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Imaging nanoparticle stability and activation *in vivo*

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***Abstract*—While liposomes and nanoparticles have been the subject of intense research for more than 40 years, few particles have been translated into clinical practice. Advantages of these particles include the potential to overcome the cardiac, renal or neural toxicity of systemic chemotherapy, the opportunities for multivalent targeting, the gradual yet significant accumulation within tumors due to leaky blood vessels and the myriad of new approaches to locally alter the properties of the particle in the region of interest. Given the complexity of the design and co-optimization of the surface architecture, shell formulation and drug loading, methods to image the pharmacokinetics of nanoparticles in living systems are an essential part of an efficient research methodology. Here, we describe our efforts to label the shell and drug core of lipid-shelled particles with a goal of facilitating translation of activatable particles.**

I. Introduction

Nanoparticles have been used as drug delivery systems to enhance drug stability, circulation time, localized delivery and efficacy. Our goal is to develop real-time minimally-invasive methods for imaging the pharmacokinetics of activatable drug delivery vehicles. By labeling the particle shell and the drug compartment, formulations can be optimized for specific molecular targeting, stealth, plasma stability and activation. We have focused on labeling particles with positron emission tomography (PET) and optical labels; PET facilitates quantitative, real-time estimates of pharmacokinetics and optical imaging facilitates the visualization of stability and activation.

II. Labeling and Resulting Images

We have considered two approaches for the incorporation of a radiolabel. First, we developed a method to conjugate a fluorine-18 label on a lipid molecule prior to its insertion within a nanoparticle (Fig. 1) [1, 2]. The radiolabel remains stably attached until the lipid is metabolized, at which time the fatty acid tails are separated from the radiolabeled-small molecule head group. This approach facilitates both the visualization of the circulating nanoparticles and detection of lipid metabolism due to the elimination of the small molecule head group. With a long-circulating particle and small bore PET scanner, high resolution images of vascular structure are obtained, as in [2]. This insertion-based labeling method avoids the inclusion of a chelator, which is desirable since the properties of the chelator can influence the biodistribution of the particle or limit the ability to label particles with a dense coating of a charged targeting moiety[1]. A disadvantage of this strategy is that the yield of radioactive particles may be lower, given the multi-step process of labeling the lipid and incorporating the lipid within the particle—each step must be optimized. Further, the multi-step process may limit the labeling of pre-loaded particles containing a drug payload.

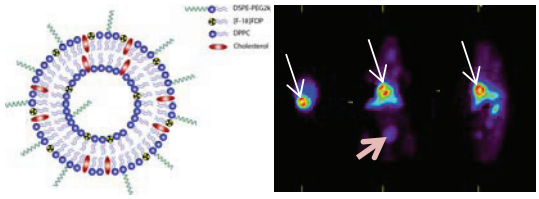

Fig. 1. F-18 labeled particle and resulting imaging. (**left**) Particle labeled with F-18 probe on the lipid head group. (**right**). Three views of PET images of a peptide-coated particle (using CRPPR peptide) that has bound to the cardiac endothelium in a normal mouse, heart indicated with white arrows, bladder with pink arrows. F-18 has been covalently bound to lipid molecules that form the vehicle by self-assembly with other lipids.

A second strategy involves the conjugation of a chelator to the shell of the particle (Fig. 2)[3]. Advantages of this approach can include a high labeling efficiency, long circulation of the labeled particle and ease of labeling pre-loaded particles.

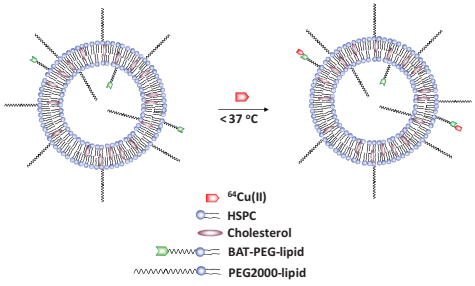

Fig. 2. The radioisotope (Cu-64 here) can be chelated onto the lipid head group. The vehicle can include a drug or optical model drug and multiple lipid components.

Vehicles containing an optical label within the particle core together with a PET label on the shell have been evaluated in tumor models, resulting in the development of a pharmacokinetic model for the uptake by the reticuloendothelial system and tumor and the stability of the

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particle (Fig. 3). We will compare and contrast results obtained with these labeling strategies. Ultrasound, microwave or other activation methods can be used to release the optical probe from the core.

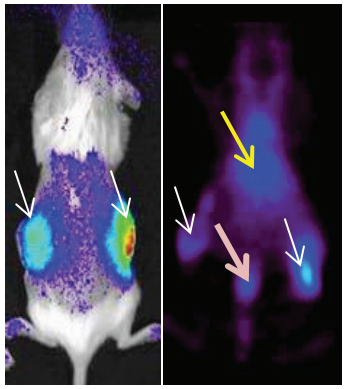

Fig. 3. Optical (**left**) and positron emission tomography images (**right**) of the circulating particle can then be visualized. Tumors are indicated by white arrows, liver by yellow and bladder by pink.

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