Modification of an Injectable, Biodegradable Polymer for Sustained Local Protein Delivery

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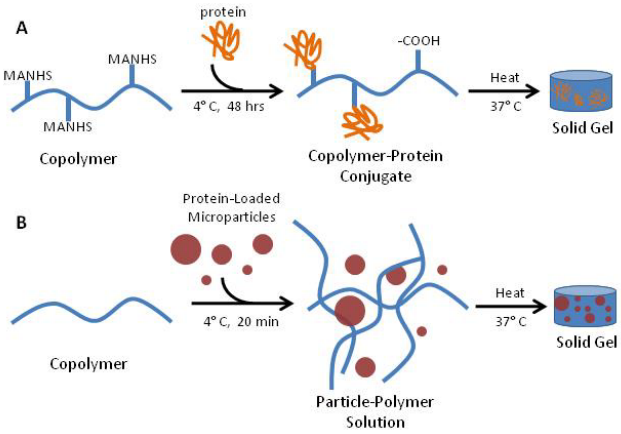
Statement of Purpose: Stimuli-responsive materials have been studied in many applications ranging from sensors to drug delivery. The advantage of such materials is that they can be designed to change behavior depending on their environment. For example, thermally-responsive materials can change from liquid to a solid structure upon heating to body temperature – giving them application as injectable biomaterials. Several groups have utilized injectable biomaterials as a means to treat ischemic cardiomyopathy with injection of both biological and synthetic materials being shown to positively impact cardiac remodeling and function. The benefits of a given material are generally greater when incorporating controlled release of growth factors.

We have previously reported on the development and application of a thermally-responsive N-isopropyl acrylamide (NIPAAm) based copolymer that, when injected into infarcted myocardium, attenuates cardiac dilation, maintains cardiac function, and elicits local muscle formation.[1] Here we report on broadening the functionality of a NIPAAm-based hydrogel by investigating two approaches to achieve a controlled protein release profile: 1) covalently attaching protein to the polymer, and 2) mixing protein-loaded microparticles within the hydrogel.

Methods: Hydrogel 1 consisted of NIPAAm, 2-hydroxyethyl methacrylate (HEMA), and biodegradable polylactide-methacrylate (MAPLA) at a molar ratio of 80:10:10, synthesized by free radical polymerization. Hydrogel 2 was similar to Hydrogel 1 but with the addition of 1 mol% methacrylate-N,hydroxy succinimide (MANHS) to facilitate covalent attachment to proteins. Hydrogel 3 also contained 1 mol% MANHS but additionally had 1 mol% acrylic acid (AAc) to increase protein delivery rate. All polymers were dissolved at 16.7 wt% into a cold protein/PBS solution. Attachment of protein to Hydrogels 2 and 3 occurred for 48 h at 4 °C in PBS. Additionally, protein-filled microparticles of poly(lactide-co-glycolide) (PLGA, 75:25) were synthesized using a double emulsion technique and mixed with Hydrogel 1 – **Scheme 1**. BSA-I^{125} (Perkin Elmer) was used in all studies. Hydrogels with and without microparticles, and microparticles alone were kept in PBS at 37° C. Incubation fluid was changed at designated time points and protein content measured using a gamma counter (Cobra II, Packard Instruments).

Results: Protein released during hydrogel transition from liquid to gel was highest in Hydrogel 1 at 78.0%, indicating a loading efficiency of 22.0%. The loading efficiency improved to 45.5% and 40.6% in Hydrogel 2 and, 3 respectively ($p<0.05$), showing the beneficial effects of covalent protein attachment. After gel formation the release rates were near zero-order for up to 3 months, with AAc increasing the delivery rate. For particle-loaded gels 96.2% of protein remained following gel formation. Both burst release and the extended release rates of BSA were slowed when particles were contained in the hydrogel structure - providing a more linear release profile (**Fig. 1**).



Scheme 1. A) Approach 1: Covalent attachment of protein to copolymer, B) Approach 2: Mixing of microparticles into hydrogel solution before gelling

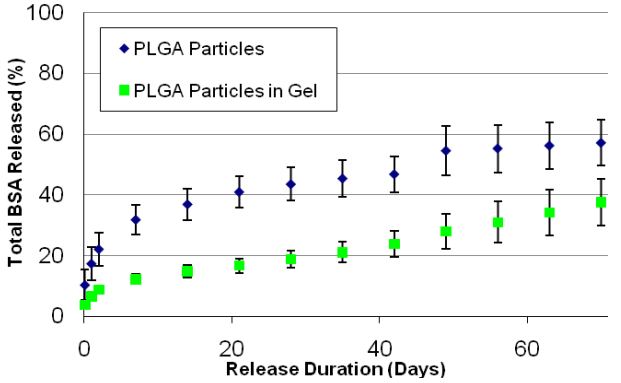


Fig. 1 BSA release from PLGA particles with/without gel

Conclusions: The biodegradable hydrogels discussed here can provide extended protein delivery at near zero order release rates *in vitro*. The rate of delivery can be altered through covalent protein attachment to the polymer and through inclusion of protein-loaded microparticles to the gel solution. These results suggest long-term delivery of proteins such as growth factors may be possible at a site of polymer injection.

References:

1. Fujimoto KL. Biomaterials 2009;30:4357–68.