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Injectable Biodegradable Polymer Depots For Minimally Invasive Delivery Of Peptides and Proteins

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Introduction

With hundreds of biotechnological drugs, mostly peptides and proteins, undergoing clinical trials, there is an urgent need for improved methods to deliver these magnificent biomacromolecules. Unfortunately, when administered to the body by noninvasive routes, most peptide and protein drugs suffer from significant biological barriers such as poor drug permeability across intestinal mucosa and susceptibility to proteolysis, which in turn, prevents the drug from reaching the systemic circulation and the desired pharmacological target. Therefore, this class of drug molecules is most commonly delivered by injection (e.g., subcutaneously (s.c.) or intravenously (i.v.)) allowing immediate (i.v.) or easy (s.c.) access to the blood circulation. However, even when administered i.v., the body normally eliminates the drug rapidly, requiring the drug be injected repeatedly (e.g., multiple times a day or week). Frequent injections often result in significant pain, poor patient compliance, physiological stress, and peak-and-valley drug levels in the bloodstream.

Several promising methods to overcome difficulties with peptide and protein delivery have been pursued, such as a) altering the chemical structure of drug molecule to increase plasma half-life (e.g., PEGylation), b) utilizing mucosal routes with improved drug bioavailability (e.g., pulmonary and intranasal), and c) extending drug release by microencapsulating the drug in biodegradable polymer depots. Injection frequency can be reduced from daily injections to 1–6 months for current marketed controlled-release peptide formulations, which are prepared from the most common biodegradable polymer for long-term controlled release, poly(lactic-co-glycolic acid) (PLGA). PLGA as a polyester degrades by nonenzymatic hydrolysis to yield nontoxic byproducts while slowly and continuously releasing the medication. PLGA depots have successfully been used to treat a variety of diseases, including those afflicting children and young adults, e.g., central precocious puberty (leuprolide acetate) [1], acromegaly (octreotide acetate) [2], disorders of short stature (human growth hormone) [3], and endometriosis (leuprolide) [1]. In addition to systemic delivery of polypeptides, injectable PLGA depots are also used for drug delivery directly to the target (i.e., so-called “site-specific drug delivery”), e.g., allowing novel growth factors to promote growth of new blood vessels (e.g., basic fibroblast growth factor) or bone (e.g., bone morphogenetic proteins). PLGA depots are not limited to drug delivery and are among the most sought after alternative to aluminum-based adjuvants, which are the only adjuvants approved for human use in the US, and are used to deliver childhood vaccines. Finally, novel peptide antigens, which currently cannot be delivered effectively with aluminum compounds may also benefit from this safe and flexible dosage form.

Despite tremendous progress in development of injectable peptide depots, several issues have impeded their more widespread development. Important obstacles include: a) stability of the peptide/protein when encapsulated in the polymer, b) a difficulty to microencapsulate simply and inexpensively, c) poor control of drug release over the first day following administration (i.e., the initial burst release), and d) a low immune response to PLGA-encapsulated peptide antigens. We summarize here two examples of our approaches to overcome these limitations, namely: a) stabilization of PLGA-encapsulated bFGF to promote blood reperfusion and rescue ischemic SCID murine hindlimbs [4, 5], and b) the strong and unexpected enhancement of the antibody response to a birth control peptide antigen encapsulated in pH-modified PLGA [6].

Results and Discussion

During biodegradation of PLGA, the polymer becomes an acid producer as ester bonds are cleaved to yield the corresponding carboxylic acid and alcohol moieties. Whereas an acidic pH (as low as < 3 [7]) in the aqueous peptide-containing PLGA pores is often desirable for stability of many peptide drugs, most protein molecules cannot survive this microenvironment. For example, bovine serum albumin (BSA) undergoes extensive noncovalent aggregation and peptide bond hydrolysis and heparin-stabilized bFGF similarly loses immunoreactivity during one-month release from PLGA 50/50 (D,L-lactic/glycolic acid ratio) [4]. Formulations involving the co-incorporation in PLGA poorly soluble bases (i.e., antacids) such as $Mg(OH)_2$ have been shown to prevent this pH drop homogeneously throughout the polymer and facilitate stabilization and controlled release of BSA and bFGF in vitro [4].

