Bio-Inspired Assembly for Surface Localization of Gadolinium to Improve Relaxivity of an MRI Contrast-Enhancing Liposome

Cartney E. Smith, Prof. Hyunjoon Kong.
Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign.

Statement of Purpose: The next generation of nanoparticle-based delivery systems pursues methods to include both diagnostic and therapeutic components in one assembled structure. For example, the magnetic resonance imaging (MRI) contrast agent, gadolinium (Gd), may be incorporated with a drug in a lipid vesicle to enhance retention of Gd and provide a means to simultaneously monitor and treat disease (Tagami T. Biomaterials. 2011;32:6570-6578.). Current fabrication strategies, however, are plagued by difficult synthesis and purification steps, as well as low encapsulation efficiencies when multiple components are used (Kamaly N. Chem Soc Rev. 2012;41:2971-3010.). Additionally, Gd chelates have been found to interact with various biomolecules, which could negatively impact their delivery if co-encapsulated (Wang Y. Magn Reson Med. 2010;63:609-616.). Furthermore, the T_1 relaxivity provided by chelated Gd has been shown to significantly decrease when the contrast agent is encapsulated in the vesicle core (Ghaghada K. Acad Radiol. 2008;15:1259-1263.). We therefore investigated an advanced method to localize Gd on the surface of pre-formed liposomes via hydrophobic association. The technique takes its inspiration from the way in which various transmembrane proteins and viruses form associations with the cell bilayer through electrostatic assembly and insertion of a hydrophobic domain (Gallusser A. Embo J. 1990;9:2723-2729.).

Methods: Chitosan, a biodegradable polysaccharide, was reacted with the Gd chelate diethylenetriaminepentaacetic acid (DTPA) through carbodiimide-mediated amide formation. It was further modified with a C_{18} alkyl chain to enhance hydrophobic interactions. Separately, liposomes were formed from dipalmitoylphosphatidylcholine (DPPC) lipids using a film hydration method. The formed liposomes were functionalized by incubation with the modified chitosan. The association between modified chitosan, termed DTPA-chitosan-g-C_{18}, and liposome was analyzed by isothermal titration calorimetry (ITC) to determine the effect of alkyl chain on surface assembly. Additionally, DTPA-chitosan-g-C_{18} was labeled with rhodamine dye for visualization by confocal microscopy and for quantitation of adsorbed chitosan in the presence of Gd. Finally, the effectiveness of the system as a T_1 MRI contrast agent was evaluated with a 3T Siemens Magnetom Allegra MR Headscanner (Siemens AG, Erlangen, Germany).

Results: Chitosan was modified with C_{18} at a degree of substitution (DS_{C18}) of 0, 2.3, and 4.2%, and a DS_{DTPA} of approximately 15% across all samples as confirmed by TNBS and xylenol orange colorimetric assays. The interaction between DTPA-chitosan and liposomes was found to be endothermic yet favorable, as indicated by a decrease in Gibbs free energy. Additionally, the entropy-driven binding was enhanced with the inclusion of C_{18} chains, which served to introduce stabilizing hydrophobic interaction. As supported by ITC and fluorescence assay, DTPA-chitosan-g-C_{18} and liposomes interacted with a 1:1 binding stoichiometry of glucosamine unit of chitosan and exposed lipid, regardless of DS_{C18}. However, without the stabilizing alkyl graft, Gd induced desorption of the modified chitosan from the liposome surface.. Finally, as shown in Figure 1, the relaxivity of MR phantoms of liposome-adsorbed DTPA-chitosan-g-C_{18} was enhanced beyond that of clinically used DTPA. Furthermore, surface absorption proved beneficial in enhancing relaxivity compared to traditional encapsulation.

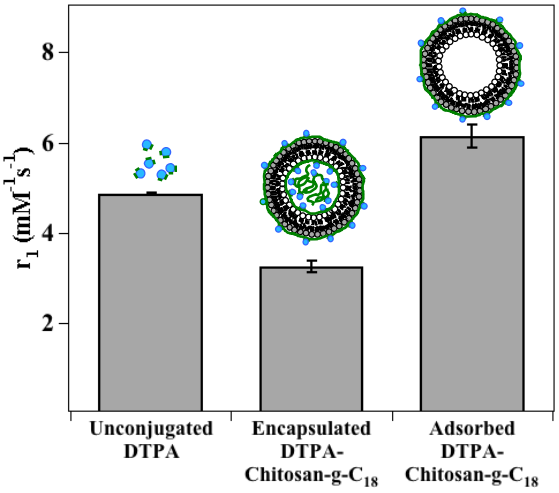

Figure 1. Relaxivities of Gd Chelates

Conclusions: We have presented a simple, but advanced method for surface modification of a liposome that overcomes the drawbacks of encapsulation of imaging contrast agents and the challenges of chemical reactions post-fabrication. The molecular design of the modified chitosan allowed for stable association with the liposome, and was able to mitigate the desorption triggered by Gd chelation. Additionally, by localizing Gd to the outer leaflet of the liposome, we demonstrated enhanced T_1 relaxivity. This approach will allow for greater ease of future optimization since it provides a way to decouple particle formation from functionalization. . Additionally, other functionalities, such as secondary imaging agents or targeting ligands, could be conjugated to the chitosan backbone and adsorbed to the liposome surface. Taken together, this study demonstrates a fabrication strategy that will be highly useful in the formulation of future multifunctional nano- and microparticles.