MR Detection of Tumor Cells Labeled with USPIO using DOPS Liposomes

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Introduction: Liposomes have been widely used to deliver drugs to tissue *in vivo*. They can also be used to label cells with MR contrast agents such as Ultrasmall SuperParamagnetic Iron Oxide (USPIO) nanoparticles. However, labeling of non-phagocytic cells with USPIO for MR detection requires that the liposomes encapsulate and deliver sufficient quantities of the contrast agent. Tumor-specific liposomes could potentially be used to deliver USPIO to the tissue, aiding in earlier detection and better visualization using MRI. Delivery and uptake of targeted drugs can also be estimated using contrast enhanced MR microimaging, by using liposomes as dual carriers for the drug and the contrast agent. In this preliminary study, we have adapted a method for encapsulating Combidex® (Advanced Magnetics, MA, size ~20nm) in liposomes made of dioleylphosphatidylserine or DOPS (Avanti Polar Lipids, Alabaster AL) and demonstrate that these liposomes can be effectively delivered to human neuroblastoma cells *in vitro* for detection using MRI.

Materials & Methods: ***a) Preparation of liposomes*:** Sonication of dextran coated USPIO particles in aqueous solution with DOPS does not yield sufficient encapsulation in the liposomes. In order to increase USPIO content in liposomes, a chemical coupling method as described by Bogdanov et al [1] was used with minor modifications. Briefly, the dextran coating on the USPIO particles is oxidized to generate aldehyde groups. Aldehydes form a covalent Schiff bond at high pH with amines of DOPS. Liposomes obtained have a mean size of 150nm as confirmed by $N4^+$ Particle Sizer (Beckman Coulter, CA) analysis. The liposome solution is dialyzed against a low pH solution to detach USPIO bound to the external layer of the liposomes. Unencapsulated USPIO are removed by affinity chromatography using a Con-A Sepharose 4B column (Amersham Biosciences Corp., NJ). The USPIO-DOPS liposome structure was confirmed by conventional electron microscopy. A standard R2 relaxivity curve generated using known quantities of free USPIO and DOPS liposomes mixture was used to estimate the iron concentration in the DOPS liposomes (Fig. 1). A maximum content of 32μg Fe/ml was achieved using 1 mM DOPS concentration.

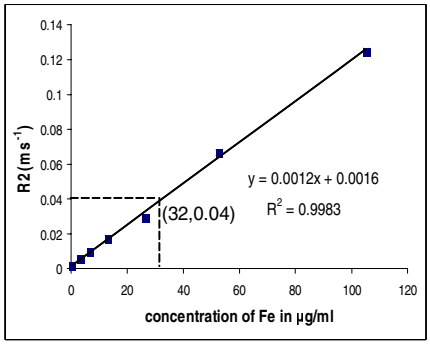

Figure 1: Standard R2 plot of USPIO and liposomes in PBS

b) Uptake in neuroblastoma cells: Four samples of neuroblastoma cells were prepared with approximately 10,000 cells per group. The first and second samples were incubated with a 100 μM and 300 μM USPIO-DOPS liposome preparation in growth medium respectively. The third sample contained liposome-USPIO solution prepared by sonication without chemical coupling and the fourth sample contained cells with no USPIO or liposomes. After incubation for 36 hours, the cells were washed 4 times, trypsinized and fixed in a mixture of 0.5% agarose solution and growth medium (1:1) in 4ml glass vials.

c) Imaging: High resolution MR imaging of the cells was performed using a 7T Bruker Biospec scanner using gradient echo methods optimized for T2* weighting. A 3D FLASH imaging sequence with TR/TE/θ of 200ms/35ms/10° and a 320×320×64 matrix was used for a 3.2cm×3.2cm×0.64cm FOV resulting in an isotropic 100μm resolution.

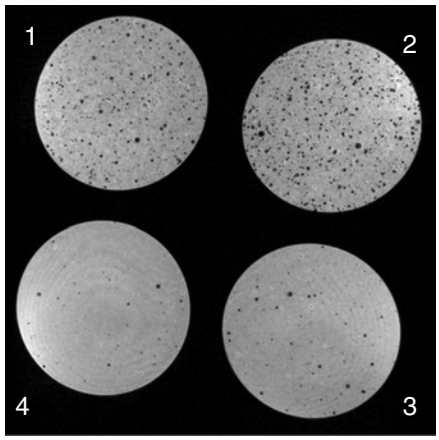

Figure 2: MR image of vials containing cells fixed in agarose

Results & Discussion: The MR images (Fig.2) indicate uptake of USPIO particles by cells in samples 1 and 2, with sample 2 showing an increased uptake corresponding to the higher concentration of USPIO-DOPS liposomes. A much lower number of cells was detected in sample 3, containing cells incubated with liposome-USPIO solution prepared by sonication. An estimate of the number of cells detected in each vial was obtained using a post-processing algorithm written in IDL®. Number of cells detected in vial 2 was approximately 1.4 times compared to vial 1. The average contrast-to-noise ratio (CNR) between the gel and hypo-intensity regions representing cells was 20.15 (std. dev 11).

Conclusion: At this stage, we have successfully demonstrated that USPIO can be coupled with DOPS in sufficient quantities to form liposomes that are internalized by neuroblastoma cells *in vitro*. The cells can be detected using high resolution gradient echo imaging methods. DOPS liposomes are also being used as carriers for a drug currently being investigated for its effect in inducing apoptosis in human neuroblastoma cells. In future studies, this drug will also be incorporated into the liposome and targeted to tumor cells. Ultimately, we aim to test this approach of using DOPS liposomes for the combined delivery of the drug and USPIO to tumors *in vivo* and use high resolution MR imaging to estimate the delivery efficacy and effect of the drug.

References: 1. Bogdanov, A.A., Jr., et al., Trapping of dextran-coated colloids in liposomes by transient binding to aminophospholipid: preparation of ferrosomes. Biochim Biophys Acta, 1994. 1193(1): p. 212-8.