Prolonged Release of Ibuprofen from a Nanofibrous Delivery System Under Physiological Conditions

Brittany Taylor, PhD[1,2]
Dong Hwa Kim, PhD[1,2]
Corinne Riggin, PhD[1,2]
Julianne Huegel, PhD[1,2]
Andrew Kuntz, MD[1,2]
Louis Soslowsky, PhD[1,2]
Robert Mauck, PhD[1,2]
Joseph Bernstein, MD[1,2]

[1]McKay Orthopaedic Research Laboratory
University of Pennsylvania

[2]Veterans Affairs Medical Center
Philadelphia, PA

Introduction

Using non-steroidal anti-inflammatory drugs (NSAIDS) to mitigate inflammation may represent a promising approach to modulate the tendon healing environment. In particular, biodegradable nanofibrous delivery systems offer an optimized architecture and surface area for cellular attachment, proliferation, and infiltration while releasing soluble factors to promote tendon regeneration [1–3]. Previous work confirmed the sustained release of ibuprofen (IBP) from Labrafil-modified poly(lactic-co-glycolic) acid (PLGA) microspheres *in vitro* in PBS and characterized a bilayer delivery system (BiLDS) incorporating these microspheres for localized delivery of therapeutics [4]. Nonetheless, the behavior of ibuprofen-releasing BiLDS in more physiologically relevant conditions and their influence on tenocytes *in vitro* is unknown. Therefore, the objective of this study was to evaluate the release profile of IBP from both PLGA microspheres alone and within BiLDS in serum and to elucidate their effect on primary tenocytes. We hypothesized that IBP would release at a faster rate from both the free microspheres and the BiLDS in serum than in PBS and the direct and indirect delivery of IBP *in vitro* would not have an adverse effect on cellular viability or morphology.

Methods

Microsphere and BiLDS Fabrication

PLGA microspheres with varying concentrations of Labrafil® M1944CS oil ranging from 0 to 600 μL (P0, P30, P300 and P600) and 30mg/mL of IBP were created using an oil-in-water emulsion technique with an external phase of 1% poly(vinyl alcohol). The microsphere solution was stirred for 4 hours at room temperature. The BiLDS were created by entrapping 10mg of the P300 microspheres, without and with IBP (BiLDS_MS and BiLDS_IBP), between two sintered 6 x 8mm electrospun poly(ε-capalactone) (PCL) scaffolds. The microspheres and BiLDS were then SEM imaged for morphological analysis.

In Vitro Release Studies

In the first release study, 20 mg of free microspheres were submerged in 5mL of normal rat serum or PBS and incubated at 37°C. The quantity of total IBP released (μg/mL) was measured over 14 days using a competitive ELISA assay and UV spectrophotometer (λ= 223nm). In the second release study, BiLDS_MS and BiLDS_IBP were incubated on a shaker at 37°C in 5mL serum up to 14 days. In the one group, BiLDS_IBP and BiLDS_MS, the total 5mL of serum was collected at 0.5, 3, 7, 14 days and in the 'continuous' groups (BiLDS_MS_C and BiLDS_IBP_C), 2mL of serum was collected at the same time points and replaced with fresh serum to assess the effect fresh serum had on the release of IBP from the BiLDS over time.

In Vitro Cell Study: Primary Achilles tenocytes were isolated from Sprague-Dawley rats and cultured in DMEM with 10% FBS and 2X penicillin-streptomycin (P/S) for one week. The tenocytes (passage 1) were seeded at 5 x 10^3 per construct on empty BiLDS (BiLDS), BiLDS_MS and BiLDS_IBP for the direct cell study. The cells were maintained in media with 1% FBS. For the indirect study, the same BiLDS groups were submerged in culture media for one week and the conditioned media was then added to cells cultured on TCP at 5 x 10^3 cells per well.

Tenocytes were cultured with normal culture media as the control. MTT cell proliferation assay and Alexa Fluor 488 phalloidin fluorescence staining for actin were used to quantify metabolic activity and visualize cell morphology over 14 days for both studies.

Statistics: Comparisons between groups at each time point were assessed using two-way ANOVAs with post-hoc Bonferroni tests. Significance of differences was set at $p<0.05$.

Results

Qualitatively, the microspheres in each group were consistent in morphology and similar to the representative image of P300 microspheres in Figure 1A. SEM images of the BiLDS confirmed the presence of microspheres between the two nanofibrous scaffolds (Fig. 1B). The IBP released in a linear manner from all the microsphere groups in serum (Fig. 2A), whereas there was an initial burst in release in PBS (Fig. 2B).

