Multimodal in vivo evaluation of a surface-switching nanoparticle platform

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Target audience: Researchers interested in novel, non-invasive imaging techniques to assess the efficacy of new drug delivery nanoparticle platforms/probes to image/treat cancers.

Purpose: Surface functionalization of nanoparticles (NPs) with targeting ligands such as antibodies, peptides or nucleic acids has shown significant advantages in preclinical cancer nanotherapy studies[1]. However, those moieties may also cause elevated NP recognition by the mononuclear phagocyte system and off-target binding. To overcome these limitations, we have developed a matrix metalloproteinase-2 (MMP2) cleavable polyethylene glycol (PEG) coating to prevent NP/cell interaction in the bloodstream. Once exposed to MMP2, i.e. when the NPs are accumulated within the tumor microenvironment, the PEG coating will be cleaved. The resulting surface exposure of the targeting moieties (RGD peptide) facilitates NP association with αvβ3 integrin expressing tumor cells (Figure 1A).

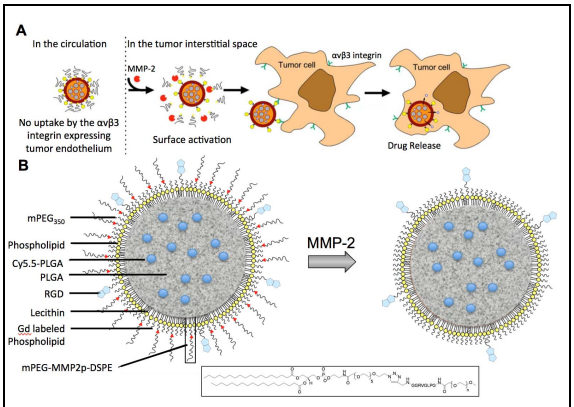

Figure 1. Concept and schematic of the surface-switchable lipid/PLGA nanoparticle platform. Structure of the MMP2-cleavable lipid is depicted in the inset.

Methods: Surface-switchable NPs are composed of a near infrared fluorescent (NIRF) poly(lactic-co-glycolic acid) (PLGA) core and a paramagnetic PEG-lipid coating (Figure 1B). These hybrid NPs were synthesized by dripping a PLGA/acetonitrile mixture into a hot water/ethanol solution containing various combinations of PEG350 phospholipids (mPEG350-DSPE), RGD functionalized PEG-phospholipids (RGD-PEG750-DSPE) and a custom made cleavable PEG-phospholipid[2] in which PEG2000 is conjugated to the phospholipid via an MMP2 cleavable peptide unit (mPEG-MMP2p-DSPE) (Figure 1B, inset). Paramagnetic properties were introduced by the inclusion of 25 mol% Gd-DTPA-DSA at the expense of mPEG350-DSPE. Once purified, nanoparticles were incubated with various cell lines, and cell uptake was measured *in vitro* by fluorescent microscopy, flow cytometry and cell pellet magnetic resonance imaging (MRI). Nanoparticles were then injected in mice bearing orthotopic MDA-MB-231 human breast tumors and biodistribution of the NPs was assessed by MRI and NIRF imaging. MRI data were acquired on a 7 Tesla MRI system before and 24 hours after the administration of the NPs to allow the generation of T_1-and T_2-maps. T_1-weighted images were obtained with a fast spin-echo, Rapid Acquisition with Relaxation Enhancement and short TEs (RAREst) sequence (RARE factor=2), with a TE=5.6 ms and 7 varying TRs (ranging from 325ms to 3500ms) and 1 signal average. T_2-weighted images were obtained using a multi-slice multi-echo sequence with a TR=2000 ms and 16 varying TEs (ranging from 8 ms to 128 ms) and 2 signal average. All images were acquired at the same geometry: 13 slices, thickness 0.5 mm, field of view 3×3 cm, matrix size 256×256.

Results: *In vitro* fluorescence microscopy (Figure 2A) demonstrated that unshielded RGD decorated nanoparticles (unshielded + 5% RGD) presented a stronger interaction with αvβ3 integrin expressing MDA-MB-231 breast cancer cells compared to non decorated (unshielded) or decorated but shielded (Shielded + 5% RGD) nanoparticles. Additionally, internalization of the nanoparticles and subsequent endosomal/lysosomal accumulation was observed. Flow cytometry data (Figure 2B) corroborated the aforementioned and confirmed the specificity of the mPEG-MMP2p-DSPE shielding. The inclusion of Gd-DTPA-DSA in the NP corona allowed cell pellet MRI (Figure 2C) and the generation of T_1-maps (Figure 2D). Similar to studies with liposomal NPs, we observed relaxivity "quenching" due to NP endosomal/lysosomal accumulation[3]. *In vivo* MRI revealed T_1 and T_2 to be lowered from 2148 to 1877 ms and 66 to 60 ms respectively (Figure 2E). *Ex vivo* NIRF imaging (Figures 2F & 2G) disclosed NP biodistribution and accumulation in the rim of the tumors.

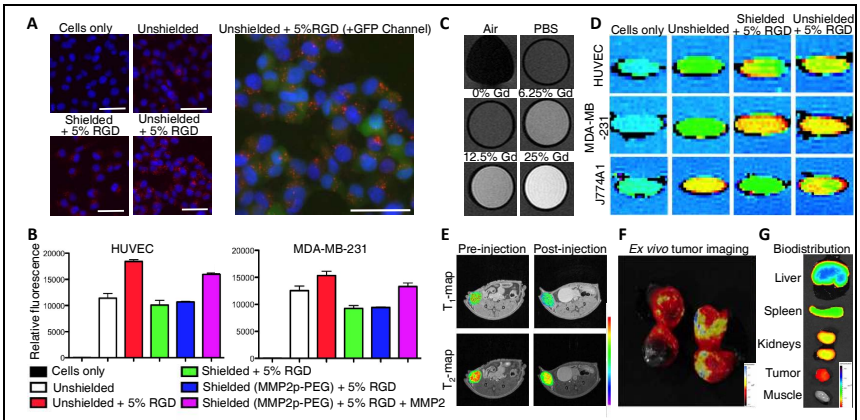

Figure 2. *In vitro* and *in vivo* assessment of the platform

Conclusion: We developed a hybrid nanoparticle platform with a MMP2 cleavable PEG-lipid corona. *In vitro* assays demonstrated that upon incubation with MMP2 the PEG coating is cleaved and targeting ligands become available to bind to αvβ3 expressing cells. The inclusion of gadolinium allowed *in vivo* MR imaging of tumor accumulation. Our new surface-switching coating approach ensures a high NP targeting specificity without compromising favorable NP pharmacokinetics.

References: 1) Fay et al, Immunotherapy 2011; 2) Gianella et al, Chem Commun. 2013; 3) Strijkers et al, Magn Reson Med. 2009.