosan is made using 1% acetic acid. The Chitosan solution is added to round bottom flask with a condenser and constantly stirred. The solution is then brought up to a constant temperature between 60-65 °C. Nitrogen gas is added to the flask to have the environment to remove ambient O_2. Methacrylic anhydride is added to the chitosan to a concentration of 2.7 vol%, giving a molar ratio of chitosan to methacrylic anhydride of 1:4. The system is covered to keep out light, and allowed to stir for 12 hours. The pH brought to 6 using a 1M solution of $NaHCO_3$ (sodium bicarbonate). DI water is used to double the volume. The N-MAC was dialyzed in DI water for 7 days to remove the unreacted reagent then lyophilized.



Bio-ink preparation

To create the bio-ink, a 2.5 wt% solution of N-MAC is made with DI water containing a 0.2 wt% of lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator. Once the chitosan has dissolved, 8 wt% of PEGDMA is added to the solution. Solution is made in a low light environment to prevent polymerization.

Bio-ink Testing

The N-MAC product was evaluated using Fourier-transform infrared spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR).

To measure the curing time, a 365 nm wavelength light was used to irradiate bio-ink which was sandwiched between two glass slides. After a time period, a hydrogel pattern can be visually observed, and the time for light irradiation is recorded as the curing time.

The solution was cast into cylindrical test specimen for compression testing in a Instron mechanical tester.

RESULTS

N-MAC Photopolymerization Rate

Photopolymerization of the N-MAC into stable hydrogel layer was found to be less than 10 seconds. During this time, the polymerization partially bonded the glass slides together.

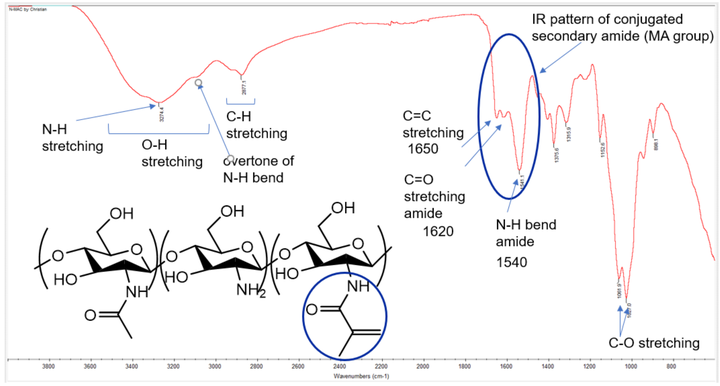


Figure 1. FTIR of the methacrylated chitosan

N-MAC FTIR Evaluation

MA was confirmed by FTIR spectrum. Circled group is ‘conjugated amide’ (double bond attaching to C=O) which is different from simple amide (chitin side group).

N-MAC NMR Evaluation

Percent of substitution evaluated using NMR (Fig. 2). The signals at 5.22 and 5.54 ppm represent vinyl protons, which was introduced by methacrylic anhydride. It was calculated that 35% of chitosan monomer unit had a MA group.

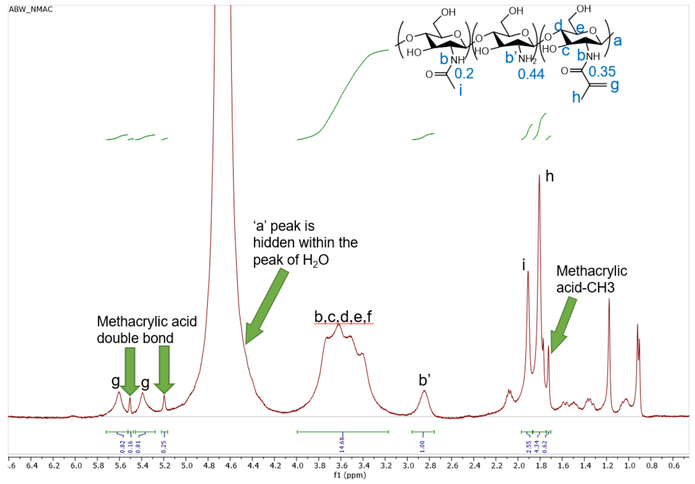


Figure 2. NMR of the methacrylated chitosan

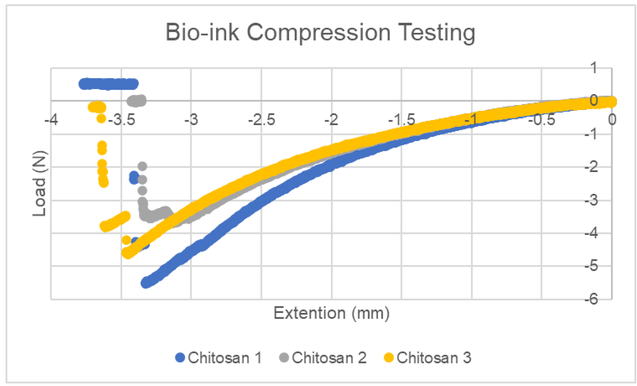


Figure 3. N-MAC-PEGDMA bio-ink replicated test. Rate of compression at 1 mm per min

Compression testing

Compressive testing showed similar results of failure. The average failure of the samples were at 4.63 N of load and 30% compression of sample.

CONCLUSIONS

Our method of N-MAC synthesis was successful at producing a photopolymerizable chitosan. However, the presence of MA residue show a need for increased purification during dialysis of the N-MAC. Photopolymerization rate is within a reasonable short time period for the use in 3D printing. After further refinement of the hydrogel’s mechanical properties, cytocompatibility of hydrogels will be evaluated.

References
[1] Dang, J M., Advd drug delvry rvws. 2006; 58.4: 487-499 .
[2] Lin, CC., Pharm Rsrch. 2009; 26.3: 631-643.
[3] Killion, J A., J of Mech Behavior of Bmed Mats. 2011; 4.7: 1219-1227