Electrospun PLGA Nanofiber Scaffolds Release Ibuprofen Faster and Degrade Slower after In Vivo Implantation

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are often prescribed, and are effective, for pain relief following tendon repairs. While improved healing after delayed delivery of NSAIDs has been demonstrated,1 these drugs have also been shown to impair healing in early stages of tendon repair by inhibiting inflammatory responses.[1,2] Therefore, to support the use of NSAIDs after tendon repair, it is imperative to identify a dose, timing, and mode of delivery that provides pain relief but does not impede tendon healing. Recent tissue engineering work has demonstrated that nanofibrous electrospun scaffolds may be useful in this realm.[3] Release of factors that are directly incorporated into nanofibers can be modulated by selecting polymers with appropriate degradation properties.[3] Therefore, the objective of this study was to develop a scaffold that would allow for local controlled release of NSAIDs during tendon healing. We further aimed to characterize the release profile and scaffold degradation properties both in vitro and in vivo.

Methods

Scaffold Fabrication: Poly(lactic-co-glycolic acid) (PLGA) scaffolds were fabricated with and without the incorporation of ibuprofen (IBP) using standard electrospinning techniques.[4] Solutions of 35% w/v 75:25 PLGA, with 5% w/w ibuprofen (IBP) or without IBP (blank), were dissolved in 1:1 tetrahydrofuran and N,N-dimethylformamide and then electrospun on a rotating mandrel to create aligned nanofibrous scaffolds. In Vitro IBP Release in PBS: IBP scaffolds were placed in phosphate buffered saline (PBS) at 37°C on a shaker. At designated time points, the PBS solution was removed and centrifuged and the IBP concentration in the supernatant quantified by measuring optical absorbance and normalized to scaffold weight. In Vivo IBP Release: With IACUC approval, 8mm diameter PLGA scaffolds were implanted subcutaneously in Sprague Dawley rats (4/animal). Rats were sacrificed at 0.5, 3, 7, and 14 days after implantation, and scaffolds harvested for subsequent ibuprofen quantification, continued in vitro release in PBS, histological analysis, and SEM imaging. IBP remaining within the scaffolds was determined by dissolving them in dimethyl sulfoxide (DMSO) and measuring absorbance. In Vitro Release in Serum: To more accurately replicate in vivo conditions in vitro, 10 IBP-containing and 5 blank scaffolds per time point were incubated in either PBS or rat serum for 0.5, 3, 7, 14, and 21 days at 37°C on a shaker. Scaffolds were removed after these incubation times for SEM (n = 1), mechanics (n = 3, retained for future analysis), and IBP quantification (n = 3, IBP only). For three IBP and one blank scaffold, the serum was replaced with PBS for continued release and analysis of degradation. Histology: Scaffolds explanted from rats were immediately placed in formalin, soaked in sucrose, flash frozen in embedding compound, cryosectioned at 10 μm, and stained with hematoxylin and eosin. SEM: Samples were flash frozen immediately after in vitro or in vivo incubation, lyophilized, mounted, and imaged at 1000× (not shown) and 5000x. Mechanical Testing: As-spun IBP and blank scaffolds (day 0) were cut into 60 × 5 mm strips with the fibers oriented along the long axis and mechanically evaluated using a ramp to failure test (0.5%/second).

Results

In Vitro Response in PBS: A distinct and reproducible release profile of IBP was observed in PBS: a burst phase over the first 3 days (releasing ~10% of the total IBP), followed by a lag phase from days 3-10, and then a linear phase during which roughly 1 g/mg scaffold/day was released (Figure 1—green plot). Macroscopically, the scaffold began to degrade and lose its shape after ~20 days (Figure 2A). SEM demonstrated that the fibers swelled immediately upon hydration, coalesced by day 14, and then disintegrated by day 21 (Figure 2A). In Vivo Response: After removal from subcutaneous implantation for 0.5, 3, 7, and 14 days, all scaffolds retained only 10-20% of the original IBP load (Figure 1—red plot). Macroscopically, there was no degradation apparent and little to no tissue adherence. Histologically, there was only scant cellular infiltration into the scaffold by day 14 (Figure 2D). After further incubation in PBS, the scaffold showed a rapid but small burst release of the remaining 10% of IBP, and failed to degrade even after nearly 3 months of incubation in PBS (data not shown). SEM demonstrated the maintenance of a fibrous structure throughout, with minimal fiber swelling compared to the PBS incubated

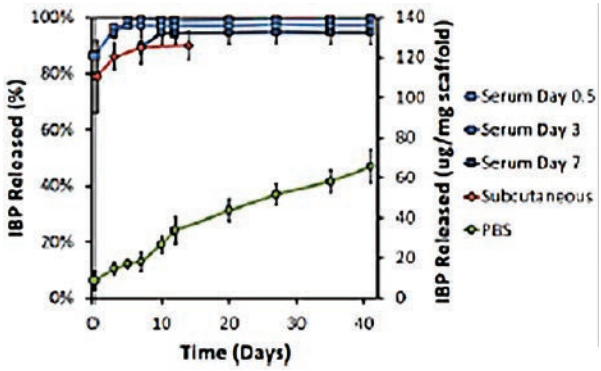

Figure 1. IBP release data represented as % release from the total (left y-axis) and ug/mg scaffold (right y-axis) after incubations in serum (blue) for 0.5, 3, and 7 days and PBS (green), as well as in vivo subcutaneous incubation (red).

