Imaging Macrophage Activity in Vulnerable Atherosclerotic Plaques With Functionalized Iron Oxide Nanoparticles

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Statement of Purpose: Ruptured atherosclerotic plaques are a leading cause of strokes and heart attacks, but there is currently no robust, noninvasive method to discriminate stable plaques from "vulnerable" plaques. In addition to macrophage infiltration, vulnerable plaques exhibit pathologic concentrations of matrix metalloproteinase-9 (MMP-9)[1]. We have, therefore, synthesized and characterized "proximity-activated" ultrasmall superparamagnetic iron oxides (PA-USPIOs) coated with two ligands: MMP-9-degradable polyethylene glycol (PEG) and ligands targeting the macrophage scavenger receptor (SR). In circulation, the MMP-9-degradable PEG masks the SR-targeting ligands. In the presence of macrophage activity and a proteolytic environment, characterized by a pathologic concentration of MMP-9, the PEGs are cleaved, unveiling the underlying SR-targeted ligands.

Methods: The behavior of PA-USPIOs co-incubated with or without enzymatically active MMP-9 was characterized by FT-IR, dynamic light scattering (DLS), and TEM. Using flow cytometry, fluorescence imaging, and MRI, MMP-9-dependent PA-USPIO binding was demonstrated *in vitro* in cell cultures of human THP-1 monocytic leukemia cells.

Results: The protease-dependent response of the proposed dual-ligand nanoparticle was analyzed via DLS (Figure 1). The data confirms that treatment of the nanoparticles with MMP-9 results in the loss of ~10nm of hydrodynamic diameter by the nanoparticles.

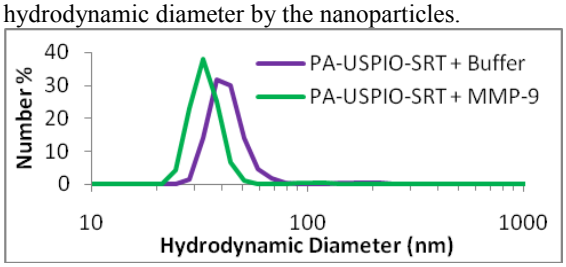

Figure 1. DLS size-number distributions of dual-ligand nanoparticles validates MMP-9-dependent shedding of polymeric shell from the nanoparticles.

We then incubated THP-1 monocyte/macrophage cells with the nanoparticles in the absence of MMP-9 in order to assess the kinetics of nonspecific uptake. At specific timepoints, cells were lysed and iron was measured via a colorimetric method, and normalized to cell number indirectly through protein content (Figure 2). As a positive control, larger, 100nm micelles of iron oxide were also studied. By mass, the 30nm nanoparticles were less avidly uptaken by the macrophages than were the 100nm nanoparticles. Further, nanoparticle uptake can be blocked at 4°C (not shown) or with co-administration of fucoidan (Figure 3), results that support the idea of specific uptake via scavenger receptor-mediated mechanisms. Peptide-mediated targeting of the nanoparticles to the scavenger receptor produced mixed results as a function of micelle size (not shown). To improve specificity, we have synthesized acetylated LDL (acLDL)-encapsulated USPIOs to serve as the "hidden package" beneath the

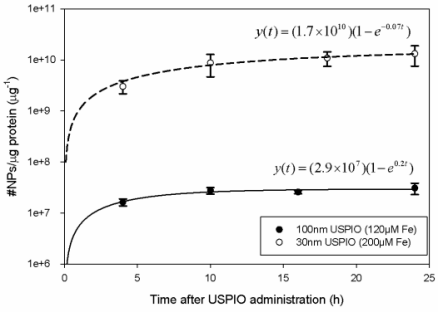

Figure 2. Analysis of nonspecific uptake of PEGylated iron oxides by THP-1 macrophages. Cells were incubated with iron oxide micelles of different sizes in order to assess their nonspecific uptake. Regression equations to fit data to a first-order saturation curve are included.

MMP-9-cleavable PEG shell. Preliminary results suggest that the acLDL improves the kinetics of macrophage targeting. Therefore, encapsulation of these acLDL-USPIO particles inside nanoparticles of MMP-9-cleavable PEG hydrogels appears promising as a means to image macrophage activity in vulnerable atherosclerotic plaques.

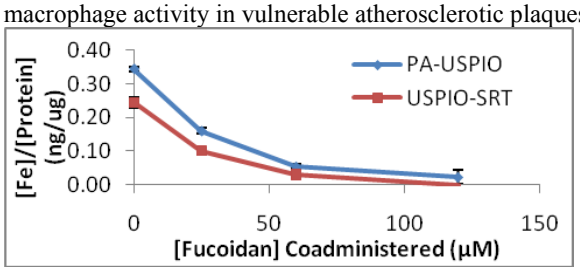

Figure 3. Nanoparticle binding (with or without SR-targeted peptide) can be competed away by co-incubation with fucoidan, supporting a scavenger receptor-mediated uptake mechanism for the nanoparticles.

Conclusions: Dual function nanoparticles exhibit the ability to unveil ligands for cell targeting in the presence of proteolytic activity. However, macrophages uptake nanoparticles non-specifically regardless of the presence of a targeting peptide. Ongoing experiments have suggested that acLDL displaying nanoparticles possess faster uptake kinetics that may enable differential uptake despite the nonspecific endocytosis. Taken altogether, MMP-9-unveilable acLDL-USPIOs appears to be a promising approach for imaging of macrophages in vulnerable atherosclerotic plaques.

References:

[1] Galis ZS. J Clin Invest. 1994; 94: 2493-2503.