Tuning the Strength & Swelling of an Injectable Polysaccharide Hydrogel, and the Subsequent Release of Nisin, a Broad Spectrum Bacteriocin

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Background: Controlling the release, swelling, mechanical properties and inhibitory activity of an antimicrobial peptide (AMP) from an injectable hydrogel could enable prolonged local treatment of infections. Here, it was hypothesized that tuning the composition of a dextran alginate crosslinked hydrogel via addition of glycol chitosan (GC) while reducing the concentration of alginate-hydrazine would control the release of the AMP from the resulting porous gel network, while preserving the gel strength and integrity.

Methods: Hydrogels of different compositions of dextran (Dex), alginate (Alg) and GC were made via injection of the two polymers through a double-barrelled syringe with crosslinking *in situ* occurring in an attached 21 G needle into a mold. Nisin was encapsulated such that 1 mg was contained in each gel. The release of nisin was studied into mFaSSGF (pH 1.6) and quantified using rp-HPLC. The swelling of the gels was studied in the same FaSSGF media where gels were submerged, taken out and blotted dry at different time points and weighed. Young's modulus was calculated using compression testing with a force of 100 kPa applied to each gel. The in vitro biocompatibility was determined using MTT assay on human embryonic kidney cells (HEK293), and *in vitro* antimicrobial activity was determined against *S. aureus* (20231 DSM).

Results: Gels were successfully formed with concentrations of 0, 3 and 6 % GC, when combined with 3, 1.5 and 0.5% Alg (respectively) and 6% Dex. Increasing the concentration of GC and subsequently reducing the concentration of Alg modulated the Young's modulus of the gels from 0.18 kPa at 0% GC to 19.8 and 37.3 kPa at 3 and 6% GC respectively. It was also found that the degree of swelling increased as GC increased, where 0% GC gels didn't swell, whereas 6% GC gels swelled by 250%. Increasing GC also slowed the release of nisin into FaSSGF from a max release of 70% (0% GC, day 12) to 25% (6% GC, day 12). Gels showed inhibition of *S. aureus* for at least 10 days. Interestingly, nisin and GC showed synergistic antimicrobial activity. The addition of GC didn't impact the *in vitro* biocompatibility of the gels (HEK293).

Conclusions: The incorporation of glycol chitosan into an injectable polysaccharide gel allows for modulation of the gels swelling and mechanical properties without sacrifice of physiologically appropriate conditions for an encapsulated antimicrobial peptide. In previous investigations, the mechanical strength of hydrogels has been modulated through means of varying ionic concentrations, rendering the gels unsuitable for sensitive biologics. By introducing GC into these gels, the mechanical strength of the gels was increased through varying GC concentrations. The incorporation of glycol chitosan also does not affect the biocompatibility, and it has been found to act synergistically with nisin in the inhibition of the growth of *S. aureus*. This study introduces a highly tuneable platform for the encapsulation and subsequent release of the AMP nisin for at least 10 days.

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