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A biomimetic approach to active self-microencapsulation of proteins in PLGA

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ABSTRACT

A biomimetic approach to organic solvent-free microencapsulation of proteins based on the self-healing capacity of poly (DL)-lactic-co-glycolic acid (PLGA) microspheres containing glycosaminoglycan-like biopolymers (BPs), was examined. To screen BPs, aqueous solutions of BP [high molecular weight dextran sulfate (HDS), low molecular weight dextran sulfate (LDS), chondroitin sulfate (CS), heparin (HP), hyaluronic acid (HA), chitosan (CH)] and model protein lysozyme (LYZ) were combined in different molar and mass ratios, at 37 °C and pH 7. The BP-PLGA microspheres (20–63 μm) were prepared by a double water–oil–water emulsion method with a range of BP content, and trehalose and $MgCO_3$ to control microclimate pH and to create percolating pores for protein. Biomimetic active self-encapsulation (ASE) of proteins [LYZ, vascular endothelial growth factor165 (VEGF) and fibroblast growth factor (FgF-20)] was accomplished by incubating blank BP-PLGA microspheres in low concentration protein solutions at ~24 °C, for 48 h. Pore closure was induced at 42.5 °C under mild agitation for 42 h. Formulation parameters of BP-PLGA microspheres and loading conditions were studied to optimize protein loading and subsequent release. LDS and HP were found to bind >95% LYZ at BP:LYZ > 0.125 w/w, whereas HDS and CS bound >80% LYZ at BP:LYZ of 0.25–1 and <0.33, respectively. HA-PLGA microspheres were found to be not ideal for obtaining high protein loading (>2% w/w of LYZ). Sulfated BP-PLGA microspheres were capable of loading LYZ (~2–7% w/w), VEGF (~4% w/w), and FgF-20 (~2% w/w) with high efficiency. Protein loading was found to be dependent on the loading solution concentration, with higher protein loading obtained at higher loading solution concentration within the range investigated. Loading also increased with content of sulfated BP in microspheres. Release kinetics of proteins was evaluated *in-vitro* with complete release media replacement. Rate and extent of release were found to depend upon volume of release (with non-sink conditions observed <5 ml release volume for ~18 mg loaded BP-PLGA microspheres), ionic strength of release media and loading solution concentration. HDS-PLGA formulations were identified as having ideal loading and release characteristics. These optimal microspheres released ~73–80% of the encapsulated LYZ over 60 days, with >90% of protein being enzymatically active. Nearly 72% of immunoreactive VEGF was similarly released over 42 days, without significant losses in heparin binding affinity in the release medium.

1. Introduction

The rapidly emerging classes of bio-pharmaceutical products present increasingly complex challenges to the traditional pharmaceutical formulation paradigms. New delivery strategies and technologies have been developed to address these needs, although a vast majority of the new approaches have yet to produce a significant number of commercially viable products. Numerous local/regional and targeting modalities/strategies have also been used in conjunction with these systems to enhance delivery, efficacy and reduce costs, but better drug delivery methodologies need to be developed to help increase the use of biologics [1,2].

Biodegradable microspheres composed of natural or synthetic polymers have emerged as suitable delivery systems for controlled release of proteins, peptides, growth factors, small molecules and chemotherapeutic agents [3,4]. Poly(lactic-co-glycolic acid) (PLGA)-based polymers possess highly desirable qualities such as biodegradability and biocompatibility, when employed to fabricate systems for drug delivery [5]. PLGA has been incorporated in numerous products approved by the United States Food and Drug Administration, and this established use makes it an attractive polymer for developing new delivery systems. One major drawback of the polymer is the commonly observed acidic microenvironment, due to the build-up of degradation products in the polymer [6,7]. However, this issue has largely been overcome by

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