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Hybrid nanoparticles improve targeting to inflammatory macrophages through phagocytic signals

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ABSTRACT

Macrophages are innate immune cells with great phenotypic plasticity, which allows them to regulate an array of physiological processes such as host defense, tissue repair, and lipid/lipoprotein metabolism. In this proof-of-principle study, we report that macrophages of the M1 inflammatory phenotype can be selectively targeted by model hybrid lipid–latex (LiLa) nanoparticles bearing phagocytic signals. We demonstrate a simple and robust route to fabricate nanoparticles and then show their efficacy through imaging and drug delivery in inflammatory disease models of atherosclerosis and obesity. Self-assembled LiLa nanoparticles can be modified with a variety of hydrophobic entities such as drug cargos, signaling lipids, and imaging reporters resulting in sub-100 nm nanoparticles with low polydispersities. The optimized theranostic LiLa formulation with gadolinium, fluorescein and "eat-me" phagocytic signals (Gd-FITC-LiLa) a) demonstrates high relaxivity that improves magnetic resonance imaging (MRI) sensitivity, b) encapsulates hydrophobic drugs at up to 60% by weight, and c) selectively targets inflammatory M1 macrophages concomitant with controlled release of the payload of anti-inflammatory drug. The mechanism and kinetics of the payload discharge appeared to be phospholipase A2 activity-dependent, as determined by means of intracellular Förster resonance energy transfer (FRET). In vivo, LiLa targets M1 macrophages in a mouse model of atherosclerosis, allowing noninvasive imaging of atherosclerotic plaque by MRI. In the context of obesity, LiLa particles were selectively deposited to M1 macrophages within inflamed adipose tissue, as demonstrated by single-photon intravital imaging in mice. Collectively, our results suggest that phagocytic signals can preferentially target inflammatory macrophages in experimental models of atherosclerosis and obesity, thus opening the possibility of future clinical applications that diagnose/treat these conditions. Tunable LiLa nanoparticles reported here can serve as a model theranostic platform with application in various types of imaging of the diseases such as cardiovascular disorders, obesity, and cancer where macrophages play a pathogenic role.

1. Introduction

Macrophages play a key role in initiating the inflammatory and immune responses that often becomes uncontrolled in diseases such as atherosclerosis, myocardial infarction, obesity, and cancer[1,2]. It has been shown that the number of inflammatory macrophages in atherosclerotic plaque correlates with plaque rupture[3]. In obesity, macrophages infiltrate the adipose tissue during weight gain and contribute to local and systemic inflammation eventually causing insulin resistance (IR) and type 2 diabetes mellitus. Medical imaging and therapeutic strategies that target tissue macrophages are intensively sought after due to their significant relevance in the etiology of cardiovascular and metabolic disease. Successful human translation of strategies honed in mouse models may allow for non-invasive detection of macrophage burden.

Nanoparticle-based probe delivery systems offer selective and effective detection of targeted cells. Nanoparticles labeled with imaging probes are often used for selective macrophage recognition. It is well established that circulating monocytes, splenic macrophages and disease-associated macrophages (for example in atherosclerotic plaque and tumor) rapidly engulf the nanoparticles by virtue of their intrinsic phagocytic ability[4]. It is clear that macrophage-targeted agents are well known, however, our ability to selectively image detrimental ("bad") macrophages is very limited. New nanoparticle formulations are needed to overcome the hurdle of cell specificity manifested in

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the affinity of nanoparticles to multiple macrophage sub-types. For example, many currently available nanoparticles target both anti-inflammatory and pro-inflammatory macrophages and tend to be engulfed by other phagocytic cells such as dendritic cells and neutrophils. Time consuming screening studies are sometimes needed to obtain nanoparticle that target to specific cell population, or to devise the optimal physicochemical characteristics of the cell-targeted carrier such as size, charge, and shape[5]. Macrophage-targeted theranostics currently focus on macrophage reduction strategies such as selective killing of plaque macrophages by photodynamic therapy (PDT) and photothermal therapy (PTT) using photosensitizers such as dextran coated gold nanorods or single walled carbon nanotubes[6]. Although somewhat effective, such therapies have limited light penetration and can cause significant collateral tissue damage. In contrast, macrophage selective interventions that deliver small-molecule therapeutics, oligonucleotides or biologics can avoid potential tissue damage, diminish unwanted side effects, and provide opportunity to manipulate the cells' immune response[7].

Our laboratory, among others, has been working on the detection of inflammatory cell populations in experimental disease models such as atherosclerosis and type 2 diabetes[8–10]. We set out to develop a "universal" nanoparticle probe capable of incorporating a variety of imaging probes and therapeutic drugs with targeted delivery to M1 macrophages. Towards this goal, we turned our attention to commercially available polystyrene latex as model nanoparticles and fine research tool. Polystyrene nanoparticles have been used as model nanoparticles to study mechanism of nanoparticle drug delivery carrier uptake and processing in macrophages[11–14]. Latex nanoparticles are inexpensive, stable at high salt and protein content, and can be easily modified via passive adsorption or covalently bound to biomolecules[15,16]. Importantly, bare latex nanoparticles have been shown to selectively label inflammatory monocytes and macrophages in pathologies such as atherosclerosis, diabetes, and airway infection though the efficiency was relatively low[17,18].

