Development of Super-Lubricious Platelet-Rich Plasma Loaded Microgels for the Treatment of Knee Osteoarthritis

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INTRODUCTION: Knee osteoarthritis (OA) is a debilitating disease that significantly alters the microenvironment of the knee, increasing inflammation and reducing lubrication. This increased friction leads to significant wear and tear of articular cartilage, causing excruciating pain. Cartilage degeneration and inflammation proceed until a complete knee replacement is necessitated. It is currently estimated that over 27 million people in the United States alone suffer from varying degrees of OA, totaling ~$185 billion in socioeconomic costs. Furthermore, it is anticipated that 1 in 2 people will develop OA by the age of 85, resulting in over 67 million cases by 2030. As such, innovative treatments are needed to slow the inflammation and articular cartilage degeneration that characterize OA and to potentially restore joint homeostasis. One such approach is the delivery of platelet-rich plasma (PRP). PRP has shown mixed efficacy in delaying OA progression by releasing over 300 bioactive molecules that are critical to tissue regeneration and cellular recruitment. However, PRP delivery is hampered by rapid clearance from the synovial cavity. As such, we propose the use of polyethylene glycol (PEG) microgels to serve as delivery depots to prolong the half-life of PRP within the target area. PEG offers tunable swelling, mechanical, and degradation properties, allowing for intentional vehicle design while maintaining guest molecule bioactivity. Furthermore, our PEG microgels are coated with a custom-synthesized copolymer of dopamine methacrylate (DMA) and sulfobetaine methacrylate (SBMA), offering a layer of polymer brush coating to improve the lubricity of the synovial fluid in the OA knee.

METHODS: PEG microgels (~100 µm) were fabricated using a modified electrospraying setup, where gelation was induced following droplet formation through the addition of triethanolamine to the oil bath containing the PEG droplets. This setup allowed for longer run times than traditional methods, where gelation occurred within the syringe after 10 min. Following washing and buffer exchange into phosphate buffered saline (PBS), PEG microgels were dip-coated in 10 mg/mL custom poly(DMA-co-SBMA) copolymer for 30 min. Spectroscopy and confocal microscopy confirmed successful adsorption of copolymer onto PEG microgels. To evaluate the mechanical properties of the microgels, a custom tribo-rheology setup was developed to form a ball-on-three-ball setup, allowing for the measurement of the coefficient of friction. Microgels with and without copolymer coating were compared to PBS and simulated osteoarthritic fluid. Coated PEG microgels were evaluated in vivo in a murine model for their stability and for pain. Microgels with encapsulated fluorescent particles were measured using an in vivo imaging system (IVIS) over 12 d and Von Frey and static weight-bearing tests were performed for mice injected with microgels or PBS sham. PRP was encapsulated within PEG microgels and release was followed via Bradford assay for 14 d. Additionally, fluorescence correlation spectroscopy (FCS) was used to probe the diffusivity of PRP-related proteins, allowing for the calculation of release kinetics on the basis of protein molecular weight in various PEG and PEG + PRP conditions. This study was approved by IACUC.

RESULTS SECTION: PEG microgels with diameter of ~100 µm were successfully and consistently fabricated using the modified electrospraying setup, allowing for high-throughput fabrication (Figure 1A). Custom synthesized poly(DMA-co-SBMA) copolymer showed nuclear magnetic resonance (NMR) and infrared peaks consistent with each of the monomers being incorporated into one cohesive structure. Furthermore, the copolymer with integrated rhodamine B exhibited strong fluorescence that was correlated with copolymer concentration. PEG microgels swelled (20% increase in diameter) following buffer exchange into PBS but did not show a significant change in size following coating with copolymer. Copolymer coating on PEG microgels persisted for at least 14 d. In vivo testing revealed coated PEG microgels were stable for at least 30 d and did not significantly change mice behavior or pain threshold (Figure 1B). Importantly, coated microgels exhibited reduced coefficient of friction when compared to uncoated microgels and simulated OA synovial fluid (Figure 1C). Microgels were also shown to be easily injectable through a 27G needle. Following encapsulation within PEG microgels, PRP exhibited prolonged release over 14 d (Figure 1D), with release of specific proteins contingent on protein size. These trends were corroborated using FCS, where the presence of PRP significantly reduced protein diffusivity. Using these directly measured diffusion coefficients, a mathematical model was developed to predict the release kinetics of these proteins from microspheres of varying diameters (Figure 1E).

DISCUSSION: Together, these results demonstrate the tunable nature of these PEG microspheres to form a two-pronged treatment method for OA by improving lubrication and sustained delivery of PRP to modulate the local inflammatory response. The intentional design of these microgels allows for optimization with regard to both lubrication and PRP delivery kinetics in an easily injectable device. While the polymeric microgels themselves can aid in reducing friction in the synovial cavity, coating the microgels further improved lubricity. By encapsulating PRP within PEG microgels, slowed release was possible due to hindered diffusion within the PEG mesh network as well as the formation of an interpenetrating network formed by PRP itself. The prolonged release of PRP allows for fewer injections than traditional bolus dosing of PRP. Future work will focus on determining the in vivo efficacy of the PRP-loaded super-lubricious microgels.

SIGNIFICANCE/CLINICAL RELEVANCE: By developing dual-action hydrogel microspheres that restore joint lubrication and slow cartilage degeneration through the release of PRP, the progression of OA may be slowed, delaying the need for total joint replacement.

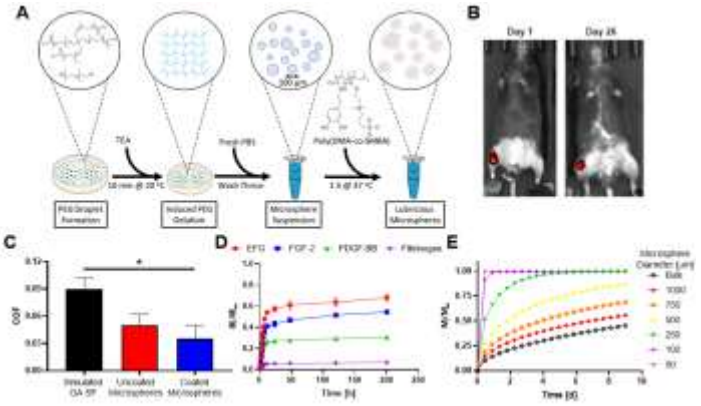

Figure 1: A. Schematic of PEG microgels coated with super-lubricious copolymer poly(DMA-co-SBMA). **B.** In vivo confirmation of microsphere localization and stability within a mouse knee. **C.** Dynamic coefficient of friction of simulated OA synovial fluid compared to uncoated microgels and microgels coated with lubricious copolymer. * indicates statistically significant difference ($N = 5$, $p < 0.05$.) **D.** Release profiles of PRP-related proteins from PEG + PRP microspheres in vitro. **E.** Predicted release kinetics of PDGF-BB from PEG + PRP microspheres of varying diameter and from 5 mm bulk hydrogel.