Sustained Release of Ibuprofen from Labrafil-Modified PLGA Microspheres

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Introduction

Sustained delivery of non-steroidal anti-inflammatory drugs (NSAIDs) is an attractive method for long-term management of pain and inflammation after tendon repair. Delivery systems have been developed to enable the controlled or targeted delivery of drugs and biofactors, using micelles, emulsions, and micro/nanoparticles.[1-3] The rate of release from micro/nanoparticles depends on several factors, including the type of polymer used, the molecular weight of the drug, and others that can be controlled during fabrication.[4] While NSAIDs like ibuprofen (IBP) can be incorporated into poly(lactic-co-glycolic acid) (PLGA) microspheres,[5] previous studies indicate that this system exhibits a high initial burst release.[6,7] We hypothesized that this characteristic burst release could be attenuated by including excipients to the polymer solution during microparticle formation.[8,9] In this study, we employed Labrafil, a PEG derivative that is biocompatible and biodegradable, as a non-ionic amphiphilic excipient to attenuate the burst release of IBP from PLGA microspheres.[11] The objective was to investigate the effect of Labrafil concentration on the release kinetics of IBP. Furthermore, we sought to couple these IBP-loaded microparticles with a nanofibrous scaffold[12] to generate a delivery vehicle for localized and sustained release.

Methods

Four different microsphere formulations were investigated with varying Labrafil® M1944CS oil concentrations: PI 0 (PLGA-IBP-Oil free), PI 30 (PLGA-IBP-Oil 30 μL), PI 300 (PLGA-IBP-Oil 300 μL), and PI 600 (PLGA-IBP-Oil 600 μL). In each formulation, 75:25 PLGA was used (0.15 g/mL, Mw = 70 kDa) and the IBP content was maintained at 30 mg/mL. Labrafil oil was dissolved in l mL of dichloromethane and added to the polymer solution prior to microsphere formation. The external phase of the emulsion consisted of 5 mL of a 1% Poly(vinyl alcohol) aqueous solution. The emulsion was sonicated, added to distilled water, and stirred for 2 min. Formed microspheres were suspended in distilled water and stirred continuously for 4 h. Finally, the microspheres were washed and dried under vacuum for 48 h, followed by SEM imaging. To measure IBP release, microspheres (20 mg) were suspended in 20 mL of phosphate buffered saline (PBS) at 37°C on a shaker. The supernatant (5 mL) was withdrawn (and replaced with fresh PBS) at various time intervals and centrifuged. IBP content was determined by UV spectrophotometry at λ = 223 nm. To determine the biocompatibility of delivery, tendon fibroblasts (2×10^4 cells/well in a 24-well plate) were cultured in media pre-treated with or without microspheres that had released for 18 hours. Cell adhesion and proliferation over 7 days were evaluated via light microscopy and the MTT assay. Next, composite electrospun scaffolds were formed with PI 300 microspheres. For this, 0.2 g microspheres (0.05 g/mL) were dispersed in 10% poly(ethylene oxide) (PEO), which was co-electrospun (2.5 mL/hr) with 14.3 % w/v poly(ε-caprolactone) (PCL) (3 mL/hr, in 1:1 tetrahydrofuran and N,N-dimethylformamide) onto a common collecting mandrel. After fabrication, scaffolds were hydrated to remove the PEO. Scaffolds were imaged via SEM and IBP release evaluated over 21 days.

Results

SEM images revealed that Labrafil-modified PI microspheres were larger than those without Labrafil (PI 0) (Figure 1). Inclusion of Labrafil attenuated the initial burst release and resulted in release profiles that became more linear with increasing Labrafil concentration. In PI 0 microspheres, 100% of the encapsulated IBP was released within 26 days, with 63% released over the first 2 h. Conversely, Labrafil-modified microspheres had a much slower release profile, with 72% and 61% of IBP released from PI 300 and PI 600 microspheres over 102 days, respectively. Importantly, the initial burst release was greatly reduced (3% for PI 600 in the first 2 h, Figure 2A). The MTT assay showed that, compared to proliferation on TCP, cells proliferated more slowly when more IBP was released (i.e., at lower Labrafil concentrations) (Figure 2B). For composite nanofibrous scaffolds containing IBP microspheres, SEM images after PEO removal showed that microspheres remained entrapped

