Hexamethyldisiloxane-based dual-modality dual-functional nanoprobes for cellular and molecular imaging

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Introduction: There has been a growing interest in developing MR contrast agents for cellular and molecular imaging especially in the last two decades. Gadolinium chelates, superparamagnetic iron oxide (SPIO) particles and perfluorocarbon (PFC) nanoemulsions have all been used as imaging agents to non-invasively monitor cellular processes or the behavior of macromolecules *in vivo* [1]. Previously, PFC nanoemulsions were used in ^{19}F MR oximetry [2] while it was recently demonstrated that nanoemulsions encapsulating hexamethyldisiloxane (HMDSO) reporter molecules can be used to quantitatively measure oxygen tension (pO_2) in tissues using ^{1}H MRI [3]. In this study, HMDSO based dual modality (MRI/Fluorescence), dual-functional (oximetry/detection) nanoemulsions were prepared for cellular and molecular imaging.

Methods: HMDSO-based nanoemulsions (NEs) were prepared by a single emulsion method in which a solution containing HMDSO, de-ionized water and surfactant mixture (Polyethylene glycol hydroxystearate (HS-15) and soybean lecithin) in ratios of 40:55:5 % v/v respectively were homogenized using an ultrasonic homogenizer. The sample was then extruded through a Lipofast extruder (polycarbonate membrane filter diameter - 50 nm), to obtain uniform particle size distribution. Characterization of these NEs was done using DLS, TEM and. Shelf life and particle size variations were assessed in DI water and human plasma. Longitudinal relaxation rate (R_1) vs pO_2 calibration curve of the NE was determined using a spin-echo based pulse sequence by spectroscopy in a Varian 4.7 T scanner at 37 ^{o}C. *In vitro* cytotoxicity of NEs and surfactant combination on 3T3 fibroblasts were studied using MTS assay. Intracellular localization and time-dependent uptake of Nile red-doped NEs by MCF-7 cells transfected with GFP (Green Fluorescent Protein) was determined using confocal microscopy. Additionally, the dual-functionality of the NEs was tested by fluorescence and PISTOL[4] imaging of a sample containing separate layers of basement membrane matrigel containing MCF7 and Nile red-doped NEs.

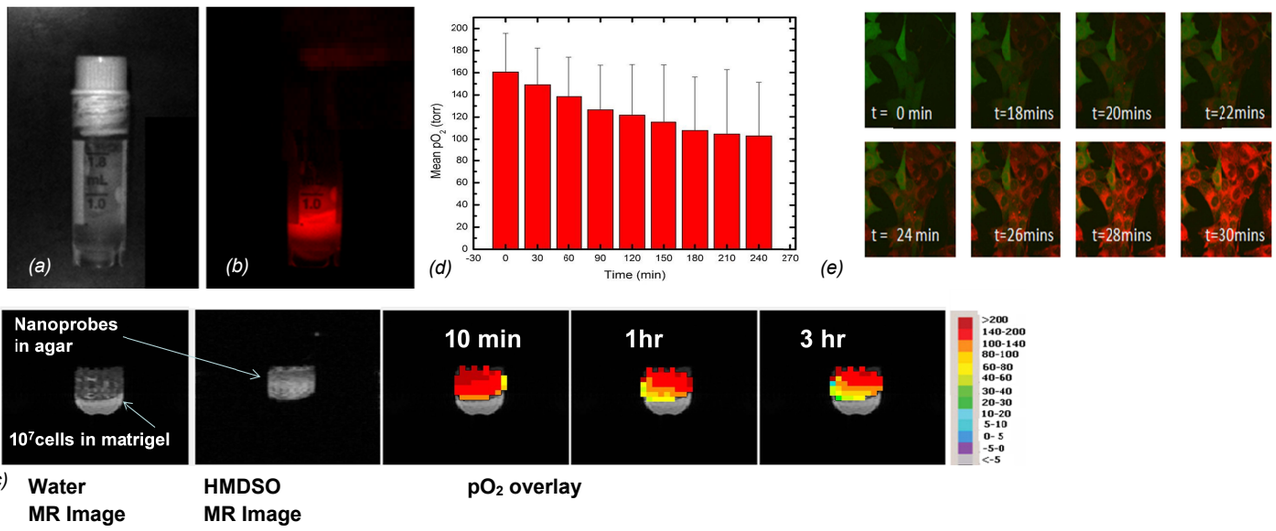

***Figure 1**: **(a)** White light and **(b)** fluorescence images of a sample containing Nile-red doped dual functional nanoprobes and MCF7 cells in different layers of matrigel **(c)** Time-course pO_2 maps showing oxygen consumption in the same sample **(d)** Mean pO_2 timecourse showing oxygen consumption. **(e)** Maximum intensity projection of confocal microscopy composite images showing time-dependent uptake of the nanoprobes by MCF7-GFP cells over a period of 30 mins.*

Results: The NEs were measured to have an average % mass weighted radius of about 70.62 nm. The nanoprobes maintained consistent particle size in DI water and 50% human plasma for 24 hours and showed cytocompatibility up to 0.4%(v/v) concentration (IC_{50}). Dual modality imaging was successfully performed on matrigel phantoms to show fluorescence (Figure 1 (a), (b)) and measure oxygen consumption by MCF7 cells (Figure 1 (c), (d)), using these nanoprobes. A pO_2 drop of 60 torr was observed over 4 hours with an increasing pO_2 gradient across the labeled matrigel. Additionally, Nile red fluorescent dye-doped nanoemulsions were successfully visualized in the cytosol of MCF7-GFP breast cancer cells and uptake was observable within 18 minutes of incubation and progressed over the observation period (Figure 1 (e)).

Conclusion: Dual-modality dual-functional HMDSO-based nanoprobes were successfully synthesized and characterized for ^{1}H MRI based imaging and oximetry and fluorescence imaging. As proof of principle, these were successfully used for studying oxygen consumption in MCF-7 cells. These results indicate that the HMDSO-based nanoemulsions can be effectively used for oximetry and fluorescence detection simultaneously.

References: 1) Bulte JW *et al*., NMR Biomed 17(7):p 484-499 (2004). 2) McIntyre *et al*. Curr. Sci. 1999; 76: 753–762. 3) Gulaka PK *et al*., NMR in Biomedicine, epub ahead of print DOI:10.1002/nbm.1678. 4) Kodibagkar VD *et al*. NMR Biomed. 2008; 21(8): 899–907.

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