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An in situ cross-linking hybrid hydrogel for controlled release of proteins

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ABSTRACT

There is a clear need for methods to provide a safe controlled release of therapeutic proteins, either to achieve and maintain high local protein concentrations, or for sustained systemic delivery. We have developed a protein delivery system that combines in situ cross-linkable polysaccharide hydrogels with gelatin. This formulation is injectable, easy to apply, and obviates the need for organic solvents or potentially toxic cross-linking agents in the formulation process. The cross-linked polysaccharides themselves (comprising hyaluronic acid, dextran and/or carboxymethylcellulose) provided prolonged release of fluorescently labeled albumin (FITC-albumin). The duration of release was markedly extended by the incorporation of gelatin into the formulation: FITC-albumin and interleukin-2 (IL-2) were released over the course of more than 3 weeks. The IL-2 maintained >70% activity throughout that time. Gelatin also accelerated the gelation time of the hydrogels, and reduced their swelling in phosphate-buffered saline. The composite hydrogel (dextran–carboxymethylcellulose–gelatin) showed minimal cytotoxicity in vitro, and benign tissue reaction after subcutaneous injection in rats.

1. Introduction

The proliferation of protein-based therapeutics has created broad interest in the development of controlled release technologies for proteins, either to achieve and maintain high local protein concentrations, or for sustained systemic delivery. Proteins can benefit greatly from controlled release systems, to overcome problems with bioavailability and short plasma half-lives [1,2]. Therefore, a number of controlled release systems, including osmotic pumps [3,4], Pluronic gels [5], gelatin [6], collagen [7,8] and biodegradable polymeric particles [9], have been used to prolong the systemic serum presence of proteins.

Recently, there has been increasing interest in highly localized protein delivery over extended periods, particularly since systemic distribution of many proteins can create toxic side effects. For example, polymer-based microsphere formulations have been developed to provide sustained, localized, cytokine delivery [9]. Hydrogels can be excellent candidates for protein delivery [6,10] since they may not have the pro-inflammatory effects of polymeric systems [11] and tissue reaction to them tends to be benign [12,13]. Furthermore, unlike polymeric systems, there may be no need for surfactants, organic phases or sonication which can reduce the biological efficacy of the protein [2,14]. Protein-based hydrogels may be able to control the rate of release of proteins drugs through electrostatic interactions as well as through degradation, resulting in quite extended release profiles, as was the case with gelatin-based systems [2,14]. Disadvantages of the gelatin hydrogels included the fact that formation required a cross-linker such as glutaraldehyde [15], and that as a consequence, the gels had to be cast in advance. While they could be produced as microspheres, that still does not allow the flexibility upon application of in situ cross-linking hydrogels in terms of reliably coating complex surfaces (e.g. the peritoneal cavity, or specific portions thereof) in an ad hoc manner during procedures [16].

We have previously shown that hyaluronic acid hydrogels [17] that cross-link in situ by hydrazone bond formation have excellent biocompatibility in the peritoneum—a relatively sensitive anatomic location [18]—and that when optimized those hydrogels can release a protein such as tissue plasminogen activator (tPA) with great effectiveness in preventing adhesions [19]. Although the released tPA was biologically effective, approximately 80% of it was released within 2 days [19].

Here we have hypothesized that combining in situ cross-linkable polysaccharide hydrogels with gelatin would provide the ease of application and biocompatibility of in situ cross-linking hydrogels with the prolonged protein release of gelatin-based systems, without needing surfactants and toxic cross-linkers.

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