Nanoengineered particles for enhanced intra-articular retention and delivery of therapeutic proteins

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Statement of Purpose: Synovial inflammation has emerged as a promising target for therapeutic intervention in osteoarthritis (OA). Interleukin (IL)-1 and TNF-α have been implicated in OA pathogenesis by promoting synovial inflammation and activating chondrocytes and synovial fibroblasts. These cytokines stimulate their own production and induce synovial cells and chondrocytes to express IL-6, IL-8, and other inflammatory mediators as well as proteases and prostaglandins [1, 2]. Anti-inflammatory mediators, such as the Interleukin-1 receptor antagonist (IL-1Ra), reduce inflammation and slow down OA progression in animal models. Intra-articular delivery of therapeutics to modulate osteoarthritis (OA) is challenging and bolus protein injections of IL-1Ra suffer from rapid clearance and reduced potency over time. We recently demonstrated a RAFT-chemistry based self-assembly nanoparticles (300 nm) that efficiently bound IL-1Ra, targeted synoviocyte cells and inhibited IL1-beta mediated signaling [3]. These nanoparticles demonstrated significantly longer retention time of IL-1Ra in the rat stifle joint compared to that of soluble IL-1Ra and no adverse effect on the cartilage structure in knee joints. We then hypothesized that larger sized nanoparticles in the 500-1000 nm range will prolong the retention in rat intra-articular knee space. We have now engineered a new class of self-assembly polymer which contains a poly-hydroxyethylmethacrylate (pHEMA) backbone with a functionalized side chain that allows easy control over the particle size.

Methods: pHEMA-Pyridine was synthesized by reacting pHEMA with nicotinoyl chloride hydrochloride in tetrahydrofuran and pyridine. We studied a model protein Bovine Serum Albumin (BSA) for engineering self-assembled nanoparticles. Intra-articular delivery and retention in a rat knee was studied using Fluorescence molecular tomography. Briefly, endotoxin-free BSA was conjugated with VivoTag®-S 750, an amine-reactive near infrared (NIR) fluorochrome as per manufacturers' protocol. Male Lewis rats (10-12 week old, n = 5) received 50 µL of either protein loaded particles or soluble protein (500 µg VivoTag®-S 750-tagged BSA) via intra-articular injection to the right stifle joint space, while the left stifle served as a contralateral control. Fluorescence molecular tomography was performed on rat knees and average fluorescence efficiency within a region of interest centered on the knee normalized to their individual day 0 values and fitted using a one-phase exponential decay.

Results: Here we report a new class of self-assembly polymer which contains a poly-hydroxyethylmethacrylate backbone with a functionalized side chain to vary the particle size range and demonstrate the effect of nanoparticle size on retention time in rat intra-articular knee space. The polymer assembled into particles with average diameters that varied between each protein:polymer ratio of reactants in the range of 500 - 1000 nm. Using fluorescence molecular tomography, we evaluated the relationship between size of the nanoparticles and retention time in rat intra-articular knee space. As seen in Figure 1, protein-loaded larger nanoparticles (900 nm) demonstrated to 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).

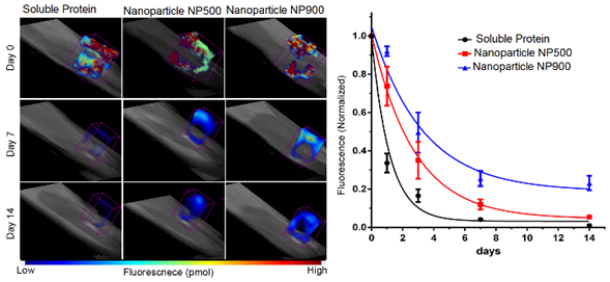

Fig. 1 Nanoparticle size controls retention in the intra-articular spaces in rat knee. *Fluorescence molecular tomography of rat knees injected with bolus VivoTag®-S 750-BSA protein and nanoparticle complexed protein. Half-life: NP500 > soluble $p<0.025$; NP900 > soluble $p < 0.005$; Plateau: NP900 > soluble $p < 0.05$; NP900 > NP500 $p < 0.05$*

Conclusions: Creating localized drug delivery systems can reduce inflammation associated with OA treatment but poses a challenge because of rapid clearance. Using protein-polymer complexes at different weight ratios and reaction stirring rates, we have engineered particles ranging from 500-900 nm and demonstrated prolonged retention with larger nanoparticles compared to smaller nanoparticles or bolus protein over a period of 14 days. Future studies will focus towards studying the retention of IL-1Ra protein in rat knee and treatment of OA in rats with induced knee injuries. We anticipate that disease conditions will further enhance retention due to receptor ligand interactions and therefore IL-1Ra intra-articular injections could emerge as a safer and more effective treatment for OA.

References:

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