On-demand Immunomodulation Centers for Reducing Inflammation in Osteoarthritis

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Introduction: Osteoarthritis (OA) affects nearly 27 million Americans. OA is characterized by progressive degradation of cartilage. Cellular changes include decreased chondrocyte viability and increased proliferation, altered matrix synthesis, and increased levels of pro-inflammatory cytokines, resulting in elevated production of matrix-degrading enzymes. OA is often localized to less vascularized joint spaces such as the knee, limiting the efficiency of systemically delivered anti-inflammatory therapeutics. Therefore, localized intra-articular administration of drugs has emerged as a promising strategy for therapeutic intervention in OA. However, a major limitations is the low retention of bolus administered therapeutics in knee joints (1-5 hr). To overcome this limitation, we have recently engineered self-assembly nanoparticles that prolong the retention of therapeutic proteins over 14 days. In this study we report an "on-demand" protein delivery system that retains the anti-inflammatory therapeutics until exposed to matrix-degrading enzymes (matrix-metalloproteinase (MMPs)). Such on-demand immunomodulatory systems are expected to retain therapeutics over several months and release drugs every time the disease resurfaces, for example in patients exposed to repeated joint loading (e.g. running) or trauma.

Methods: Poly(2-hydroxyethyl methacrylate) (pHEMA) - pyridine was synthesized by conjugating pHEMA with nicotinoyl chloride hydrochloride, catalyzed by 4-dimethylaminopyridine (DMAP). Polymer modification was confirmed via NMR. The nanoparticles were fabricated by dripping a small volume of polymer solution into a solution of protein (BSA, FITC-BSA), which was vortexed. Unbound protein was removed by centrifugation. Intra-articular delivery and retention of nanoparticles without hydrogels have been previously reported by us [1] in rat knee studies using Fluorescence molecular tomography (**Fig. 1**). We next established the "on-demand" delivery approach by encapsulating the nanoparticles within MMP degradable PEG-Maleimide hydrogels. Release studies at various collagenase concentrations (5-15 µg/mL) were performed using BD Accuri flow cytometer.

Results: The polymer pHEMA-pyridine was successfully synthesized, confirmed with NMR. We have previously shown that the nanoparticles are non-toxic and maintain protein bioactivity. As seen in Figure 1 and reported earlier, protein-loaded larger nanoparticles (900 nm) have a longer half-life (2.5 days) as compared to the 500 nm nanoparticles (1.9 days) and bolus protein with a half-life 0.63 days over a period of 14 days (**Fig 1**). For on-demand release studies, we chose 900 nm nanoparticles encapsulated in PEG-MAL hydrogels cross-linked with di-thiolated VPM peptides that degrade in presence of MMPs such as collagenase. Recent studies in OA using gelatin-based system showed a release of 50% drug in

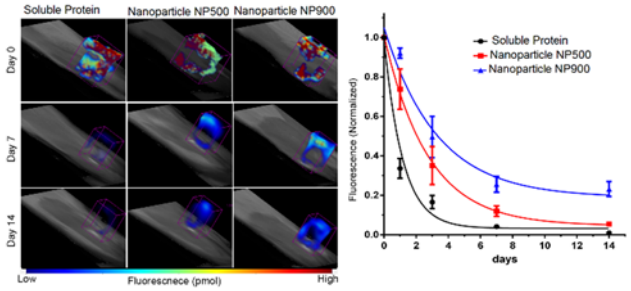

Fig. 1 Nanoparticle size controls retention in the intra-articular spaces in rat knee. *Fluorescence molecular tomography of the rat knee joints injected with bolus VivoTag®-S 750-BSA protein and nanoparticle complexed protein. Male Lewis rats (10-12 week old, n = 5) received 50 µL of either protein loaded particles or soluble protein via intra-articular injection to the right stifle joint space, while the left stifle served as a contralateral control. Half-life: NP500 > soluble p<0.025; NP900 > soluble p < 0.005; Plateau: NP900 > soluble p < 0.05; NP900 > NP500 p <0.05. Adapted from Singh et al 2014.*

PBS prior to enzyme exposure at 5 µg/mL [2]. Therefore, we tested our delivery platform for nanoparticle-protein retention between (5-15 µg/mL concentration of collagenase and observed 4.6 ± 1 % release at 5 µg/mL to 14.6 ± 1 % release at 15 µg/mL collagenase (**Fig. 2**). These studies simulated the in vivo situation in which the same hydrogel would be exposed to varying levels of MMP enzymes over multiple days. The untreated hydrogels only released approximately 1 % nanoparticles which was markedly lower than previously reported systems releasing ~20-40% of therapeutics in 2-4 hrs [2].

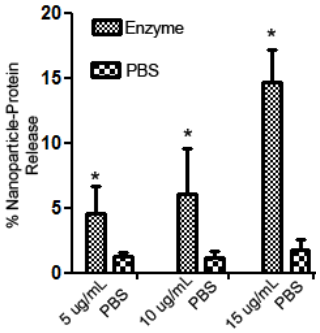

Fig. 2 On-demand release of protein complexed nanoparticles. *The on-demand immunomodulation center released protein loaded nanoparticles as a function of time and enzyme concentration.* P<0.05 compared to PBS on that particular day.*

Conclusion: We have engineered an on-demand therapeutic delivery platform for intra-articular delivery of therapeutic proteins. Our results establish that the immunomodulatory platform is sensitive to the presence and absence of enzyme concentrations, and retains nanoparticle-protein inside hydrogels by releasing 5-15% protein between 5-15 µg/mL enzyme concentrations. Future studies will establish the response to lower enzyme doses and efficacy in non-traumatic OA models [3].

Reference:

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