Injectable Enzymatically Crosslinked Hydrogels: A Unique Platform with Tunable Degradation Properties

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Statement of Purpose: Injectable hydrogels have recently emerged as promising biomaterials due to their minimally invasive application. Chitosan is a widely investigated biocompatible polymer with potent wound healing properties [1]. This has been attributed to its ability to modulate the phenotypic polarization of macrophages to an anti-inflammatory, wound healing M2 phenotype [2]. The inadequate solubility of chitosan at physiological pH however, limits its use as an injectable hydrogel. We have developed an aqueous, enzymatically cross-linkable chitosan hydrogel to address this limitation. Biodegradation of carrier also plays a significant role in determining its suitability for a wide spectrum of biomedical applications. The objective of the present study is to develop a chitosan-based injectable hydrogel platform with controllable *in vitro* and *in vivo* degradation properties and to evaluate the *in vivo* cellular response to these hydrogels.

Methods:

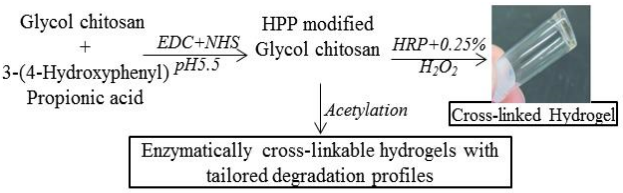

Figure 1. Schematic showing development of enzymatically crosslinkable chitosan hydrogels with tailored degradation

Glycol-chitosan was modified with 3-(4-hydroxyphenyl) propionic acid (HPP) by carbodiimide coupling. The acetyl content of the modified polymer was varied by reacetylating the polymer with increasing concentrations of acetic anhydride (Fig.1). ^{1}H-NMR was used to confirm phenol modification and changes in degree of acetylation (DA). Gelation was initiated by enzymatic crosslinking of aqueous polymer solution in presence of horse radish peroxidase (HRP, 20 U/mL) and hydrogen peroxide (H_2O_2). The gels were characterized by rheology. The *in vitro* accelerated gel degradation was evaluated in PBS containing 50µg lysozyme at 37°C, by weight and morphology changes (SEM). *In vivo* gel degradation was evaluated by implantation of 20µl gel in subcutaneous (SC) dorsal sites of mice. The *in vivo* biocompatibility, degradation and local cellular response were analyzed by histomorphometry.

Results:

The %DA of the acetylated polymers was calculated from the ^{1}H-NMR data, using the following equation,

$$DA(\%) = 100 - \left\{\left[1 - \left(\frac{\frac{1}{3}HAc}{\frac{1}{6}H2-6}\right)\right] \times 100\right\} \quad \text{(Fig.2).}$$

Rheological characterization studies showed an increase in hydrogels' storage modulus with increasing DA. The accelerated *in vitro* degradation of the hydrogels was found to be directly proportional to the DA of the polymer. Fig.3A&B shows the feasibility to modulate hydrogel degradation by varying the chitosan DA. The 7%DA gels showed slowest degradation and minimal weight loss during the duration of the study. The 27%DA gels were completely degraded within 120h, whereas 38%DA gels were completely degraded in <65h. The histological sections of SC implanted hydrogels showed the presence of non-degraded hydrogel (arrow) in 7%DA group at 14 days (Fig.3C). The 38%DA hydrogel showed complete degradation at that time. Significant differences were observed in the cellular response to implanted gels with varying DA.

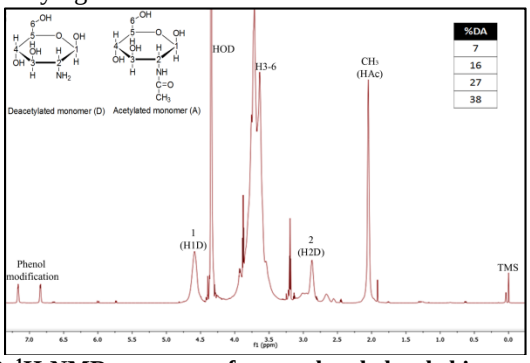

Fig 2. ^{1}H-NMR spectrum of reacetylated glycol chitosan.

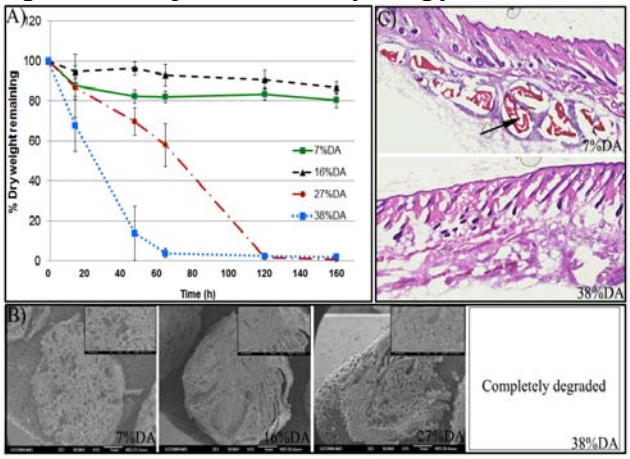

Figure 3. *In vitro* accelerated gel degradation showing changes in A) Dry weight with time and B) Morphology at 65h; C) H&E showing *in vivo* degradation at 14 days.

Conclusions: The study demonstrated for the first time, the feasibility of developing an enzymatically cross-linkable hydrogel platform with a wide range of degradation characteristics. The tunable degradation, together with the good biocompatibility and aqueous solubility of this hydrogel platform makes it a potential candidate for a broad range of biomedical applications.

Reference: [1]. S.V. Gohil, L.S. Nair; in *"Biomaterials Science: Processing, properties and applications IV: Ceramic Transactions"*, 2014, 251, 95–104.

[2]. M. I. Oliveira *et al*, European Cells and Materials, 2012, 24, 136-153.

Acknowledgement: W81XWH-10-1-0653