A Protease-Activatable Nanoparticle Contrast Agent for Molecular Imaging by Dual Energy Computed Tomography

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Statement of Purpose: X-ray computed tomography (CT) is one of the most useful imaging tools for modern clinicians. However, molecular imaging with CT is not feasible with existing contrast agents, so clinicians must instead rely on other modalities which are more expensive and less readily available. Dual energy CT, a relatively new technique using two x-ray energies for a single scan, can provide valuable information about material composition. We have previously shown that dual energy CT can accurately differentiate and quantify gold and iodine in vivo[1] (see Fig. 1). Two-material differentiation can potentially be used for molecular imaging if it is coupled with appropriately-designed contrast agents. We seek to develop for the first time an activatable dual energy CT contrast agent for the molecular imaging of protease activity. This agent is a composite probe consisting of iodine-containing liposomes (~120 nm) linked to multiple small (~5 nm) gold nanoparticles (AuNPs) by a protease-cleavable peptide. Cleavage of the peptide within tumor tissues will allow the small AuNPs to diffuse away from the much larger liposomes. Separation of the gold and iodine signals, detected by dual energy CT, can then be used to measure protease activity.

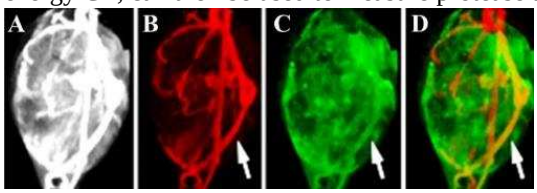

Figure 1. **(A)** Raw CT image of soft-tissue sarcoma after contrast agent injection. **(B)** Iodine map after dual energy decomposition shows iodine within the blood vessels. **(C)** Gold map shows gold accumulated within the tumor. **(D)** Overlay of separated gold and iodine maps.

Methods: AuNPs were produced by reduction of gold chloride by citrate and tannic acid. AuNPs underwent ligand exchange with a mixture of PEG-thiol and azide-PEG-thiol to produce a biocompatible surface with functional groups available for further conjugation. A protease-cleavable peptide, with the sequence GGGPQGIWGQGGCG, was synthesized. This peptide is sensitive to cleavage by matrix metalloproteinases 2 and 9, which are upregulated in many tumor types. The N-terminal amine of the synthesized peptide was then modified with DBCO-PEG-NHS (Dibenzocyclooctyl-PEG-N-hydroxy succinimide). Iodine-containing liposomes were produced by extrusion of phospholipids hydrated in concentrated iodixanol. The cleavable peptide was then linked to the liposome surface via a reaction between the peptide thiol and maleimide groups on the liposome surface. The peptide-modified liposomes were then linked to the AuNPs via an azide-alkyne reaction between the peptide DBCO and AuNP azide groups. The AuNPs, liposomes, and composite nanoprobes were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential, and size exclusion chromatography (SEC). Stability of the AuNPs and the liposomes in physiological salt conditions and serum was confirmed by UV-Vis spectrophotometry and DLS. Cleavage of the peptide linker in a solution of collagenase was quantified using a fluorescamine assay.

Results: AuNPs were synthesized with an average diameter of 5.0 nm and zeta potential of -33 mV, which increased to -16 mV following ligand exchange. The resulting AuNPs showed no aggregation in 1 M NaCl or serum. The synthesized peptide linker was rapidly degraded by collagenase, while a control peptide sequence showed only minimal degradation. Iodine-containing liposomes were produced with an average hydrodynamic diameter of 120 nm. The liposomes were stable in physiological conditions and showed no leakage over 2 weeks. In vivo studies demonstrated high CT enhancement following liposome injection with a blood half-life of ~72 hours. Conjugation of the gold nanoparticles to the liposomes increased the diameter of the probe to 220 nm (see Fig. 2A). Successful conjugation was confirmed by SEC. TEM imaging of the combined nanoprobe (see Fig. 2B) shows liposomes surrounded by a layer of AuNPs. The combined probe shows no size change when exposed to solutions of high salt or serum and is stable for >2 weeks. The combined probe contains a gold:iodine ratio of 0.5 and can be concentrated up to 100 mg/mL iodine for in vivo injection.

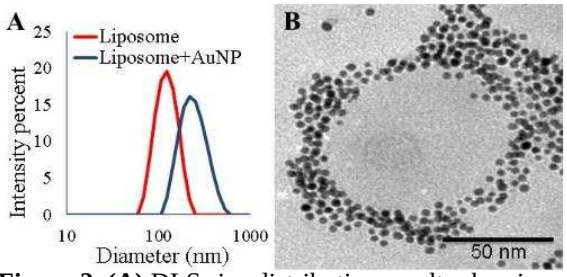

Figure 2. **(A)** DLS size distribution results showing an increase in diameter following conjugation of AuNPs to the liposomes. **(B)** TEM image of liposome conjugated to multiple gold nanoparticles. Scale bar represents 50 nm.

Conclusions: We have successfully synthesized and characterized a CT probe consisting of iodine-containing liposomes conjugated to gold nanoparticles by a protease sensitive peptide linker. Each of the probe components shows high stability and biocompatibility. This is the first demonstration of an activatable molecular imaging CT probe. Future studies will test the use of this probe for dual energy CT imaging of protease activity in vivo. This probe has the potential to significantly improve CT imaging for a variety of disease processes with high proteolytic activity, including cancer and atherosclerosis.

References:

1. Clark DP. Phys Med Biol.2013;58(6):1683-1704.