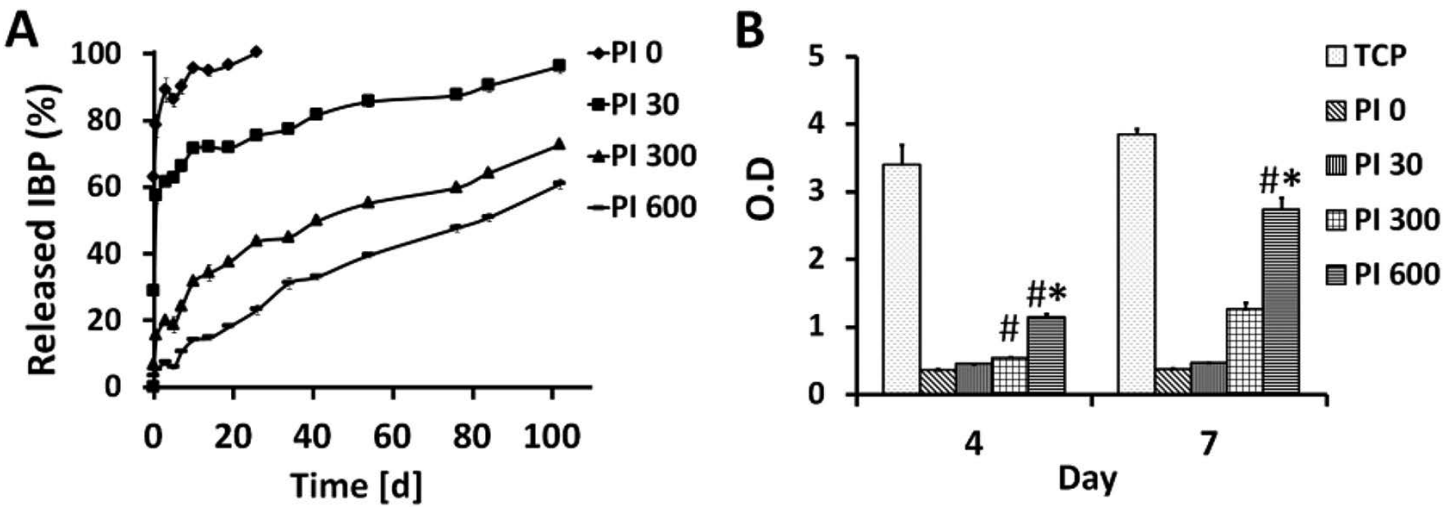

Figure 1. SEM images of IBP-loaded PLGA microspheres with/without oil. (A) PI 0 (PLGA-IBP-Oil free), (B) PI 30 (PLGA-IBP-Oil 30 µl), (C) PI 300 (PLGA-IBP-Oil 300 µl), (D) PI 600 (PLGA-IBP-Oil 600 µl) (scale bar = 10 µm, ×5000), (E) PI 300 (scale bar = 5 µm), (F) PI 600 (scale bar = 100 µm).

