Surface Modification of Plaque-Targeting Nanoparticles Allows for Control of Cell Internalization

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Statement of Purpose. Superparamagnetic iron oxide (SPIO) contrast agents provide exciting new opportunities to improve the sensitivity and specificity of magnetic resonance imaging (MRI) for assessment of plaque composition and morphology. Targeting agents for atherosclerotic plaque could provide early detection by specific binding to cell surface markers of endothelium covering atherosclerotic plaque. These multi-functional particles could also be engineered to deliver desired drugs to the site of plaque. This study focuses on time and surface dependent uptake of superparamagnetic iron oxide nanoparticles by human aortic endothelial cells (HAECs). These magnetic nanoparticles are composed of iron oxide crystals embedded in a dextran matrix. Surface modifying agents (polyethylene glycol (PEG) and a targeting protein ligand (TPL)) are conjugated to the dextran matrix via hydroxyl oxidation and $NaCNBH_3$ reduction.

Methods. Derivatized superparamagnetic iron oxide nanoparticles (Miltenyi Biotec) were characterized for size, surface composition and cellular interactions with human aortic endothelial cells (HAECs). Surface modification was confirmed using Fourier-transform infrared spectroscopy. Dynamic light scattering was used to determine the hydrodynamic diameter of underivatized and modified nanoparticles. HAECs were seeded on 4-well chamber slides (Fisher) and incubated at 37°C in a humidified atmosphere with 5% CO_2 with PEG- and TPL-conjugated nanoparticles for incubation times of 15, 30 and 60 minutes. For control experiments, cells with no nanoparticles were used. The cells were washed with a 0.1 M phosphate buffer/0.1 M sucrose solution and post fixed in buffer containing 1% osmium tetroxide for 30 minutes at room temperature. For TEM analysis, the fixed cell samples were molded into capsules with epoxy resin, cut into 70 nm thin sections and stained with 2% uranyl acetate and Reynolds lead citrate. A Philips CM 12 transmission electron microscope was used to collect high resolution images.

Results / Discussion. The mean hydrodynamic diameter of basic nanoparticles was 82 nm, while the mean diameter of the PEG- and TPL-conjugated nanoparticles increased by 5-20 nm. This increase in particle size is consistent with the length of the polymer or protein chains. Results from the TEM studies showed marked differences between the internalization of the passive PEG-nanoparticles and the targeting TPL-nanoparticles. The PEG-nanoparticles were found inside the HAECs within 15 minutes, with substantial accumulation in the perinuclear region. Also, these nanoparticles were mostly seen as individual nanoparticles. After 15 minutes, the PEG-nanoparticles showed little change in distribution, although some accumulation of nanoparticles into vesicles after 15 minutes suggested intracellular shuttling mechanisms. Alternatively, the TPL-nanoparticles were predominantly found at the outer cell membrane after 15 minutes, with some aggregates seen (Figure 1). After 30 minutes, these nanoparticles were seen within the cell cytoplasm, but none were seen at or near the nucleus. Aggregation within vesicles was observed at this time frame (Figure 1). At the one hour point, nanoparticles were no longer observed within vesicles, but were seen as individual nanoparticles within the cytoplasm, suggesting an escape mechanism from the vesicles or rapid degradation within the vesicles.

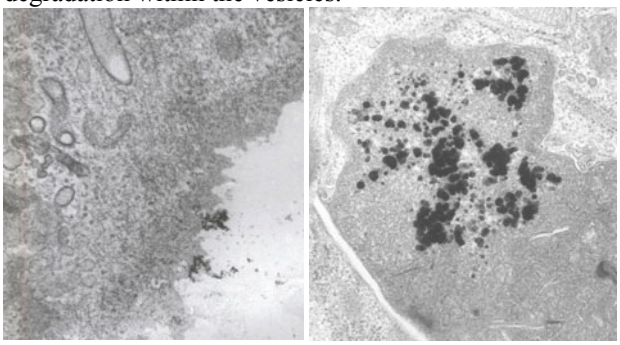

Figure 1. TEM images showing characteristic views of TPL-derivatized superparamagnetic iron oxide nanoparticles. After 15 minutes, small clusters are seen at cell membrane (Left). After 30 minutes, aggregates within vesicles are observed (Right).

These results demonstrate the ability to manipulate cellular internalization and response by modification of the nanoparticle surface. The PEG-conjugated particles likely provided an easier route of internalization due to the neutral, lipophilic PEG chains that could penetrate the cell membrane. Conversely, the TPL-nanoparticles were likely held up at the cell membrane from targeting of the cell surface and electrostatic charge interactions at the cell surface. Once inside the cell, the tethered molecules elicited different responses from the cell, which could be important for drug delivery applications.

Conclusion. The uptake of PEG- and targeting ligand superparamagnetic iron oxide nanoparticles by human aortic endothelial cells was time and surface dependent. Each surface generated a unique cellular response and internalization effect. Tailoring the nanoparticle surfaces with a combination of targeting and passive biomolecules will likely afford an ideal candidate for plaque targeting and drug targeting either within the cell or at the cell surface.

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