Furthermore, the P0 microspheres released 74% more IBP than the P600 microspheres through Day 14, indicating the total amount of IBP released was inversely related to the

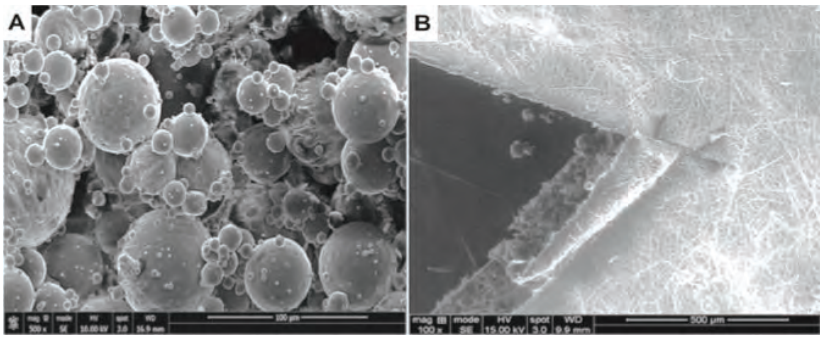

Figure 1. SEM images of **(A)** P300 microspheres at 500× and **(B)** the cross-section of a BiLDS at 100×

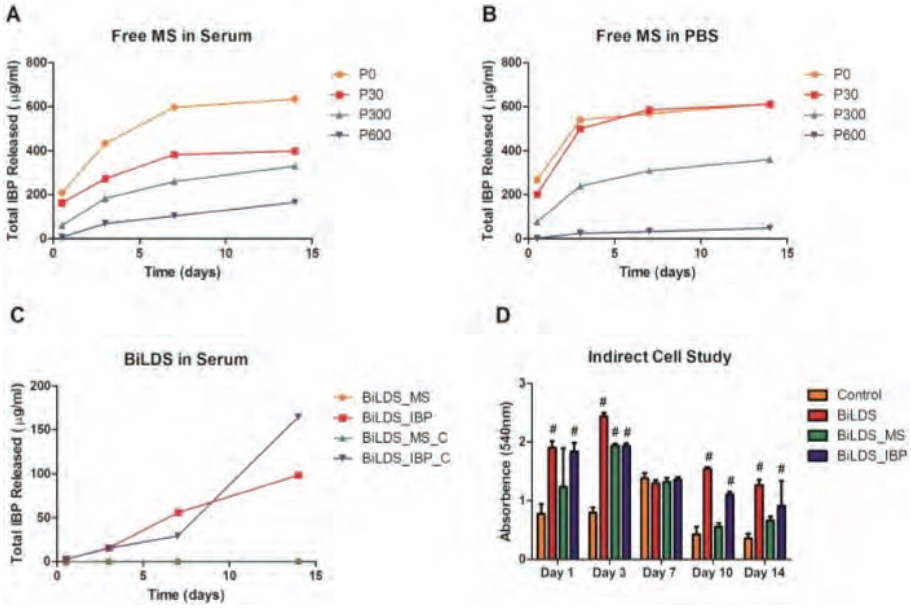

Figure 2. Release of IBP from free microspheres in **(A)** serum and **(B)** PBS over 14 days **(C)** Release of IBP from the BiLDS in serum over 14 days. **(D)** MTT cell proliferation assay results of primary Achilles tenocytes cultured in conditioned medium from control (TCP), BiLDS alone, and BiLDS with and without IBP.

concentration of Labrafil oil used to create the microspheres. At Day 14, 165 μg/ml of total IBP was released from the BiLDS in serum, which was about 70% less than the free P300 microspheres in serum. The BiLDS with IBP in the continuous group, BiLDS_IBP_C, had a significant increase in total IBP released from Day 7 to Day 14 (Fig. 2C). There were no differences in cell viability over 14 days for all the BiLDS groups in the direct cell study (data not shown). However, when the cells were cultured in conditioned media for the indirect cell study, there was a significant increase in cellular viability at Day 1, 10 and 14 for the BiLDS and BiLDS_IBP groups and at Day 3 for all the groups in comparison to the cells cultured in control media (Fig. 2D).

Discussion

Similar to previous studies, the addition of Labrafil oil slowed the release of IBP over time. The release of the ibuprofen was delayed and sustained from the P300 microspheres enclosed in the bilayer scaffold design in serum, confirming the prolonged behavior of the microspheres in physiologically relevant conditions. This is a desirable characteristic in modulating inflammation during the tendon healing phase *in vivo*. Also, the addition of fresh serum over time, resembling *in vivo* conditions of exchange of serum, increased the release of IBP from the BiLDS. This could be due to the frequent pH changes in the environment affecting the scaffold degradation or release kinetics [5]. *In vitro* biological assessment proved that all the components of the BiLDS were biocompatible. Furthermore, the concentration of IBP released did not have any detrimental effects on cellular viability or morphology. Future studies will investigate the regenerative effects of the BiLDS using an *in vitro* inflammation model. **SIGNIFICANCE:** This study identifies the therapeutic potential of a biocompatible nanofibrous bilayer delivery system for prolonged and continued released of ibuprofen to mitigate inflammation during tendon healing.

Acknowledgements

This study was supported by VA Merit Grant (O0979-R), Penn Center for Musculoskeletal Disorders (NIH/NIAMS P30 AR069619), and a fellowship for B. Taylor through the University of Pennsylvania's Office of the Vice Provost for Research.

References

1. **Huang *et al.*** *J. Hand Surg. Am.*, 31:693–704, 2006.
2. **Sahoo *et al.*** *Tissue Eng.*, 12:91–99, 2006.
3. **Reverchon *et al.*** *Muscles. Ligaments Tendons J.*, 2:181–6, 2012.
4. **Kim *et al.*** *ORS*, 2017.
5. **Riggin *et al.*** *Ann. Biomed. Eng.*, 1–12, 2017 (epub).