Synthesis of self-assembled IL-1Ra-presenting nanoparticles for the treatment of osteoarthritis

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Statement of Purpose: Intra-articular delivery of therapeutics to modulate osteoarthritis (OA) is challenging. Delivery of interleukin-1 receptor antagonist (IL-1Ra), the natural protein inhibitor of IL-1, to modulate IL-1-based inflammation through gene therapy or bolus protein injections has emerged as a promising therapy for OA. However, these approaches suffer from rapid clearance and reduced potency over time. Nano/microparticles represent a promising strategy for overcoming the shortcomings of intra-articular drug delivery. However, these delivery vehicles are limited for delivery of protein therapeutics due to their hydrophobic character, low drug loading efficiency, and harsh chemical conditions during particle processing. Here, we report a new class of self-assembly polymer which contains a poly-hydroxyethylmethacrylate (pHEMA) backbone with a functionalized hydrophobic side chain of pyridine to complex and present the therapeutic IL-1Ra protein In the present work, we focused on engineering IL-1Ra-presenting particles of various sizes by varying the protein:polymer ratio.

Methods: To prepare the nanoparticles, 50 µL of 1mg/mL IL1-Ra was added to 0.4 mL of phosphate-buffered saline in an Eppendorf to form a protein suspension. After homogenization, poly hydroxyethyl methacrylate-pyridine in dimethylformamide (DMF) at a concentration 2 mg/mL was slowly added to the protein suspension under vortex agitation. After shaking for 30 minutes, solutions were transferred to 10 kDa dialysis cassettes and dialyzed overnight against ultrapure distilled water with at least 3 buffer changes. The particles were transferred to a sterile Eppendorf tube and stored at 4°C until further use. Particle size was analyzed by dynamic light scattering using a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation, Holtsville, NY). For scanning electron microscopy (SEM), 5 µL of nanoparticle suspension was dispensed on a SEM stub, air dried for 2 hours and sputter coated with 5 nm of gold to make the sample conductive. Imaging was done with a 5 KeV electron beam.

Results: The system self assembles into nanoparticles with polymer forming the core of the particle and protein presented at the surface. In this experiment, five different protein to polymer ratios were studied: 1.0, 1.25, 1.5, 1.75 and 2.0. Figure 1 shows the measured particle size measured by DLS for each studied composition.

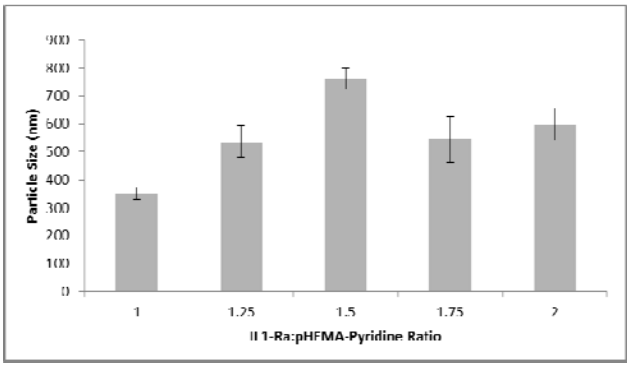

Figure 1. Particle size of the synthesized nanoparticles measured by DLS

Results show the protein:polymer ratio regulates the size of nanoparticles with a maximum size of 761 ± 38 nm for a 1.5 ratio. The particle size measured by DLS was confirmed by SEM, as showed in Figure 2.

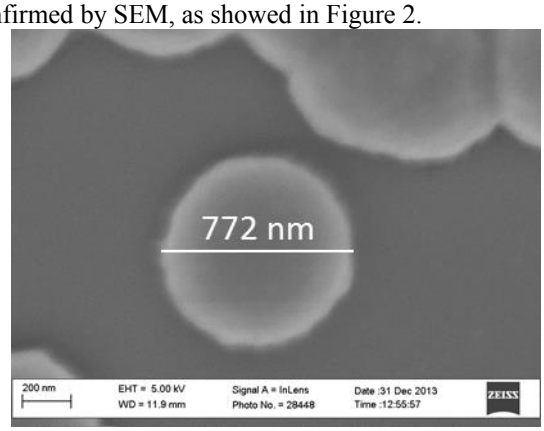

Figure 2. SEM micrograph showing spherical particle for 1.5 ratio.

We attribute the driving force for the co-assembly into nanoparticles to non-covalent interactions between the protein and polymer because pHEMA-pyridine has the ability to provide electrostatic, hydrophobicity, and hydrogen bonding. When the polymer is dissolved in an organic solvent and mixed with protein in an aqueous environment, due to presence of pyridine groups on the polymer, the polymer has a tendency to protect itself from water. Hence it self-assembles such that protein makes a layer around the polymer and after extensive dialysis against water, it forms a solid particle.

Conclusions: IL-1Ra-presenting nanoparticles of varying size were obtained by varying the protein to polymer ratio. Future studies will examine the bioactivity of the IL-1Ra and the effect of nanoparticle size on protein retention time in the rat intra-articular stifle joint space.

References: R. E. Whitmire, D. S. Wilson, A. Singh, M. E. Levenston, N. Murthy, A. J. Garcia, Biomaterials 2012, 33, 7665.