PEGYLATED NANO-PEACHES: A NOVEL MULTIMODALITY PLATFORM FOR IMAGING OF ATHEROSCLEROSIS

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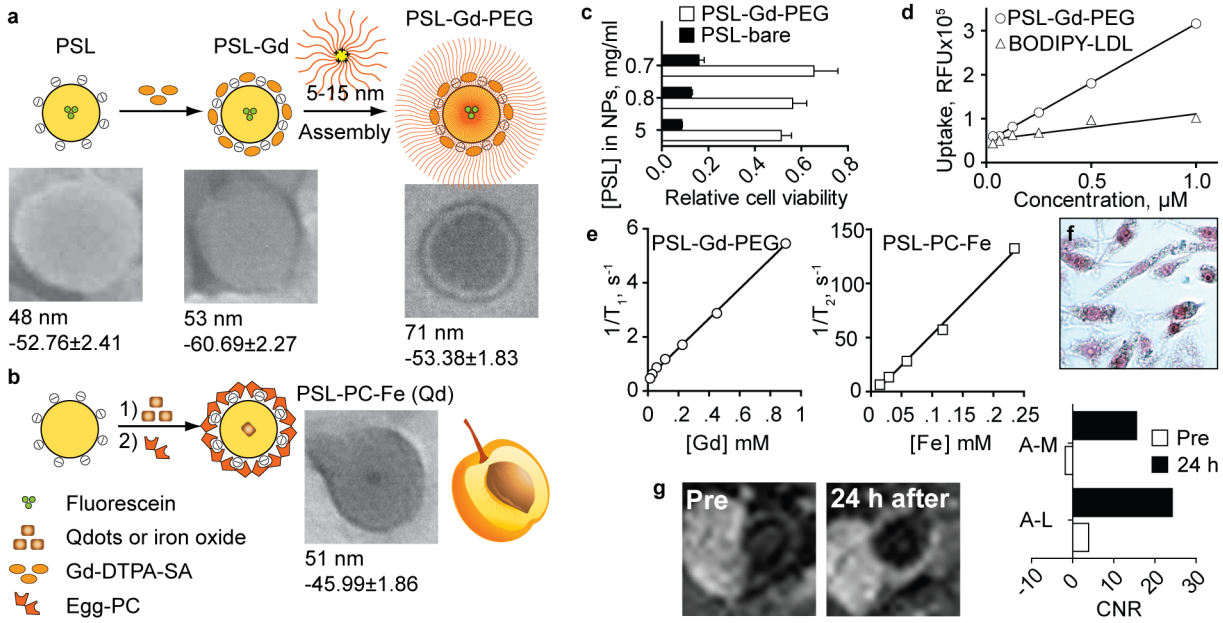
Introduction

There is a compelling need for a new class of engineered imaging nanoparticles (NPs) that possess easily controllable physicochemical parameters, allowing modifications for targeted delivery depending on the tissue and disease state. These NPs must simultaneously meet the criteria of allowing multimodality flexibility, targeting efficiency, higher plasma retention times, safety, and cost. Herein, we describe the synthesis and characterization of a novel family of multimodality polystyrene NPs that can be assembled in a "layer by layer" manner resulting in contrast agents with diverse functionality, including T_1/T_2 weighted MR and near-infrared imaging capabilities. We additionally tested some of these formulations in simple cell culture models of uptake (cultured macrophages) and *in-vivo,* using a mouse model of atherosclerosis.

Methods and Results

Carboxylated polystyrene latex (PSL) NPs were used as a core for all synthesized contrast agents. Fluorescein loaded or bare PSLs with 48 nm size were sequentially treated with gadolinium lipid (Gd-DTPA-SA), egg-phosphatidylcholine (Egg-PC) and/or PEG-micelles resulting in ≈70 nm long-circulating PEG-grafted NPs (**Fig. 1a**). In order to create contrast agents that are photo-bleaching stable, suitable for multimodal near-infrared and MR imaging, PSLs were implemented with quantum dot nanocrystals (QDot) emitting at 670 nm (**Fig. 1b**). High power sonication of PSL with oleic acid -capped CdSe QDots allowed to incorporate the latter into the hydrophobic interior of NPs. Consecutive treatment with Gd-DTPA-SA and Egg-PC resulted in lipid coated particles with ≈50 nm size. Hydrophobically coated superparamagnetic iron oxide crystals were loaded into PSL in the same manner and yielded a multimodality T_2-contrast agent of similar size. Structural morphology of all particles was studied by electron microscopy. The results showed distinctive "peach-like" structures with a highly electron-dense "pit" in case of PSL-PC-Fe(Qd) and low electron density corona ("peel") for PSL-Gd-PEG. Highly negative zeta-potentials (less than -40 mV) suggest stability of all obtained NPs in solution (values are shown on **Fig. 1a, b** under each of TEM micrographs). Nano-peaches are highly biocompatible in comparison with bare PSLs. Minimal cytotoxicity was detected in cultured RAW macrophages using MTT assay (**Fig. 1c**). Uptake of PSL-Gd-PEG by macrophages was linearly related to NPs concentrations showing no saturation and suggesting non-receptor mediated binding (**Fig. 1d**). In addition, the similar size of PSL-Gd-PEG to low-density lipoprotein (LDL) allowed us to speculate that uptake of these NPs can mimic fluid-phase pinocytosis of lipoproteins in atherosclerotic lesions and preferentially accumulate in the plaque. Magnetic resonance relaxation properties of particles were studied using a 1.5 T Siemens clinical scanner (**Fig. 1e**). As a result, r_1 (PSL-Gd-PEG) and r_2 (PSL-PC-Fe) were found to be 5.5±0.1 and 573±23 $s^{-1} \cdot mmol/L^{-1}$, respectively. Cultured macrophages effectively take up PSL-PC-Fe (Prussian blue stain on **Fig. 1f**) when incubated for 5 h. This suggests that nano-peaches are rapidly taken up by macorphages which is advantageous for certain types of T_2-imaging. *In-vivo* imaging was performed in $ApoE^{-/-}$ mice fed a high-fat/high-cholesterol diet on a 11.7 T Bruker BioSpin scanner. The results show enhancement of plaque with PSL-Gd-PEG that is stable ≥ 24 h post-injection (**Fig. 1g**). Contrast-to-noise ratios (CNR) of aorta to muscle (**A-M**) and aorta to lumen (**A-L**) were found to be more than 20 times higher when compared to baseline indicating aorta-specific uptake.

Figure 1



Conclusion

Novel multifunctional polystyrene-supported nanoparticles were fabricated, characterized and tested in *in-vitro* and *in-vivo*. NPs are powerful tools for non-invasive imaging of atherosclerosis due to rapid macrophage uptake, ability to enhance atherosclerotic lesions (long half-life), and possible fluid-phase pinocytosis in atherosclerotic lesions *in vivo*.