Localized Delivery of Ibuprofen via a Bilayer Delivery System (BiLDS) for Supraspinatus Tendon Healing in a Rat Model

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Introduction

The high prevalence of tendon re-tear following rotator cuff repair motivates the development of new therapeutics to promote improved tendon healing. Controlled delivery of non-steroidal anti-inflammatory drugs (NSAIDs) to the repair site via an implanted scaffold is a promising option for modulating inflammation in the healing environment. Previous work confirmed the *in vitro* sustained release of ibuprofen (IBP) from Labrafil- modified poly(lactic-co-glycolic) acid (PLGA) microspheres within sintered poly(ε-caprolactone) (PCL) electrospun scaffolds[1]. Biocompatibility of this bilayer delivery system (BiLDS) was also demonstrated with primary rat bicep and Achilles tenocytes *in vitro*[1,2]. However, the effect of these IBP- releasing BiLDS on tendon healing *in vivo* is unknown. Therefore, the objective of this study was to investigate the effects of sustained release of IBP from BiLDS implanted at the repair site in a rat supraspinatus injury and repair model. We hypothesized that the controlled release of IBP from BiLDS would improve tendon healing by decreasing the expression of pro-inflammatory cytokines, thus improving tendon remodeling and mechanics.

Methods

BiLDS Fabrication

PLGA microspheres with 300μL of Labrafil® M1944CS oil and with or without 30mg/mL of IBP were created as described[1]. 170μg of the microspheres, with or without IBP, were entrapped between two sintered 3x5mm scaffolds to generate BiLDS_IBP or BiLDS implants, respectively. Based on *in vitro* release studies, we predicted that the100μm thick BiLDS would deliver approximately 270μg of IBP to the injury site over 8 weeks. *BiLDS In Vivo Implantation*: 90 adult male Sprague-Dawley rats (400-450g) underwent bilateral supraspinatus detachment and repair (IACUC approved)[3]. Animals were randomly divided into groups receiving no scaffold (No_BiLDS), BiLDS with empty microspheres (BiLDS), and BiLDS with IBP-loaded microspheres (BiLDS_IBP) (n=30/group). BiLDS were secured proximally to the tendon via sutures and distally to the bone tunnel drilled through the greater tuberosity. Animals were sacrificed at 1, 4, and 8 weeks post-surgery. The right supraspinatus tendons and blood serum were collected at the time of sacrifice for biological assessment.

Biological Assessment

Sagittal sections were stained with H&E, imaged at 20X and graded for cell shape and cellularity (n = 6/group/timepoint). RNA was extracted from tendons harvested one week post-surgery (n = 6/group) and qRT-PCR was run in quadruplicate using TaqMan assays on a QuantStudio 12K Flex Real-Time PCR System[4]. Genes of interest included markers of inflammation (TNF-α IL-1b, IL- 6, and IL-10, Prostaglandin E2, CD68, CD163, and CD45), tendon repair (TGF-β1, TGF-β3, and VEGFb) and tendon remodeling (COL I, III, and IV, MMPs −2, −3, −8 and −10, tenascin, tenomodulin, and aggrecan). Expression was normalized to the internal control (GAPDH) and fold change was calculated by normalizing treatment groups to the untreated control, No_BiLDS. ELISA for TNF-α and IL-6 was performed on protein isolates from the excised tendons and for IBP in serum samples collected at 4 and 8 weeks (n = 6/group/timepoint).

Tensile Mechanical Testing

The cross-sectional area of the left intact supraspinatus tendons from animals sacrificed at 4 and 8 weeks (n = 12/group/timepoint) was measured using a custom laser device. Ex vivo tensile testing was performed as follows: preload, preconditioning, stress relaxation, and ramp to failure. Modulus, stiffness, maximum load, and maximum stress were computed.

Statistics

Two-way ANOVA and normality tests were performed on all datasets. To compare between groups at each timepoint, one-way ANOVA or Kruskall-Wallis tests were performed, depending on normality. To compare over time within each treatment group, Welch's t-tests or Mann-Whitney U tests were performed. Significance was set at $p < 0.05$ (*); ** denotes $p < 0.01$ and ***denotes $p < 0.001$.