scaffolds (Figure 2C). In Vitro Response in Serum: Scaffolds placed in serum for 0.5, 3, 7, 14, and 21 days demonstrated a pattern of release similar to those implanted in vivo, namely, an immediate burst release of nearly 100% of the IBP (Figure 1A—blue plots). Macroscopically, the scaffolds did not show any signs of degradation, even after almost 2 months of incubation in PBS (Figure 2B). SEM demonstrated that the scaffolds maintained their fibrous structure with very little fiber swelling over 21 days (Figure 2B). Blank vs. IBP Scaffolds: Incubation in PBS steadily degraded both the IBP and blank scaffolds, though the IBP scaffold degraded at a faster rate (data not shown). Interestingly, neither scaffold showed signs of degradation when incubated in serum for up to 40 days (data not shown). Additionally, mechanical testing demonstrated that as-spun IBP scaffolds had no change in stiffness, failure load, and yield strain properties (data not shown) compared to blank scaffolds. However, the IBP scaffolds showed a decreased modulus (Figure 3B), yield load, yield stress, and failure stress (data not shown),

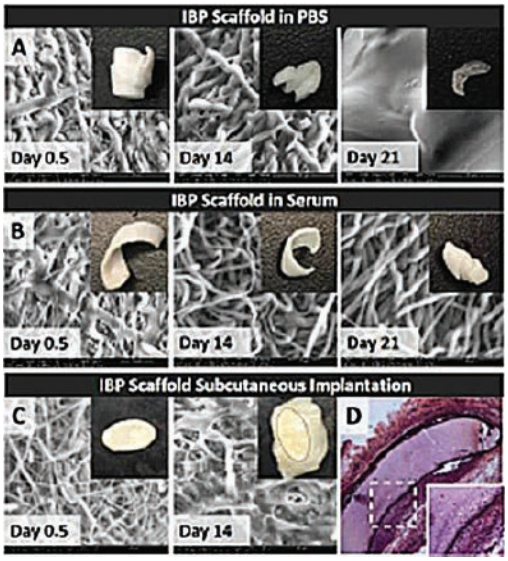

Figure 2. SEM (5000x) and macroscopic images of IBP scaffolds at 0.5, 14, and 21 days of incubation in (A) PBS, (B) rat serum, and (C) in vivo subcutaneous implantation. The macroscopic view at 14 days includes surrounding tissue, with the scaffold location outlined. (D) Histological image demonstrating sparse cellular infiltration into the scaffold at 14 days.

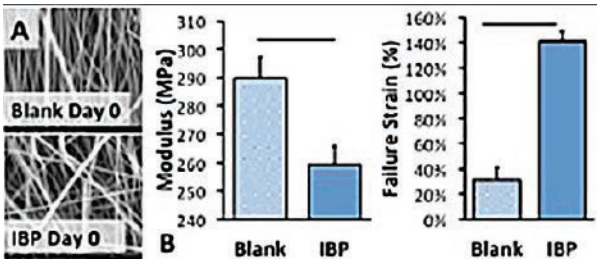

Figure 3. (A) SEM images of blank and IBP scaffolds prior to incubation. (B) Modulus and failure strain of IBP and blank scaffolds prior to incubation ($p < 0.01$).

as well as an increased failure strain (Figure 3B) compared to blank scaffolds ($p < 0.01$).

Discussion

This study demonstrated that a controlled linear release profile of IBP can be created using PLGA nanofiber scaffolds when maintained in vitro in PBS. Although this release profile is highly desirable for our clinical application, the release profile of scaffolds placed in serum or in vivo was not (i.e., we observed a burst release). This may be due to IBP's tendency to bind to serum albumin, as it does within the vascular system.[5] Additionally, serum and in vivo conditions seem to inhibit degradation of the scaffold, the reason for which remains unknown. It is known that biomaterials perform differently in vitro vs. in vivo, as these data confirm, and this study demonstrates the necessity to fully evaluate biomaterials in environments similar to the intended application. Additionally, this study supports a method for more accurately mimicking the in vivo environment, allowing a more thorough in vitro investigation prior to progressing to in vivo animal studies. Ongoing work is focused on determining the polymer molecular weight and dispersion, and evaluating the mechanical properties of these scaffolds during in vitro, in vivo, and simulated-in vivo degradation. Additionally, we are working to create a scaffold whose release profile following in vivo implantation better mirrors the more desirable in vitro results.

Significance

This work demonstrates that electrospun nanofibrous scaffolds can deliver NSAIDs, though the in vivo release profile has not yet matched the more desirable in vitro behavior. Additionally, this study supports the use of serum over saline for in vitro evaluation, to more accurately represent in vivo conditions, and thereby, reduce the number of animal subjects.

Acknowledgments

We acknowledge the VA Medical Center Merit Grant (O0979-R) and the NSF GRFP.

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