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Modulated release of bioactive protein from multilayered blended PLGA coatings

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Abstract

The objective of this study was to develop a poly(D,L-lactic-co-glycolic acid) (PLGA)-based coating system for producing biologically-inspired delivery profiles. Protein-loaded microspheres were made from PLGA (50:50) terminated with carboxylic acid groups (PLGA-2A) blended either with more hydrophobic PLGA (50:50) having lauryl ester endcaps (PLGA-LE) or with the more hydrophilic Pluronic F-127 (PF-127). Dense coatings were formed by pressure-sintering the microspheres. Altering hydrophobicity changed the water concentration within coatings, and consequently the time to onset of polymer degradation and protein release was modulated. After blending up to 8% Pluronic, degradation by-products began accumulating immediately upon incubation in saline, whereas, degradation was delayed for up to 14 days with blending of up to 30% PLGA-LE. Primary protein release peaks from one-layer coatings could be created from 7 to 20 days using 8% PF-127 or 30% PLGA-LE blends, respectively. Multilayered coatings of different blends generated several release peaks, with their temporal occurrence remaining approximately the same when layers of other hydrophobicity were added above or below. To allow design of coatings for future use, results were used to construct a model based on Fourier analysis. This polymer blend system and model can be used to mimic temporally varying profiles of protein expression.

Keywords: Polymer blending; PLGA; Protein release; Multilayered; Biodegradable

1. Introduction

The goal of many drug delivery devices has been to obtain zero-order release kinetics. For applications such as treatment of some systemic or chronic diseases, this approach, which is similar to continuous intravenous administration, may be desirable. However, wound healing is a dynamic process involving numerous biomolecules that trigger chemotaxis, proliferation, and differentiation of several cell types. For example, analysis of growth factor expression in callus during bone fracture healing has revealed a complex sequence of several biomolecules, including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), transforming growth factor (TGF), and bone morphogenetic protein (BMP) (e.g. (Bolander, 1992; Bourque et al., 1993; Bostrom et al., 1995; Yu et al., 2002)). Constant delivery of a single type of molecule may alter cell and tissue behavior, but it does not optimally coordinate appropriate responses. Additionally, sustained exposure, high concentrations of biomolecules, and/or delivery at inappropriate times can desensitize cells as they downregulate expression of receptors or lead to adverse cell behavior (e.g. (Heldin et al., 1982; Assoian, 1985; Border and Ruoslahti, 1992; Lee et al., 1997)). Delivery of growth factors with release profiles inspired by those of natural wound healing may enhance healing and improve integration of implants in bone. Ideally, devices also would be able to deliver multiple molecules with different times to onset of release, similar to those found during bone repair.

In previous work, we developed a multilayered gelatin system for the combined or sequential release of multiple osteotropic biomolecules (Raiche and Puleo, 2001, 2004a,b). By heterogeneously loading and crosslinking the gelatin, different release profiles could be achieved. Osteoblastic responses could be modulated by releasing BMP-2 and IGF-I individually, together, or in sequence. Alkaline phosphatase activity and matrix mineralization were greatest with release of BMP-2 followed by IGF-I or by

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BMP-2 + IGF-I. Recurrent concerns about glutaraldehyde used as a crosslinking agent, however, stimulated interest in developing alternate delivery systems based on synthetic polymers.

Biodegradable polyesters of lactic and glycolic acids have enjoyed remarkably broad medical application for nearly 30 years. Despite potentially negative side effects resulting from acidic degradation products, poly(D,L-lactic-co-glycolic acid) (PLGA) devices have been shown to be biocompatible for orthopedic, soft tissue, non-load-bearing, and load-bearing applications (reviewed in Athanasiou et al. (1996), An et al. (2000), Peltoniemi et al. (2002)). PLGA microspheres and films have been widely used for controlled drug release. Both small molecules, such as opioid analogs and hormones, and large proteins, such as growth factors, have been delivered from PLGA devices (e.g. (Yolles et al., 1975; Wang et al., 1996; Cleland et al., 1997; Burton et al., 2000; Charlier et al., 2000; Elkheshen and Radwan, 2000; Pean et al., 2000; Cleland et al., 2001; Han et al., 2001; Lu et al., 2001; Meinel et al., 2001; Woo et al., 2001; Kim and Burgess, 2002)).