RESULTS

There were no statistically significant differences in cell shape, cellularity, and

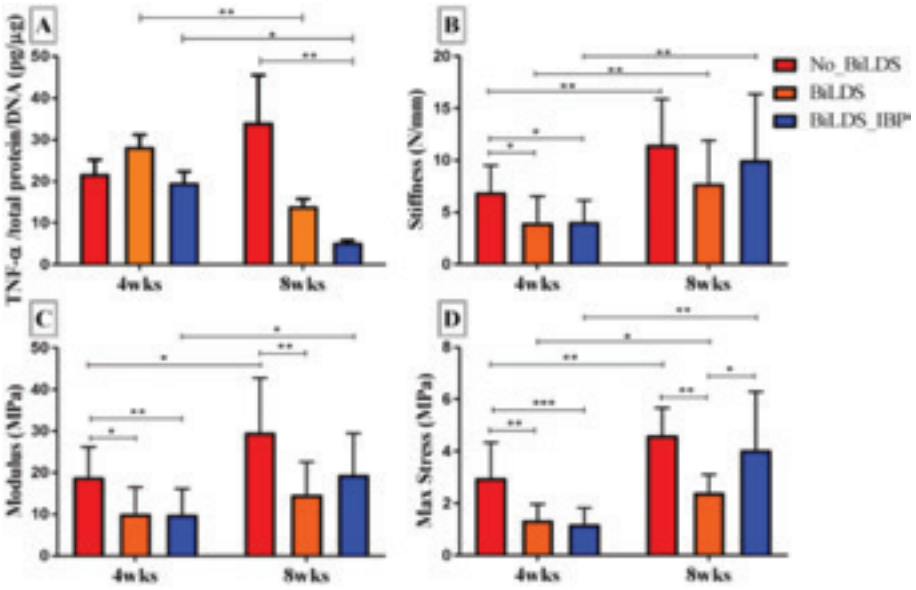

Figure 1. (A) Treated tendons BiLDS and BiLDS_IBP, significantly decreased in cytokine expression of TNF-a overtime and IBP-treated tendons expressed significantly less TNF-a than the untreated tendons, No_BiLDS, at 8 weeks. The untreated tendons No_BiLDS, exhibited significantly greater **(B)** stiffness, **(C)** modulus, and **(D)** maximum stress at 4 weeks in comparison to the treated tendons, BiLDS and BiLDS_IBP. Data presented as mean ± SD. (*$p < 0.0$, **$p < 0.01$ ***$p < 0.001$)

expression of tendon healing genes or IL-6 cytokine expression between the treatment groups at each timepoint (data not shown). IBP was undetectable in the serum of all animals at 4 and 8 weeks (data not shown). Tendons treated with BiLDS_IBP expressed significantly less TNF-α compared to untreated tendons, No_BiLDS, at 8 weeks and both BiLDS groups decreased in TNF-α at the protein level over time (Figure 1A). Stiffness, modulus, maximum stress, and maximum load of the untreated tendons (No_BiLDS) were significantly greater than in either of the treated groups, BiLDS and BiLDS_IBP, at 4 weeks (Figure 1B-D). Stiffness, maximum stress, and maximum load increased for all groups over time (Figure 1B & 2D). Modulus and maximum stress of the treated tendons in the BiLDS group were lower in comparison to the No_BiLDS group at 8 weeks, but there were no differences in these parameters between the No_BiLDS and BiLDS_IBP groups at 8 weeks (Figure 1C & 1D). There were no significant differences in stiffness (Figure 1B) and maximum load at 8 weeks or in tendon cross-sectional area at either 4 or 8 weeks (data not shown).

Discussion

Although the use of BiLDS and BiLDS_IBP was not therapeutically beneficial for rat rotator cuff healing in terms of mechanics, the release of IBP from BiLDS significantly decreased pro-inflammatory signaling in the late healing phase. There were no substantial changes in gene expression 1 week post-repair with either treatment (BiLDS or BiLDS_IBP) compared to standard surgical repair (No_BiLDS). Therefore, we are unable to conclude the biological effect of the BiLDS with and without IBP on tendon repair at this time. Further investigation is ongoing to evaluate additional tendon healing markers at the protein level up to 8 weeks post-repair. Mechanical testing results indicated both BiLDS and BiLDS_IBP were detrimental to tendon mechanics compared to surgical repair alone, especially at early timepoints. Previous work revealed no significant differences in structural properties after surgical repair with and without the implantation of a single layered PCL scaffold in a rat rotator cuff injury and repair model[5]. Therefore, the decreased mechanics seen with the use of BiLDS in this study may be due to the increased size of the BiLDS compared to a single-layer PCL scaffold. Implanting a substantially thicker scaffold into the tight subacromial space in the rat shoulder may have caused supraspinatus impingement and negatively affected early tendon healing. Despite this, the BiLDS and BiLDS_IBP constructs remained intact, led to decreased pro-inflammatory expression over time, and recovered the tendon structural properties by 8 weeks. Future studies are required to elucidate the effect of the BiLDS and BiLDS_IBP on tendon mechanics at later timepoints and in larger defects in which supplementation with a scaffold may be necessary to stabilize repair.

Significance

This study investigates a biocompatible nanofibrous bilayer delivery system (BiLDS) for localized delivery of ibuprofen to mitigate inflammation in a rat rotator cuff repair model. Further evaluation is necessary to elucidate the beneficial effects of the system in a larger animal model.

References

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