Cell labeling with Gd-based MRI agents: recent achievements using sonoporation in the presence of liposomes.

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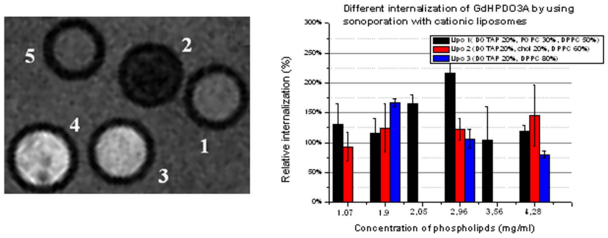
Purpose:The development of innovative strategies for labeling cells with positive Gd.-based MRI agents is still an important topic. It has been demonstrated that the ability of a labeling agent to generate a contrast when internalized into a cell is significantly higher if it is localized in the cytosol. An example is represented by electroporation, but this technique is typically associated with poor cell viability. Also Ultrasound stimulation can permeabilize cell membranes, thus allowing the passage of molecules from the external solution to the cytosol. The internalization efficiency of this procedure, named sonoporation, can be improved by using microbubbles that, upon US stimulation, can create cavitation events able to facilitate the formation of transient or permanent pores into cell membranes. The purpose of this work was to design a proper experimental set up for optimizing the cell labeling procedure via sonoporation. Furthermore, and even more important, the study was aimed at comparing the effect caused by the presence of microbubbles or liposomes on labeling efficiency and cell viability.

Methods: Murine breast cancer cells (TSA) or human promonocytic cells (U937) were placed into a PBS solution containing the clinically approved MRI agent Gadoteridol. Cells were sonoporated alone or in the presence of microbubbles or differently formulated liposomes. Sonoporation was carried by using a 1 MHz non focused US transducer with acoustic intensity of 1.3±0.2 W/cm^2, and varying the different variables involved in the protocol (e.g. duty cycle, time of insonation, voltage) to find the best set up. The amount of internalized MRI probe was evaluated relaxometrically, thereby allowing the determination of the amount of internalized probe per cell. Cell viability was evaluated by both trypan blue exclusion and Bradford assays. Intracellular localization of the labeling agent was evaluated by confocal fluorescence microscopy, and by T_1 and T_2 measurements carried out at 7 T on pelleted labeled cells.

Results: The insonation parameters by using the 1 MHz transducer were optimized to increase the loading efficiency, and a new sterile set up was realized in order to seed in plate cells after the treatment and verify their viability and functionality. Cells were able to attach to the plate and to duplicate without any morphological change after US stimulation. In addition, treated U937 cells were able to differentiate into macrophages after incubation with TPA and to duplicate. The viability of cells was well preserved after the treatment (better than electroporation) and the amount of Gadoteridol internalized showed a good reproducibility and efficiency. Interestingly, the presence of liposomes during the sonoporation increased the internalization efficiency more than what observed in the presence of microbubbles. The study was replicated in the presence of liposomal with different characteristics (membrane composition, charge, content, size) and excellent results were obtained using cationic liposomes.

Discussion: The MRI visualization of labeled cells with Gd-agents requires the internalization of 10^8-10^{10} Gd^{3+} ions per cell. As the endosomal localization of the positive agent is detrimental for the contrast generation, electroporation is preferable, but it suffers for an intrinsic high toxicity both *in vitro* and *in vivo* experiments. The herein reported work demonstrates that sonoporation has the potential to be a very efficient method for labeling cells with a positive MRI agent. Furthermore, it has been observed that liposomes can further increase the loading efficiency, thereby offering a valuable tool to reduce the detection threshold of Gd-labeled cells. Likely, the liposomes act as positive mediator of the acoustic radiation.

Conclusions: In vitro sonoporation carried out in presence of lipid-based nanoparticles improves internalization efficiency, MRI detection of Gd-labeled cells, and cell viability.



(Left)T_{1w} image of a phantom composed by: 1)Unlabelled TSA cells, 2) TSA cells labelled by pinocytosis, 3) TSA cells labelled by electroporation, 4)TSA cells labelled by sonoporation, 5) water.

(Right) Relative internalization of GdHPDO3A by applying sonoporation in the presence of different cationic liposomes