Biocompatibility and Bioactivity of an FGF-Loaded Microsphere-Based Bilayer Delivery System

Dong Hwa Kim, PhD[1,2]
Julianne Huegel, PhD[1,2]
Courtney Nuss[1,2]
Stephanie Weiss[1,2]
Louis Soslowsky, PhD[1,2]
Robert Mauck, PhD[1,2]
Andrew Kuntz, MD[1,2]

[1]McKay Orthopaedic Research Laboratory
University of Pennsylvania

[2]Veterans Affairs Medical Center
Philadelphia, PA

Introduction

Biodegradable micro-particle systems have attracted increasing interest for use as delivery vehicles for drugs, proteins, and other factors[1-2]. Several new strategies have been developed to improve protein stability within such biodegradable polymer matrices[3]. For instance, sustained release of basic fibroblast growth factor (bFGF) from microspheres can promote proliferation and differentiation processes in a wide range of cells[4]. Albumin is commonly included in such formulations, both as a model protein to monitor release and as a carrier to preserve growth factor activity and prolong shelf-time[5]. In this study, we developed microspheres (MS) containing both Alexa-tagged BSA and bFGF and incorporated them into a Bilayer Delivery System (BiLDS)[6]. This system was designed to sequester MS in a defined pocket between two nanofibrous scaffolds, where the scaffold provides a template for new tissue formation while enabling independent and local release from the co-delivered MS. The objective of this study was to evaluate the biocompatibility and bioactivity of an FGF-loaded BiLDS system in vitro and in vivo.

Methods

Microsphere and BiLDS fabrication

Microspheres were produced by combining 75:25 PLGA (0.15 g/mL, Mw=70 kDa) with/without 200 μg recombinant human bFGF and Alexa-BSA in dichloromethane. The external phase of the emulsion consisted of 5 mL of aqueous 1% poly(vinyl alcohol). To generate the bilayered delivery system (BiLDS), each MS formulation (Alexa-BSA MS and Alexa-BSA/bFGF MS) was suspended in 50 μl of PBS and placed onto the center of an aligned poly(ε-caprolactone) nanofibrous scaffold (6×8 mm)[6]. A second layer was placed on top and the two layers were sealed together by heat-annealing in a circular pattern around the microspheres using a custom heating device.

Direct/indirect tenocyte culture

For direct culture, rat tenocytes (5000 cells/BiLDS) were seeded onto the BiLDS and cultured for 18 days in 1% FBS containing DMEM. For indirect culture, each BiLDS was incubated in basal media for 1 week at 37°C. Tenocytes were seeded (3×10^3 cells/well) into a 24-well plate and the conditioned media from each BiLDS was added. At regular intervals, cell viability (via MTT assay, n=4-5) and MS and cell morphology (via actin staining and SEM, n=3) was evaluated.

BiLDS release in-vivo: BiLDS containing no MS, Alexa-BSA-MS, and Alexa-BSA/bFGF-MS (n=4/group) were fabricated and implanted into the rat's dorsal subcutaneous space. At 1, 2, and 4 weeks, samples were recovered and fluorescence images were taken to identify MS within the BiLDS and frozen sections were processed for hematoxylin and eosin (H&E) staining. Statistical analysis was performed by 2-way ANOVA with Tukey's post-hoc test.

Results

SEM images demonstrated that MS were spherical with a smooth surface. Alexa-BSA and Alexa-BSA/bFGF MS ranged in diameter from 1.5-3 μm and 1.5-4.5 μm, respectively (not shown) (Fig. 1A, B). SEM images also showed a seal along the margin that effectively localized MS within the BiLDS (Fig. 1C) and cross-sectional views of the BiLDS showed large quantities of MS within the BiLDS (Fig. 1D). In direct culture, cell viability and proliferation of Alexa-BSA and Alexa- BSA/bFGF BiLDS increased during culture and were significantly higher than control at day 18 (Fig. 2A). Cells attached and spread along the BiLDS (Fig. 2B). SEM images confirmed this finding, and cross-sectional views showed that MS remained entrapped after 18 days (Fig. 2C). In indirect culture, after 4 and 7 days, proliferation in media from Alexa-BSA/bFGF BiLDS was higher than from no MS and Alexa-BSA BiLDS (Fig. 2D, E). After implantation, fluorescent images showed that MS remained within the BiLDS (Fig. 3A) and H&E staining revealed increased cellularity at the periphery with greater infiltration into nanofiber layers of the Alexa- BSA/bFGF BiLDS (Fig. 3B).