Several approaches have been used to modulate release of drugs from PLGA. Techniques include colyophilizing amphiphilic polymers with the protein to be released (Morita et al., 2001), addition of surfactants to the aqueous, drug-containing phase of a double emulsion (Blanco and Alonso, 1998; Rojas et al., 1999), copolymerizing with poly(ethylene glycol) (Penco et al., 1996; Bae et al., 2000), blending with different molecular weights of the same polymer (Bodmeier et al., 1989; von Recum et al., 1995), or blending with polymers of different hydrophobicity/hydrophilicity (Cha and Pitt, 1990; Park et al., 1992; Yeh et al., 1996; Cleek et al., 1997; Ravivarapu et al., 2000). These approaches cannot only change the release kinetics over a period of days to years, but the bioactivity of encapsulated proteins also may be protected.

The present studies sought to build on the previously referenced work by blending hydrophilic and hydrophobic polymers to change the equilibrium water content within biodegradable coatings and therefore to change the time to onset of protein release. Specifically, the objectives of this project were to: (a) develop multilayered PLGA-based coatings that mimic time-dependent concentrations of growth factors present during natural wound healing, and (b) develop a model capable of describing drug release by manipulation of the independent variable, hydrophobicity.

2. Materials and methods

2.1. Polymers and blending

PLGA is generally made by a ring opening polymerization reaction catalyzed by stannous octanoate or zinc (Vert et al., 1998). Use of stannous octanoate and hydrophobic molecules present during purification results in polymer chains terminating with aliphatic chains. One version, which we will designate PLGA-LE (50:50, MW 63,000 Da, T_g 47 °C; Alkermes, Wilmington, OH), requires months to degrade because of its high molecular weight and hydrophobic lauryl ester endgroups.

Current processing and purification techniques allow for manufacture of PLGA terminated with carboxylic acid groups. One such polymer, PLGA-2A (50:50, MW 11,000 Da, T_g 41 °C; Alkermes), degrades after approximately 2 weeks. Its low molecular weight and hydrophilic endgroups are responsible for the relatively short degradation time. Because of its shorter degradation time, PLGA-2A was used as the base polymer for this study.

Pluronic F-127 (PF-127; Sigma, St. Louis, MO) is a triblock copolymer of polyethylene oxide (PEO) and polypropylene oxide with molecular weight of approximately 12,300 Da, 70% of which is PEO (Schmolka, 1972). PEO segments increase the overall hydrophilicity of the copolymer and make it water soluble. Pluronics are widely used for their gel forming, surfactant, and non-ionic interaction properties. They have been incorporated into microspheres as excipients because their non-ionic interactions protect proteins and drugs during double emulsion and spray-freeze drying processes (Carrasquillo et al., 2001; Giunchedi et al., 2001; Morita et al., 2001).

Blending was used to alter hydrophobicity of the coatings consisting primarily of PLGA-2A. Preliminary studies showed that blending up to 30% by mass PLGA-LE into PLGA-2A represented an upper limit to maintaining a homogeneous distribution of both polymers within a single coating layer. Similarly, up to 8% by mass PF-127 was blended in PLGA-2A to increase hydrophilicity.

2.2. Microsphere preparation

Protein-loaded microspheres were made using a conventional W/O/W process. A 1% solution of lysozyme was made in phosphate-buffered saline (PBS), pH 7.4. Lysozyme was selected as a model protein because its size and isoelectric point are similar to those of relevant growth factors. Also, being an enzyme, bioactivity of released protein can be readily measured. To reduce inactivation of encapsulated enzyme resulting from contact with hydrophobic polymer and solvent, 2% gelatin was added to the lysozyme solution. Lysozyme-gelatin solutions were added 1:9 v/v to 10 wt.% total polymer (i.e., PLGA-2A, PLGA-LE, and PF-127) in methylene chloride, were vortexed, and were sonicated at 25 W for 5 s to form the first emulsion. The W/O emulsion was transferred 1:9 v/v to a solution of 1% polyvinyl alcohol (MW 30,000–70,000 Da; Sigma) in deionized water to form the W/O/W emulsion and mixed for 4 h. Microspheres were collected by centrifugation, filtered using an 8 μm membrane, rinsed with deionized water, and dried under vacuum for 48 h. Control (blank) micropheres were prepared identically except that lysozyme was omitted from the PBS solution.

2.3. Pressure-sintered multilayered coatings

Dense coatings were made using methods adapted from those originally described by Cohen et al. (1984) and more recently by Nof and Shea (2002). By applying pressure while the polymer is above its glass transition temperature, particles are consolidated. Because the glass transition temperatures of polymer blends in the present work were in excess of 40 °C, microspheres