Liposomal Probes for Enhanced Vascular Imaging and Diagnosis via a Polymeric Fastener

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Statement of Purpose: Gadolinium chelates as magnetic resonance imaging (MRI) contrast agents are cleared rapidly from the body. As such, gadolinium (Gd) is often incorporated in the core of a nanoparticle, frequently a liposome, to enhance retention and allow for co-delivery with therapeutic components (Na K. Colloids Surf B. 2011;84:82-87.). Encapsulation however, greatly reduces the molar relaxivity of the contrast agent (Ghaghada K. Acad Radiol. 2008;15:1259-1263.). Additionally, chemical conjugation on the particle surface is inefficient and can damage the nanoparticle. We therefore present a polymeric fastener, named for its ability to self-assemble on the surface of a liposome and immobilize Gd through surface chelation (Smith CE. ACS Nano. 2013; in press.). Furthermore, as liposomes are prone to degradation *in vivo* due to several factors such as rupture and disassembly (Taira MC. Drug Deliv. 2004;11:123-128.), we present a method of stabilization via cross-linkable lipids to stablize the particle and its association with the fastener. Taken together, these strategies should enhance the diagnostic capability of MRI.

Methods: The polymeric fastener was synthesized by conjugating chitosan with the Gd chelate, diethylenetriaminepentaaceitc acid (DTPA), as well as stearic acid to act as a hydrophobic anchor. Liposomes were formed by hydration of dipalmitoylphosphatidylcholine (DPPC), and the association between liposome and fastener was evaluated by isothermal titration calorimetry (MicroCal, Northampton, MA) and confocal microscopy (LSM 700, Carl Zeiss Microimaging, GmbH, Germany). Relaxivity of the contrast agent was determined by Siemens Magnetom Allegra MR Headscanner (Siemens AG, Erlangen, Germany). The effectiveness of the particles in diagnosing cardiovascular disease was then evaluated with two different animal models: hindlimb ischemia of BALB/c mice, and renal ischemia of Sprague-Dawley rats. Finally, the liposomes and fastener were stabilized in the presence of serum by employing $DC_{8,9}PC$ lipids, which were cross-linked upon exposure to light at 254 nm.

Results: Successful grafting of DTPA and stearic acid to the chitosan backbone was verified by colorimetric assays. The surface localization of the modified chitosan on the liposome surface was confirmed by confocal microscopy of fluorescently-labeled chitosan. Additionally, the adsorption process was found to be entropy-driven, and augmented by the presence of the hydrophobic anchoring provided by the alkyl graft. The stabilizing anchor proved to be imperative in enhancing MR signal per liposome dose, as Gd chelation caused 30% desorption without the anchor. Furthermore, the strategy of using a polymeric fastener proved useful in enhancing relaxivity beyond that of clinically used Gd-DTPA chelates. In animal studies, the Gd-coated liposomes were able to accumulate exclusively in an ischemic hindlimb, thereby allowing for the local diagnosis of the disease (Figure 1a). Additionally, in the ischemic kidney model, corticomedullary differentiation was maintained with the Gd-liposomes, unlike with clinical Gd-DTPA (Figure 1b).

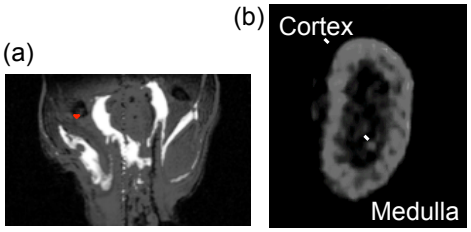

Figure 1. Diagnosis of ischemic vasculature in murine models of (a) hindlimb ischemia and (b) renal ischmia

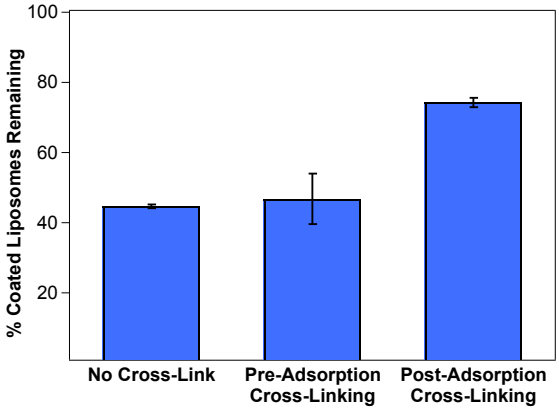

Figure 2. Percent coated liposomes remaining after 1 h incubation in human serum

To further enhance the stability of liposomes for future *in vivo* applications, the benefit of crosslinking $DC_{8,9}PC$ liposomes either before or after fastener adsorption was evaluated. Interestingly, cross-linking prior to adsorption reduced the amount of chitosan that could bind to the liposome, which was confirmed by calorimetry. As shown in Figure 2, cross-linking the liposomes after fastener adsorption greatly enhanced the amount of functionalized liposomes present after one hour of incubation in serum.

Conclusions: The chitosan fastener represents a simple method to formulate nanoparticle-based contrast agents through modular assembly for enhanced relaxivity and retention. The assembled complex can be used for diagnosis of various diseases, including cardiovascular disease. Furthermore, the fastener and liposomes are stabilized via cross-linking. Overall, the strategies presented herein will be broadly useful for formulation of next generation nano- and microparticles.

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