Gellan gum and trilysine hydrogels with tunable mechanical properties for drug delivery

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Statement of Purpose: Currently, hydrogels are being researched to achieve local delivery of therapies, as this approach has the potential to lessen systemic toxicities and increase the exposure time of the therapeutic agent in the targeted site.[1, 2] In the last decade, exploration of new biopolymers, such as gellan gum (GG), has increased for drug delivery utilizing various formats such as hydrogels, nanohydrogels, beads or films. The appeal for GG include its biodegradable nature, biocompatibility, stability under a broad range of pH and temperature conditions, rapid gelation, mucoadhesive, and tunable mechanical and physicochemical properties.[3] However, non-covalent crosslinking to form the hydrogel is temperature dependent and typically occurs at values above 40°C. Herein, we present the potential of using a peptide based molecule, trilysine, as an alternative crosslinker to traditional monovalent (Na^+) or divalent (Ca^{2+}) ions to crosslink gellan gum, with varying mechanical properties associated to both polymer and crosslinker concentration.

Methods: GG solution was prepared by adding GG powder into preheated water to ~ 70 °C and then allowed to hydrate under constant stirring for ~ 2 h, resulting in a clear and bubble free solution. The crosslinker solution was gradually added after the solution cooled down to ~ 40 °C and subsequently loaded on syringes. Injection force was characterized with an Instron 5565 instrument at a rate of 2 mm/min with a 50 N load cell. Drug release profile was evaluated for up to 28 d using PBS as the release media (1 mL) in microcentrifuge tubes containing hydrogel samples (~100 mg) and incubated at 37°C. Antibody concentration was determined via ELISA. Cell viability was assessed with normal human dermal fibroblast (NHDF) with the Resazurin test kit through indirect metabolic activity. Cells were incubated in a 24-well plate ($5x10^4$) and evaluated 48 h post exposure to hydrogel. All testing was conducted with three separate samples.

Results: Gellan gum-based hydrogels were produced after mixing trilysine under constant agitation with a bubble free solution of GG. Increasing concentrations of GG (1.0, 1.5, 2.0%) and trilysine (0.01 – 0.1%) were evaluated to determine the injection force requirements. As expected, when varying the GG concentration while keeping the trilysine concentration constant, the injection force requirement increased; when varying the trilysine concentration while keeping the GG concentration constant, the injection force requirement increased up to 0.05% trilysine, after which there was no increase in injection force (**Figure 1**). All injection force requirements were below the acceptability threshold, <38 N, for considerable effort from the user perspective as reported by Robinson T.E *et al.*[4] Additionally, from the application perspective, the ability to load the pre-gelled GG formulation into a syringe was compromised with

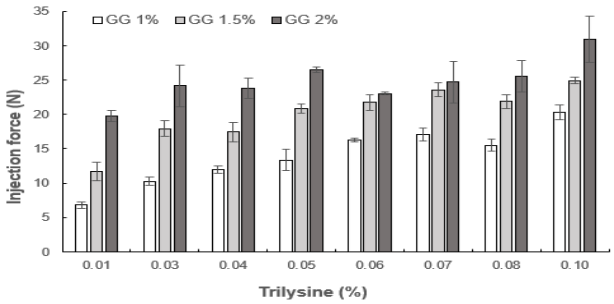

Figure 1. Injection force of gellan gum/trilysine hydrogels. Changes in GG concentration results in increasing injection forces with varying levels of trilysine

increases in trilysine concentration (> 0.05%), due to premature gelation. Drug release potential was evaluated using IgG as a surrogate molecule to antibodies. Preliminary results indicate an initial burst release with continued release over the first 24 h. This is an early indication of possible entrapment of IgG within the matrix (data not shown). Drug release evaluation will continue through 28 days to identify formulations with long-term release potential. Finally, it was confirmed that GG-based hydrogels crosslinked with trilysine would not pose toxicity when exposed to normal human dermal fibroblast cell viability (data not shown), for potential clinical applications. Data collected, presents the promise of the use of peptides in combination with gellan gum to produce biocompatible hydrogels with tunable mechanical properties.

This work serves as a foundation for the use of GG hydrogels as an antibody delivery platform utilizing a novel crosslinker that does not require high temperature processing. Future work will include *in vivo* efficacy of antibodies used for disease treatment of certain cancers as a local, sustained release platform.

References:

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