In this study, we report lipid–latex (LiLa) hybrid nanoparticles targeting inflammatory macrophages. As shown in Fig. 1, the latex core served as a model hydrophobic polymeric template and the lipids provided targeting functionality and colloidal stability. Targeting to inflammatory macrophages was achieved by coating LiLa with phosphatidylserine (PtdSer) and oxidized cholesterol ester derivative cholesterol-9-carboxynonanoate (9-CCN). These lipids, sometimes called "eat-me" signals, are efficiently phagocytized by macrophages[8,19]. We previously used PtdSer and 9-CCN in different nanoparticles to target and image macrophages in atherosclerosis, however, to our knowledge there are no prior reports of a theranostic nanocarrier using "eat-me" signals that can accommodate hydrophobic dye/drug molecules in any combination and size (latex nanoparticles and spheres are commercially available in sizes from 20 nm to 10 μm). This makes LiLa particles truly versatile and suitable for high-throughput nanoparticle screening. The selectivity of LiLa to image pro-inflammatory macrophages was tested in two disease models: (i) an experimental atherosclerosis model, where macrophages were imaged non-invasively through magnetic resonance imaging (MRI)[4,7]; and (ii) a model of diet induced obesity, where pro-inflammatory macrophages were identified using intravital fluorescence imaging of adipose pad. In addition, we tested whether LiLa nanoparticles can serve as efficient theranostics for inflammation. These prototypical LiLa nanoparticles are schematically depicted in Fig. 1.

2. Results

2.1. Synthesis and characterization of LiLa nanoparticles

LiLa nanoparticles were synthesized through a one-step self-assembly after hydration of lipid film with 4% aqueous polystyrene latex nanoparticles (40 nm in diameter) followed by fast bath sonication. All lipid films contained 2–5 mol% of PtdSer and 9-CCN, 5 mol% of phosphatidylethanolamine-PEG2000 and varying concentrations of contrast agents, drug and dyes (see below and Supporting Information). We discovered that the lipid film may include up to 7 mol% of hydrophobic entities in any combination, thus allowing for controlled synthesis of particles with desired functionality (Fig. 1). With the aim of creating theranostic MRI-visible nanoparticles, that can be tracked via fluorescence and effectively shorten longitudinal (T1) relaxation time, we prepared nanoparticles bearing gadolinium DTPA-bis(stearylamide) [Gd-DTPA-SA] using fluorescent (FITC) latex core.

The assembly of lipid layer on latex nanoparticles was tested across a number of formulations obtained by hydrating 0.2–16 mg of lipid film with 4% of latex nanoparticles for optimal size and stability of LiLa nanoparticles. As shown in Fig. 2a, dynamic light scattering measurements indicated the formation of larger, heterogeneous particles with lipid content >1.5 mg, possibly due to the formation of mixed micelles and large vesicles encapsulating multiple latex cores. A molar ratio of 1.5 mg of lipids per 1 mg of latex provided the best compromise between size (65 ± 10 nm) and polydispersity (PDI) [0.11 ± 0.05] for Gd-FITC-LiLa particle formulation (lead formulation). Cryo-transmission electron microscopy (cryo-TEM) confirmed that Gd-LiLa particles consisted of a latex core decorated with a lipid-PEG layer, as seen by the outer corona (white arrowheads on Fig. 2b). The electron-dense dark spots (yellow arrows on Fig. 2b) seen on the surface of the latex core most likely indicate the Gd-lipids. The magnetic properties of Gd-FITC-LiLa longitudinal (T1) relaxation times of protons in water solutions of Gd-LiLa were measured at a clinically-relevant magnetic field strength of 1.5 T (Fig. 2c). The r1 relaxivity for Gd-FITC-LiLa (8.3 mM^{-1} s^{-1}) was 2-fold higher than clinically approved Magnevist Gd-DTPA (4.0 mM^{-1} s^{-1})[20]. The Gd-LiLa formulation was highly

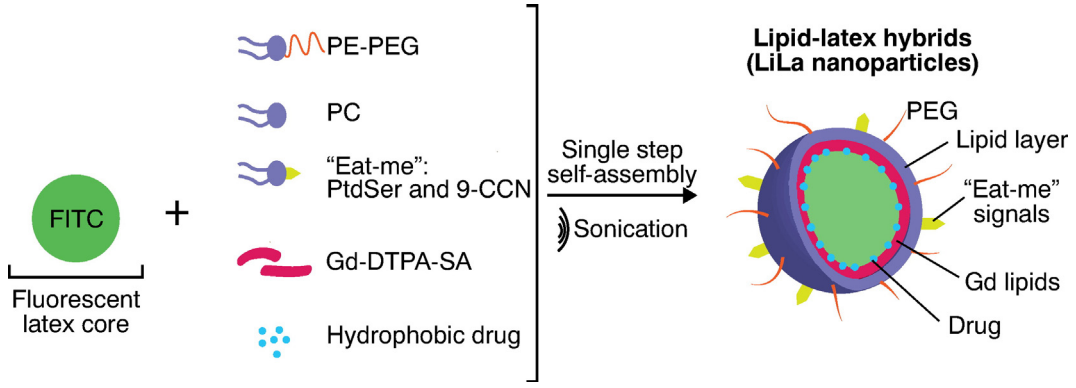

Fig. 1. Schematic illustration of components in LiLa nanoparticles. The single step self-assembly with a latex core template, lipids, dyes/drugs yields theranostic lipid–latex hybrid nanoparticles (LiLa). The targeting to macrophages is achieved by phagocytic signals phosphatidylserine (PtdSer) and cholesterol-9-carboxynonanoate (9-CCN). LiLa formulations bearing Gd or Alexa-647 imaging probes are expected to serve as contrast agents for MRI and fluorescence imaging.