Discussion

In this study, we developed a bilayered delivery system to deliver bFGF in a local manner using a clinically relevant and previously validated scaffold system. We previously showed

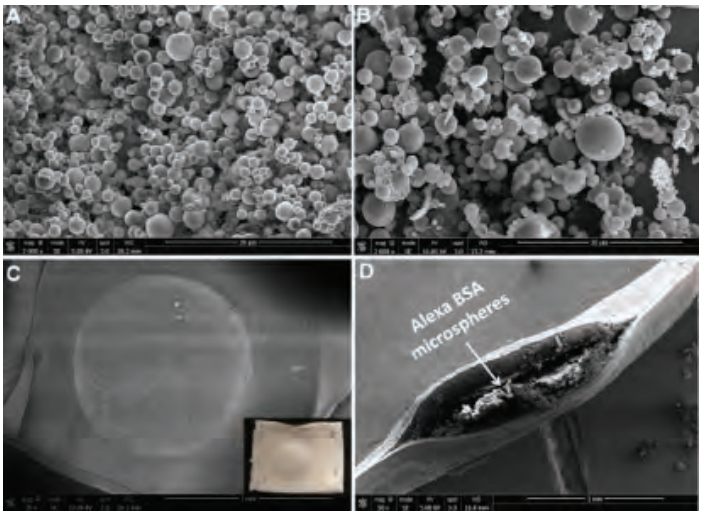

Figure 1. SEM of **(A)** Alexa-BSA and **(B)** Alexa-BSA/bFGF MS. **(C)** Top view and **(D)** cross-section showing MS within BiLDS (scale = 1mm).

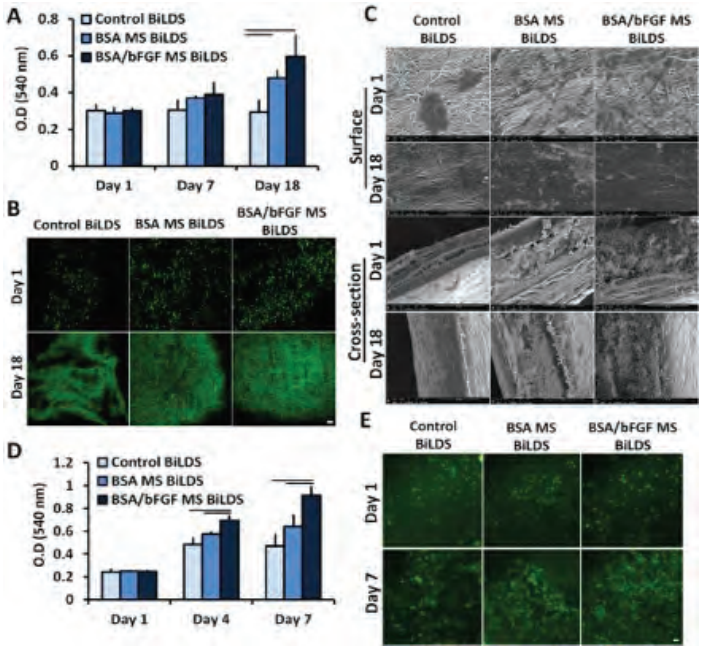

Figure 2. (A) Viability, **(B)** actin staining, and **(C)** SEM over 18 days with direct culture of tenocytes on BiLDS. **(D)** Viability and **(E)** actin staining over 7 days with indirect culture of tenocytes in media from BiLDS (Scale = 10 μm).

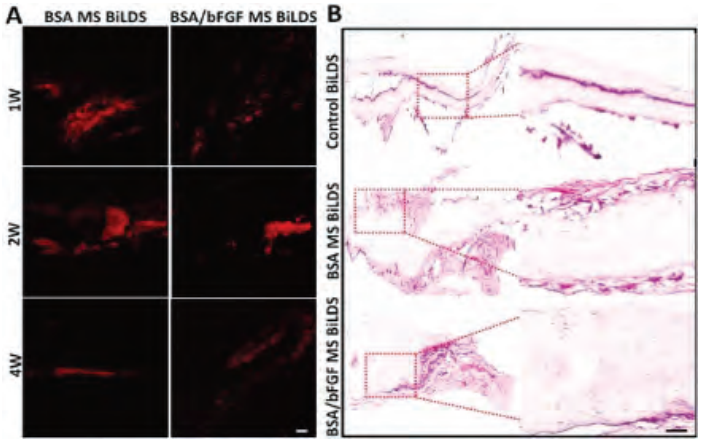

Figure 3. (A) Fluorescent images of Alexa tagged MS in BiLDS after implantation. **(B)** H&E staining after 4 weeks in vivo (scale = 200 μm).

that MS entrapped within the BiLDS system showed a somewhat attenuated release profile compared to free MS, and that protein release was sustained and continuous for up to 30 days[6]. Importantly, our new data show that cell viability and proliferation were enhanced in the context of Alexa-BSA/bFGF BiLDS, both in vitro and in vivo. MS delivered via the BiLDS system persisted in a localized area after implantation for at least 4 weeks, and bFGF release increased colonization of the implant. These data establish the BiLDS technology as a sustained in vivo drug delivery platform that can localize protein and other growth factor release to a surgical site. In future studies, we will explore the ability of this BiLDS technology to deliver growth factors to promote repair in a small animal model of rotator cuff injury.

Significance

This work establishes the biocompatibility and bioactivity of a MS- loaded bilayered delivery system (BiLDS) for sustained release in a localized and clinically relevant fashion for tissue repair and regeneration.

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