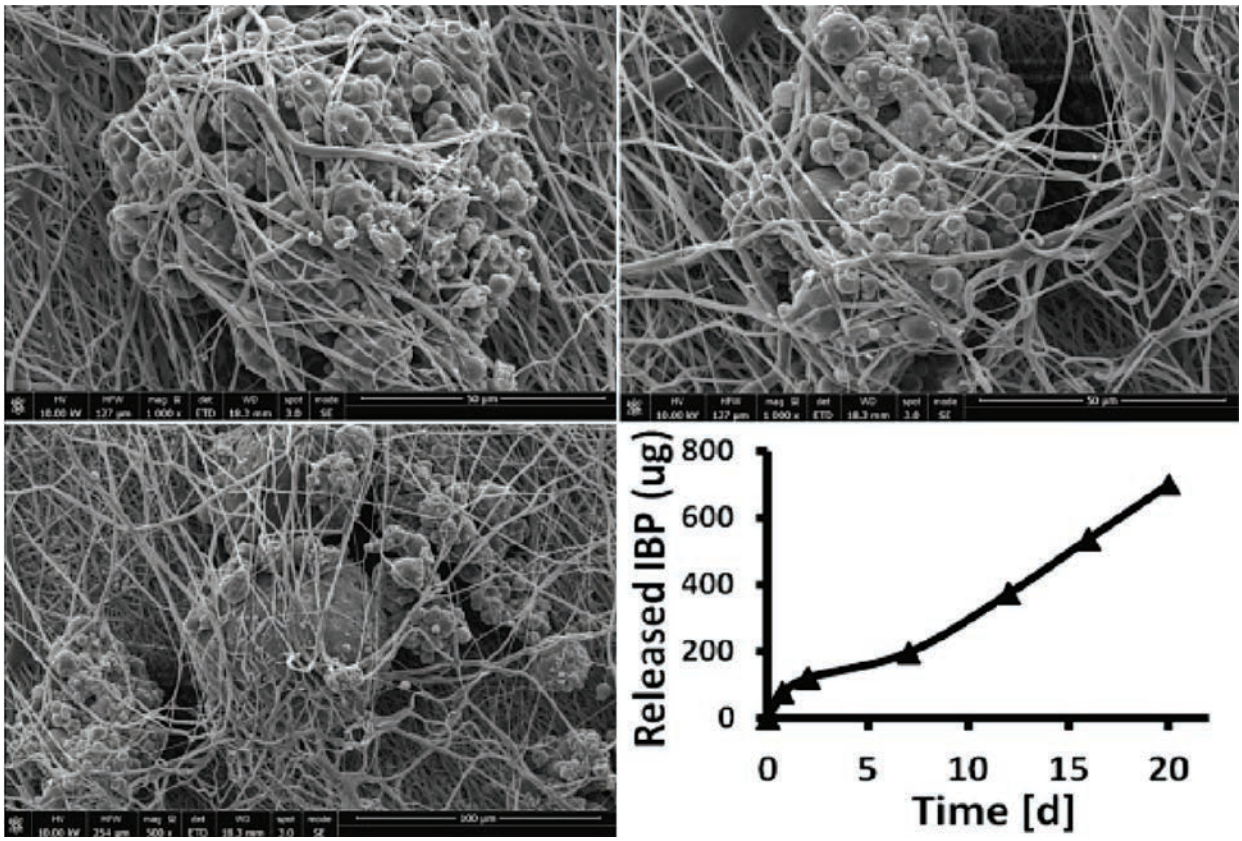

Figure 2. (A) Release profile of IBP from PLGA microspheres with/without oil in PBS at 37°C. (B) MTT assay results (*: $P < 0.05$ vs. PI 300, #: $P < 0.05$ vs. PI 0).

between the aligned fibers. These microsphere-containing scaffolds released in a continuous fashion over the first 21 days (Figure 3).

Discussion

This study demonstrated that sustained and steady release of IBP, with a reduced initial burst release, could be achieved by modifying PLGA microspheres with Labrafil oil. When a burst release was present, IBP inhibited cell adhesion and proliferation, suggesting that a more gradual release might be more effective for *in vivo* drug delivery applications. When these microspheres were embedded in a structural PCL nanofiber scaffold, microspheres remained entrapped and released at a steady rate over 21 days. Taken together, these results demonstrate that the IBP-loaded PLGA microspheres with Labrafil oil show promise as a multifunctional drug

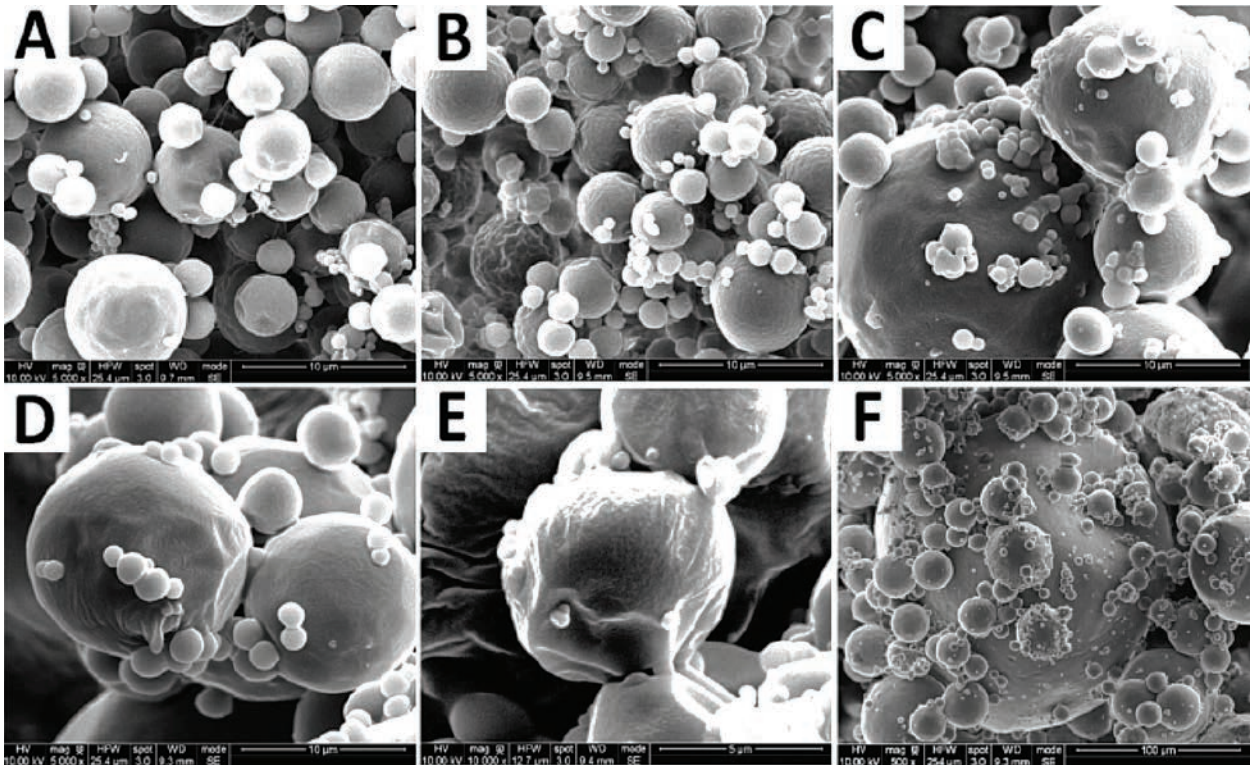

Figure 3. SEM images and release profile of IBP from PCL/PEO composite nanofiber scaffold with Labrafil-modified IBP PLGA microspheres.

delivery system. In future studies, the clinical application of these novel scaffolds will be investigated in the context of rotator cuff repair.

Significance

This work demonstrates that the controlled release of IBP can be achieved by modifying PLGA microspheres with Labrafil oil, providing a means by which the effect of prolonged release of this anti-inflammatory drug can be evaluated in tendon repair.

Acknowledgements

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