Gellan Gum And Trilysine Hydrogels with Tunable Mechanical Properties for Drug Delivery

Carolina Villarreal-Otalvaro*[1,2], Jeannine M. Coburn, Ph.D.[1]
[1]Worcester Polytechnic Institute; [2]Boston Scientific Corporation, Marlborough, MA

Introduction: Currently, hydrogels are 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. In the last decade, exploration of non-traditional 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.[1] However, non-covalent crosslinking to form the hydrogel is temperature dependent and typically occurs around 70 - 90°C. Herein, we present trilysine as an alternative crosslinker to traditional monovalent (Na^+) or divalent (Ca^{2+}) ions to crosslink gellan gum, and report on the mechanical properties, antibody release properties, degradation properties, and *in vitro* biocompatibility of the hydrogels formulated with varying gellan gum and trilysine concentration.

Materials and Methods: GG solution was prepared by adding GG powder into preheated water at ~ 70 °C and allowed to hydrate under constant agitation for ~ 2 h, resulting in a clear and bubble free solution. After the solution was cooled to ~ 40 °C, the crosslinker solution was gradually added before the formulations were loaded into syringes. Injection force was characterized with an Instron 5565 instrument (2 mm/min). Rheology testing was performed in a TA Instruments rheometer with a 60 mm cone plate at 37°C. IgG release was evaluated for up to 7 days using PBS as the release media (1 mL) in microcentrifuge tubes containing hydrogel samples (~100 mg) at 37°C. Antibody concentration was determined via ELISA. Swelling and degradation data was obtained from samples exposed to PBS for up to 2 weeks and measuring weights before and after lyophilization. Cell viability was assessed with normal human dermal fibroblast (NHDF) and murine colon adenocarcinoma (MC38) with a resazurin cell viability assay. NHDF and MC38 cells were incubated in a 24-well plate ($5x10^4$ and $5x10^3$, respectively) and evaluated 48 h post exposure to hydrogel. All testing was conducted with three separate samples.

Results and Discussion: Injection force requirements of GG-based hydrogels with varying concentrations of GG (1.0, 1.5, 2.0%) and trilysine (0.01 – 0.05%), resulted in higher injection forces when increasing both polymer and crosslinker concentration (**Figure 1**). All these pre-gelled GG formulations had the ability to be loaded into syringes without signs of premature gelation, in addition to achieving injection force values below the acceptability threshold, <38 N, for considerable effort from the user perspective as reported by Robinson T.E *et al*.[2] Storage modulus decreased with reduced concentrations of trilysine, represented by the linear viscoelastic region (**Figure 2**). Storage modules also declined with high temperatures (temperature sweep, data not shown). Drug release potential evaluated with IgG as a surrogate molecule to antibodies, indicated an initial burst release over the first 24 h with continued release up to 3 days. This is an indication of IgG entrapment within the matrix. IgG loading determined 50 % to 100% cumulative release associated to high (0.1 and 0.2 mg) and low (0.01 and 0.02 mg) IgG concentrations per 100 µL of hydrogel evaluated. Hydrogel matrix remained consistent through time as determined by swelling and degradation studies, among all varying concentrations (data not shown). Finally, it was confirmed that GG-based hydrogels would not pose toxicity when exposed to NHFD and MC38, for potential clinical applications, with 87 % and 99 % of cell viability respectively, as compared to non-hydrogel (100%). Data collected, presents the promise of the use of peptides in combination with gellan gum to produce biocompatible hydrogels with tunable mechanical properties.

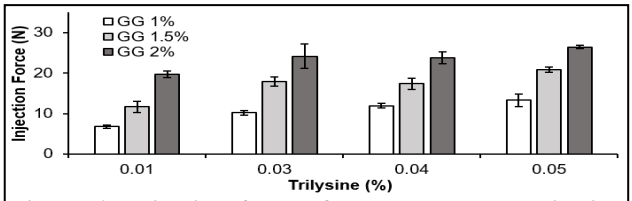

Figure 1. Injection force of gellan gum and trilysine hydrogels. Changes in GG and trilysine results in increasing injection forces below the user acceptability threshold (<38 N).

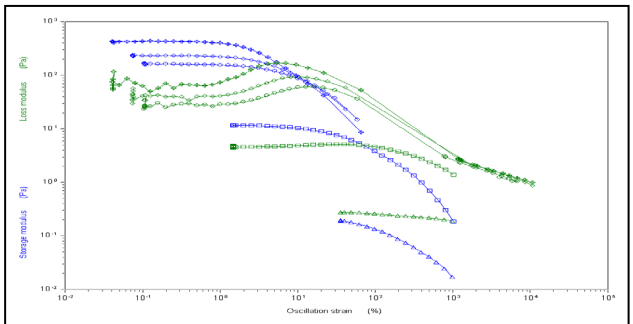

Figure 2. Amplitude sweeps of 1% GG-hydrogels with no trilysine (Δ), and increasing concentrations of trilysine, 0.01% (□), 0.03% (o), 0.04% (◊), and 0.05%

Conclusions: 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.

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References: 1. Osmałek, T *et al*, *Int J Pharm* **2014,** *466* (1-2), 328-40. 2. Robinson, T. E *et al*, *Adv H Mat* **2020,** *9* (5), 1901521.