Multifunctional SPIO Labeled Calcium Phosphate Microparticles for Localized Therapeutic Protein Delivery

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Statement of Purpose: Many strategies to improve healing involve the use of therapeutic proteins. Despite many advances in therapeutic protein discoveries, maintaining protein stability and localization remains a major challenge and limiting factor for clinical use (1,2). To address the current limitations of localized therapeutic protein delivery, we have created an injectable calcium phosphate coated microparticle decorated with superparamagnetic iron oxide (SPIO-CaP-MP) that is trifunctional; it can deliver biologically active proteins, is trackable using magnetic resonance imaging (MRI), and can be manipulated with a magnetic field. We also sought to use MRI tracking to determine the duration these microparticles remained localized in a highly dynamic tissue.

Methods: Calcium phosphate coatings were created through precipitation by incubating β-tricalcium phosphate microparticles in modified simulated body fluid (mSBF) as previously described (3). The coating was then decorated with SPIO linked to bovine serum albumin, and an additional CaP coating was formed. Coating formation was confirmed through measuring changes in calcium ion concentration in the mSBF solution along with changes in the average microparticle radius. Delivery of basic fibroblast growth factor to human dermal fibroblast was used to observe the biological activity of proteins released from SPIO-CaP-MPs. To determine magnetic manipulation, SPIO-CaP-MPs were uniformly suspended in DI water and allowed to settle in a mold or in a mold placed atop a bar magnet.

SPIO-CaP-MPs or unlabeled CaP-MPs were injected into transected rat medial collateral ligaments (MCL) and MR scans were taken for each condition immediately following surgery to observe microparticle localization within a dynamic tissue. MR scans were then taken 2,5,7,15, and 21 days post injection. A concentration gradient of SPIO-CaP-MPs was injected intramuscularly into a rat cadaver and imaged with MR.

Results: CaP coatings were formed on β-tricalcium phosphate microparticles, they were decorated with SPIO linked to BSA, and the amount of SPIO bound to the microparticle could be tailored by adjusting the concentration of SPIO in the incubation solution during fabrication. Calcium ions precipitated from the mSBF solution to form the CaP coating both before and after SPIO incubation. An increase in the microparticle radius after each step in the coating process indicated the decrease in calcium ions could be attributed to coating formation and not from new crystal nucleation.

Through *in vivo* imaging of a rat, SPIO-CaP-MP localization was visible with MRI as an area of hypointensive signal and localization at the site of application was maintained for at least 15 days. MRI

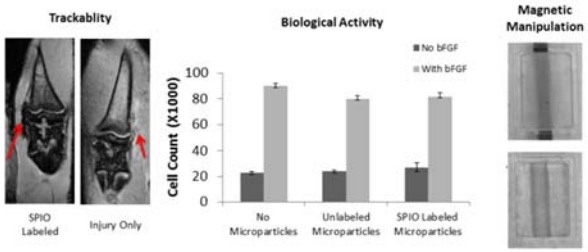

Figure 1: Trifunctionality of SPIO-CaP-MPs

imaging of an SPIO-CaP-MP concentration gradient injected intramuscularly into a rat cadaver revealed a detection limit of 1.25 mg/ml of SPIO-CaP-MPs in collagen gel, as indicated by a hypointensive area at the injection location. In order to observe the ability to distinguish SPIO-CaP-MP localization in a wound site with complicated architecture and heterogeneous composition, we implanted microparticles into a transected rat MCL. A large area of hypointensive signal along the MCL was visible in the animals that received SPIO decorated CaP microparticles when compared to the joint before injury. We also sought to observe the duration of SPIO-CaP-MP localization within a highly dynamic tissue and found that a detectable concentration of SPIO-CaP-MPs remained localized in the MCL for at least 15 days after application. We can also control localization of SPIO-CaP-MPs by manipulating them with a magnetic field. Microparticles suspended uniformly in solution localized to a magnet as the settled and could be maintained at the magnet upon further perturbation. Finally basic fibroblast growth factor released from SPIO-CaP-MPs was biologically active and resulted in an increase in human dermal fibroblast numbers. SPIO labeling did not significantly impact the biological activity of the bFGF released from the MPs

Conclusions: We have created a trifunctional CaP coated microparticle which can be tracked with MRI in complex tissues, can be manipulated with a magnetic field, and can deliver biologically active proteins. The ability to magnetically manipulate SPIO-CaP-MPs could be used to localize protein delivery by guiding particles after injection or maintaining localization using an external magnet. The ability to track and manipulated SPIO-CaP-MPs can ensure that therapeutic protein delivery remains localized for the duration of treatment, thus reducing unwanted side effects at distant sites and lowering the dose that is required to reach the therapeutic concentration range. These microparticles remain localized in highly dynamic tissues for at least 15 days and could be used to deliver therapeutic proteins in a variety of different situations where there is a need for localized delivery.

References

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