

Swollen Micelles; a Nanoparticulate Platform for the Delivery of Hydrophobic Agents

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Introduction: Nanoparticles are useful in drug delivery due to their ability to reduce drug deactivation and side effects. Furthermore, nanoparticulate emulsions can be used for the encapsulation and delivery of poorly water-soluble agents and materials. Long-circulating nanoparticles gather in tumors due to the enhanced permeability and retention (EPR) effect. To exploit the EPR effect it is desirable to synthesize emulsions of droplet diameters less than 300 nm (1). In this study we were able to meet this requirement by creating three formulations (oil in water nanoemulsions) with different and well-controlled mean sizes as established by transmission electron microscopy (TEM) and dynamic light scattering (DLS). To allow MRI detection, hydrophobically coated iron oxide particles were included into the oil phase of the droplets. Cy5.5 was coupled to the particle surface for *in vivo* fluorescence imaging as a second means to follow the fate of the particles and to determine biodistribution and pharmacokinetics (Figure 1). In the current study we demonstrate the delivery and accumulation of the nanoparticles to subcutaneous EW7 (Ewing's Sarcoma) tumors in nude mice.

Figure 1

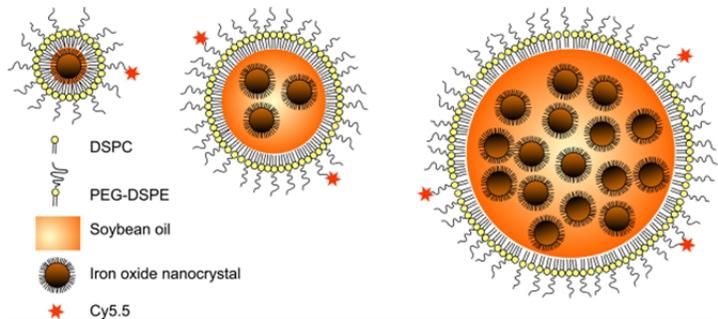
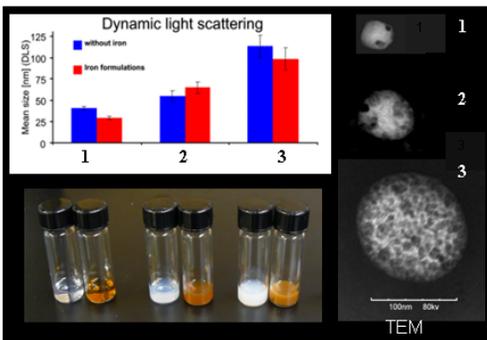


Figure 2



Materials and Methods: Increasing amounts of soybean oil were added to micellar preparations to form droplets with distinct droplet diameters. Hydrophobically coated (oleic acid) iron oxide particles (10 nm) were included into the oil phase to enable their detection by MRI. The lipid mixture consisting of DSPC and PEG-DSPE contained an amount of 0.5 mol% Cy5.5-PEG-DSPE for optical imaging. For *in vivo* experiments, swiss nude mice were inoculated with EW7 cells to form subcutaneous (s.c) tumors. Three weeks thereafter, the mice were injected intravenously (i.v.) with either formulation 1 or 3 (see Figure 2) in a volume containing an equivalent dose to 16.7 mg/kg magnetite. MRI was performed (9.4 T dedicated animal system) before and 24 hours after administration of the nanoemulsions. The tumors were excised afterwards and imaged using a Xenogen IVIS-200. In addition, after fixation of the tumors in paraformaldehyde, Perl's histological iron staining was performed to directly visualize iron oxide deposits in the tumor tissue.

Results and Discussion: The mean sizes of the three formulations were determined by DLS to be 30 nm (formulation 1), 60 nm (2) and 94 nm (3). These findings were confirmed by TEM as shown in Figure 2. The mean droplet diameter was reproducible and stable for at least three weeks without aggregation. Two of the three formulations (mean sizes 30 nm and 94 nm) were applied *in vivo* and caused a signal decrease of the s.c tumors on T2 weighted MRI, 24 hours after i.v. administration (16.7 mg/kg magnetite, TR 2500 ms/TE 53.3 ms, Figure 3A). After excision of the tumors a strong fluorescent signal originating from the Cy5.5 could be recorded, providing additional evidence for the specific accumulation of the particles in the tumor (Figure 3B). Furthermore, the presence of iron oxide was proven by using Perl's staining of histological tumor sections. In cases of a local decrease in MRI signal intensity within the tumor area, we were able to correlate the location of increased particle deposition to the corresponding histological image (Figure 4). The delivery of the particles to the tumors was clearly shown using MRI, fluorescence imaging and histological staining.

Figure 3

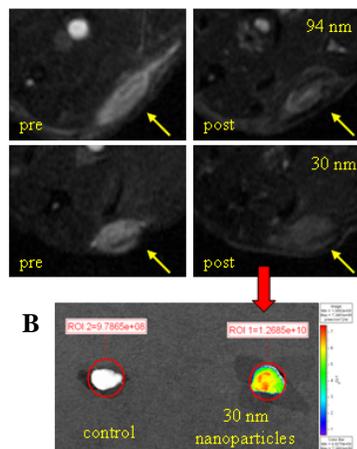
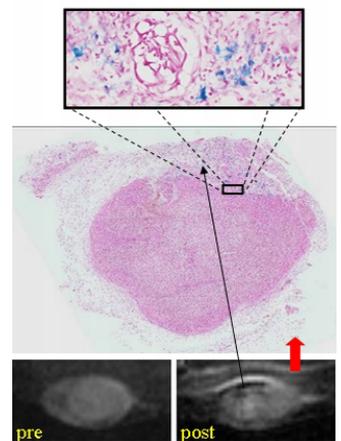


Figure 4



Conclusions: In conclusion, we were able to generate different sized nanoemulsions with a mean droplet size below 100 nm that have the capability to deliver hydrophobic agents. Moreover, by using a multimodality imaging approach it was possible to demonstrate their versatile capabilities for targeted delivery in cancer.

(1) Decuzzi P *et al.* Pharmaceutical Research. 2008.