A novel RAFT block copolymer for anti-inflammatory protein delivery

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Statement of Purpose: Inflammatory disorders have a significant socioeconomic impact in the U.S. For example, osteoarthritis (OA) affects 10% of the US population. The economic cost of this disease will continue to increase because there is no cure. OA is characterized by high levels of macrophage-produced interleukin-1 (IL-1). The IL-1 receptor antagonist (IL-1RA) has been used in animal models and human trials in

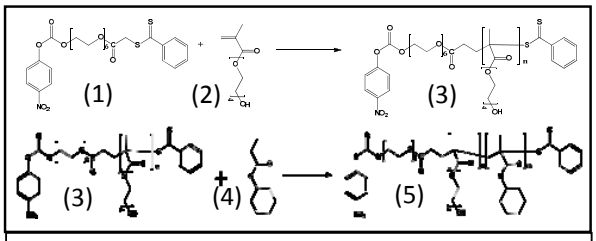

Fig. 1: Diagram of copolymer reaction scheme

an effort to competitively block IL-1 signaling in the OA joint. Obstacles to successful biomaterial-based protein delivery include low encapsulation efficiency, fast release kinetics, and harsh reaction conditions. We have synthesized an amphiphilic copolymer which forms submicron particles and efficiently tethers anti-inflammatory proteins to its surface using a novel chemistry. This technology targets IL-1-induced inflammation.

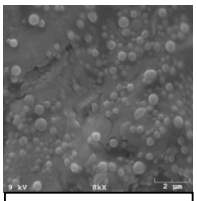

Fig 2: SEM of copolymer particles

Methods: A block copolymer was synthesized using RAFT techniques. Briefly, a commercial RAFT agent was modified to contain a paranitrophenol (pNP) group((1) in Fig. 1). This RAFT agent was then used to build our block copolymer. A ratio of 25 molar excess of tetraethylene glycol methacrylate (TEGM) monomer ((2) in Fig. 1) was added to the RAFT agent and reacted in dioxane with AIBN as the initiator under nitrogen for eight hours.

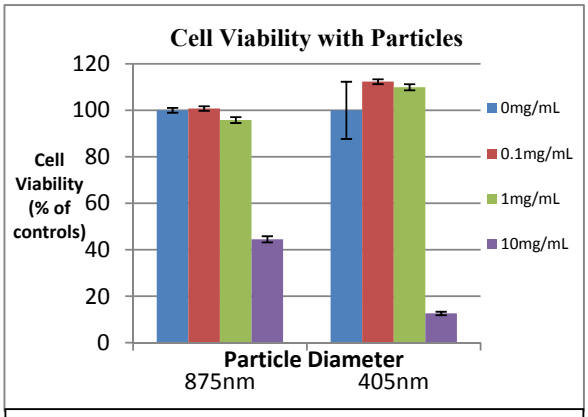

Fig. 3: Quantification of RAW264.7 macrophage cell viability using the MTT assay.

The reaction was quenched with liquid N_2 and precipitated in cold hexanes. A ratio of 5 molar excess cyclohexyl methacrylate (CHM) ((4) in Fig. 1) and AIBN was then added to the solution, and was reacted for 20 hours at 60°C. The reaction was precipitated with cold hexanes and dried under vacuum. The products were verified by GPC and H^1-NMR. To form particles, polymer was dissolved in THF at 40mg/mL. One mL of polymer solution was added to a round bottom flask. PBS, pH 6.0, was added at a rate of 1mL/min while stirring. The solution was then rotovapped for 30min to remove excess THF. To attach protein, the solution pH was raised to pH7.0. Protein was added, and the pH was raised

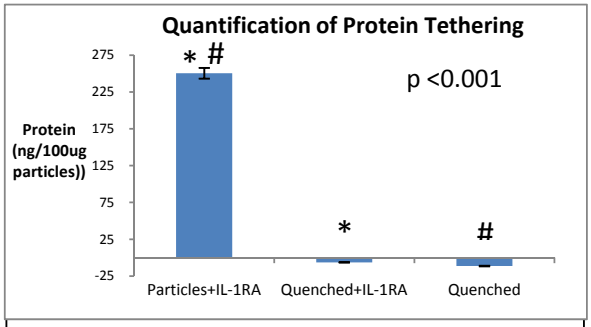

Fig. 4: IL-1RA tethering to polymer particles.

to pH8.5 and reacted for 2h. Particles were centrifuged, washed twice and resuspended in PBS.

Results: We have designed and synthesized an amphiphilic block copolymer composed of TEGM and CHM using RAFT polymerization techniques. By controlling the copolymer composition, particles on the order of 400nm can be prepared (Fig. 2). The particles' cytotoxicity is comparable to that of other microparticles, with full viability up to concentrations of 1mg/mL *in vitro* (Fig. 3). The modified RAFT agent allowed efficient attachment of IL-1RA to our particle surface under mild reaction conditions. We quantified attached protein using dot blots (Fig. 4). We are currently analyzing the ability of IL-1RA-tethered particles to block IL-1-induced cytokine upregulation in macrophage cultures and a rat model of OA.

Conclusions: We synthesized a novel block copolymer using RAFT polymerization. This copolymer spontaneously forms 400 nm particles. Using the paranitrophenol moiety on our modified RAFT agent, we can attach proteins to the particles under mild reaction conditions with high efficiency. These particles show promise for modulating IL-1-induced inflammation.

References:

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