Such protein-stabilized injectable PLGA cylindrical implants (0.8 mm diameter) containing bFGF, $Mg(OH)_2$ and other protein specific stabilizers (e.g., heparin) (stabilized—S group) were prepared by extruding a suspension of solid protein and excipients in PLGA 50/50 (inherent viscosity = 0.60 dL/g)/acetone into silicone rubber tubing before vacuum drying and removal of the solvent. Implants were placed at the site of injury in young male SCID mice whose external iliac and femoral artery and vein had been ligated and cut to create a hindlimb ischemia [5]. Limbs were visually monitored for necrosis and reperfusion of blood flow was measured noninvasively by laser Doppler perfusion imaging (LDPI) using each animal's unimpaired hindlimb as control. Polymer control groups where bFGF was unstable in vitro or absent in PLGA were also evaluated, namely: a) partially stabilized, PS, which had bFGF-specific stabilizers but no $Mg(OH)_2$; unstabilized, US, which had no stabilizer or $Mg(OH)_2$; and blank, B, which had stabilizers and $Mg(OH)_2$ but no bFGF. As shown in Table 1, after 6 weeks of implantation, nearly all ischemic limbs had recovered in the S group but underwent necrosis or exhibited negligible reperfusion in control groups (PS, US, B).

A second example to improve PLGA depot development is the unexpected finding that when an antacid ($MgCO_3$) is co-encapsulated with a human chorionic gonadotropin (hCG)-based peptide antigen to control PLGA microclimate pH, a strong and persistent antibody response is observed. Antibodies against hCG exert antifertility action without disturbance of the normal ovulation function or hormone secretion. Once the antibody level has declined

after several months, immunity can be boosted to continue contraception or discontinued to recover fertility.

The chimeric peptide consisting of a universal T-cell epitope from tetanus toxoid (TT2, residues 830–844) and a B-cell epitope from hCG (C-terminal peptide35, CTP35) was encapsulated in PLGA 50/50 microspheres (1 – 10 μ m) by the double emulsion/solvent evaporation method. One mg of antigen was administered to adult, specific pathogen-free New Zealand white rabbits in the following groups: a) a single dose of microencapsulated antigen + or – 1% MgCO₃; b) 3 doses of antigen in PBS emulsified in an unsafe oily vehicle (squalene:mannide monooleate (4:1)) and c) 3 doses of soluble antigen in PBS (negative control). As shown in Table 2, peak serum antibody levels, determined by RIA, were as high as the positive control group for microencapsulated antigen + MgCO₃, but very low for – MgCO₃ or negative controls. Similarly, the duration of the antibody response for microencapsulated antigen + MgCO₃ also mimicked the positive control (*data not shown*) [6]. Ongoing efforts are focused at understanding the mechanism of the MgCO₃ effect and its potential generality to other peptide antigens.

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Table 1^a Recovery of murine ischemic hindlimbs after 6-weeks implantation of PLGA/bFGF

^b PLGA formulation	Limb survival	^c Reperfusion in surviving limbs (%)
Stabilized – S	5 / 5	87 ± 7
Partially stabilized – PS	1 / 4	32
Unstabilized – US	0 / 4	---
Blank – B	1 / 4	21

^aData from [5]

^b All doses were 0.1 µg bFGF and protein loading was ~0.002% w/w. bFGF-specific stabilizers included (w/w): 0.01% heparin, 0.01% EDTA, 2.3% sucrose, 12.7% BSA (S), 15.7% gum arabic (PS); Mg(OH)₂ was added at 3% (S); gum arabic was substituted for BSA in PS because BSA aggregates without Mg(OH)₂.

^c mean ± SEM (n=5) for S group. PS and US had 1 surviving limb imaged and B had none

Table 2^a Peak serum anti-hCG response to PLGA/C-TT2-CTP35 microspheres

^b Peptide formulation	Peak serum anti-hCG level (nM)
Soluble antigen (neg. control)	21 ± 6
Antigen in squalene (pos. control)	730 ± 290
Encapsulated antigen + MgCO ₃	720 ± 190
Encapsulated antigen – MgCO ₃	15 ± 3

^a Data from [6]; experimental values represent mean ± SEM (n=5)^b Antigen dose was 1 mg; soluble and emulsified antigen groups were boosted at 4 and 10 weeks; positive control group also contained 25 µg nor-MDP