Injectable chitosan hydrogels with tailored degradation and release properties for localized biomolecule delivery

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Statement of Purpose: Chitosan is a biocompatible and biodegradable polysaccharide, widely investigated for drug/protein delivery and tissue engineering applications [1]. Injectable hydrogels are attractive alternatives to implantable carriers as they can provide a mild environment and allow for minimally invasive administration. The poor solubility of chitosan at physiological pH presents challenges in developing injectable chitosan gels. The purpose of this study is to develop an injectable chitosan hydrogel as localized protein delivery vehicle *via* a mild enzymatic crosslinking process. We investigated the ability of the hydrogel to retain the encapsulated protein in a bioactive form and to support localized bone formation at the injection site *in vivo*. We further investigated the feasibility to modulate hydrogel gel degradation time and protein release kinetics by varying chitosan acetyl content.

Methods:

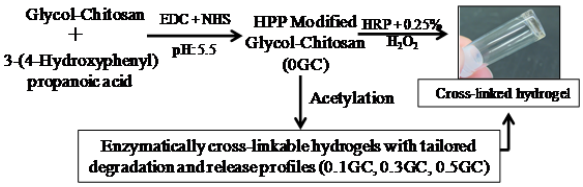

Figure 1. Schematic showing the development of enzymatically crosslinkable chitosan hydrogels

Glycol-chitosan was modified with 3-(4-hydroxyphenyl) propanoic acid (HPP) using standard carbodiimide chemistry. The HPP modified glycol chitosan (0GC) was highly soluble in aqueous media. Addition of horse radish peroxidase (HRP, 20 U/mL) and hydrogen peroxide (H_2O_2) to 0GC induced polymer gelation with a gelation time less than 1 min. *In vitro* rhBMP-2 release from the gel was followed *via* ELISA. Ability of the gel to support bone formation in the presence of rhBMP-2 was evaluated using a bilateral calvarial bone defect model in transgenic Col3.6 osteoblast reporter mouse. The acetyl content of 0GC was varied by reacting with increasing concentrations of acetic anhydride (0.1GC, 0.3GC & 0.5GC). The degradation and release characteristics of the gels were evaluated in PBS containing lysozyme at 37°C.

Results: *In vitro* release study showed the ability of the chitosan gel to retain rhBMP-2 with only ~ 11% released in 400h **(Fig 2)**. To better understand the ability of the gel to activate the regenerative process locally and to support confined bone formation at the injection site *in vivo* Col3.6Tpz osteoblast reporter mice were utilized. In a two hole calvarial model, the hydrogel loaded with 2µg rhBMP-2 (0GC+rhBMP-2) showed localized bone formation (DIC) with strongly GFP+ve osteoblasts and a few TRAP+ve osteoclasts (TRAP), along with ALP+ve osteoblasts at the site of injection, with no osteogenic cellular activity in the neighboring site injected with hydrogel alone (0GC). The data demonstrates the ability of the gel to retain rhBMP-2 bioactivity and restrict rhBMP-2 mediated osteogenic cellular activity at the site of injection **(Fig 3)**.

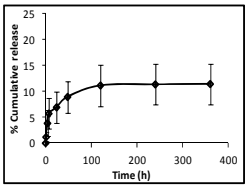

Figure2. *In vitro* rhBMP-2 release from 0GC in PBS

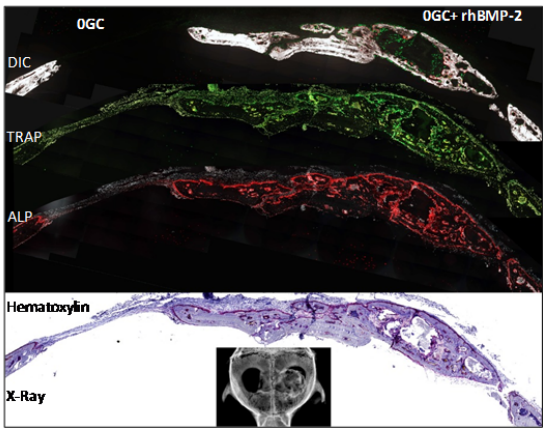

Figure 3. Histology and x-ray image of whole calvaria after 4 weeks of implantation with HPP modified glycol chitosan hydrogels with or without rhBMP-2

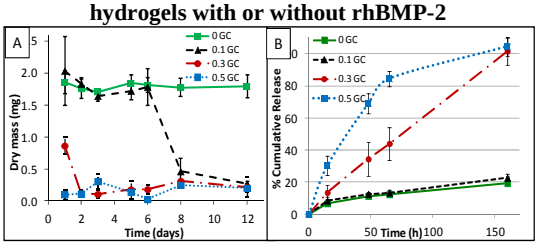

Figure 4. A) Gel degradation in PBS with 200µg lysozyme B) *In vitro* release of FITC-albumin in PBS with 50µg lysozyme

However, at 4 weeks post implantation non-degraded gel residues were still evident necessitating faster gel degradation. Acetylation of 0GC significantly changed the degradation profile. Higher the degree of acetylation, faster was the degradation rate **(Fig 4A)**. The degradation rate was found to have significant impact on protein release from the gels *in vitro* **(Fig 4B)**. The data demonstrates the feasibility to fine tune the hydrogel degradation and protein release kinetics by acetylating HPP modified glycol chitosan without compromising its solubility and ability to undergo enzymatic crosslinking.

Conclusions: The study demonstrated the feasibility of developing enzymatically crosslinkable hydrogels capable of supporting localized bone formation in the presence of rhBMP-2. The gel degradation and protein release can be further tailored *via* polymer modification. Due to the chemical versatility, these gels have the potential to serve as a carrier platform with tunable degradation and release kinetics for localized drug/growth factor delivery.

Reference: Dash M, Prog Poly Sci, 36, 2011: 981-1014

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