Hypotonic swelling: a soft route for ex vivo cellular labeling with Paramagnetic complexes

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Purpose: Different strategies to label cells with paramagnetic MRI contrast agents have been exploited in the last years like pinocytosis, electroporation, receptor/transporter mediated endocytosis, external labeling (1). Herein we show that hypotonic swelling of cells is a new efficient route to label cells with paramagnetic Gd-complexes. In fact, the cytoplasmatic uptake shifts to much higher loadings the "quenching" effect on the relaxivity observed when the paramagnetic Gd-complexes are compartmentalized into endosomal vesicles.

Methods: J774A.1 murine macrophages and k562 human myelogeneous leukemia cells have been incubated with the commercial contrast agents Gd-HPDO3A. The herein reported method exploits de-structuring phenomena occurring at the cellular membrane in the presence of a large osmotic gradient between the intracellular and the extracellular compartments. In particular when the osmotic pressure of the extracellular compartment is lower than the intracellular one, the net result is the swelling of the cells as consequence of the large uptake of water molecules in the inner compartment. During swelling, the membrane is less efficient in acting as barrier to the molecular transits. Thus, small hydrophilic molecules, such as Gd-HPDO3A, can freely cross the "temporarily -injured" membranes. Cells have been placed into a hypotonic solution (varying from 130 to 200 mOsm/l) containing the contrast agent to be loaded. After the incubation the physiological functionality of cells has been restored by changing the osmolarity of the external solution to an isotonic condition (280 mOsm/l). The restoring of cell shape is easily assessed by microscopy. The amount of internalized Gd-HPDO3A by osmotic swelling has been compared with the corresponding amounts taken up by using pinocytosis and electroporation.

Results: By applying this technique high amount of Gd-HPDO3A (in the order of 10^9-10^{10} Gd^{3+} per cell, higher than the threshold for MRI visualization) can be easily loaded into cells without any appreciable change in cell shape and function. The internalization efficiency depends on the incubation time, the concentration of the probe in the hypotonic solution and the temperature.The cytoplasmatic localization of the internalized molecules has been assessed by adding the fluorescent Carboxyfluorescein dye to the incubation medium used in the hypotonic swelling experiment. Table 1 summarizes the results obtained by comparing the three internalization routes when the concentration of Gd-HPDO3A in the incubation medium is 50mM.

Discussion: The hypotonic swelling technique appears an easy method to internalize paramagnetic Gd-complexes inside cells without causing changes in viability, morphology and functionality of cells. By this procedure the labeling molecules are confined in the cytoplasmatic compartment. One may expect that this compartmentalization results in a higher stability of the added Gd-agent as it avoids the most "aggressive" environment that it would have been met along the endosomes/lysosome pathway. Finally, also the "relaxivity-quencing" issue is definitely less important as it has been shown that it occurs when the amount of internalized Gd-complexes is >5 fold higher than the amount causing it when the internalization route is represented by pinocytosis.

Conclusion: An efficient soft route for MRI cellular labeling has been herein reported. This method has been tested for the internalization of Gd-HPDO3A and carboxyfluorescein but it is expected that this methodology will be analogously efficient to load other molecular systems.

	K562			J774A.1		
	Pinocytosis	Electroporation	Hypotonic swelling	Pinocytosis	Electroporation	Hypotonic swelling
N° Gd/cell	$5x10^9$	$2x10^{10}$	$1x10^{10}$	$5x10^{10}$	$2x10^{10}$	$9x10^9$
Cell viability	100%	65%	92%	100%	68%	95%
Proliferation rate	preserved	Reduction of proliferation	preserved	preserved	Reduction of proliferation	preserved

1) Gianolio E, Stefania R, Di Gregorio E, Aime S. Eur. J. Inorg. Chem. 2012, 1934–1944