A Microfluidic Micro-MRI Set-up to Assess the Specificity of Targeted Contrast Agents on a Living Cell Monolayer

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INTRODUCTION

The development of targeted contrast agents (CA) is of great interest to increase the specificity of MRI and allow early diagnosis and therapy follow-up of diseases. Proof of concept studies are required to establish the detectability of the CA and analyze the role of molecules and cells in the contrast mechanisms. This study investigates the ability to detect a monolayer of KB cells, a line from a human carcinoma of the nasopharynx which overexpress the folate receptor [1], by micro-MRI in a microfluidic channel allowing to carefully control the state of the cells and the CA administration. The feasibility to monitor the cell uptake of P866 (Guerbet Research, France), a high relaxivity CA which targets folate receptors [2], is evaluated.

MATERIAL & METHODS

Cells: KB cells were grown in a RPMI 1640 medium containing folate or not, with 1 % Glutamine and 10 % Newborn Calf Serum Heat-Inactivated, at 37°C and 5 % CO_2. For the uptake experiments, 2 millions cells were incubated for 17 h at a 2.7 mM Gd concentration with culture medium containing either P866 or Gd-DOTA (Dotarem®, Guerbet, France) as a control. Then, the cells were washed twice with PBS, trypsinized, and transferred in CA free medium; finally, 300.000 of them were seeded into the microfluidic channel (dimensions $0.4 \times 5 \times 50$ mm^3) of a µ-Slide I 0.4 Luer (Ibidi, Germany). After 4 h of incubation at 37°C and 5 % CO_2, the cells became adherent and formed a 15 µm thick monolayer onto the bottom of the channel. The Gd contents per cell were measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

MRI: Experiments were carried out on a 2.35 T scanner (Bruker, Germany), with a flowing air regulation to maintain the gradient bore temperature at 37°C. A 6 mm diameter microfabricated Multiturn Transmission Line Resonator [3] was used as a transmit/receive surface coil; it was placed under the µ-Slide, 180 µm far from the cell layer. A RF-spoiled 3D FLASH sequence was applied with FOV of $0.6 \times 1.9 \times 0.8$ cm^3, in-plane resolution of 160×160 µm^2 and a resolution of 12 µm in the read direction, perpendicular to the cell layer. Acquisition time was 15 min with TR/TE of 74.7/3.7 ms and a 12.5 kHz bandwidth. The transmit level was empirically adjusted to optimize the contrast to noise ratio between the folate deprived cell layer labeled with P866 and the medium; it was kept constant for all experiments. Two orthogonal 3D-FLASH scans were previously acquired in order to adjust the µ-Slide orientation. At the end of the acquisition protocol with seeded cells, 5 mL of 38 g.L^{-1} NaClO were injected into the µ-Slide channel followed by CA free medium to lyse and wash the cells away. This allowed to get reference images with the free medium alone.

Data processing: For each acquisition, the most appropriate sub-voxel shift was applied along the read direction to minimize the truncation artifact occurring at the bottom edge of the channel. A 1D signal profile orthogonal to the cell layer was then extracted with a 2D projection of the image matrix along planes parallel to the bottom of the channel. The spatial extent of the projection was limited to a region of interest where the radial sensitivity pattern of the surface coil was not varying by more than 10 %.

RESULTS & DISCUSSION

The profiles (Fig. 1) display significant signal enhancements at the level of the cell monolayer compared to the culture medium alone (the standard deviation of the signals measured with the culture medium alone was 0.16). A +5 % increase is already visible with unlabelled cells, and can be explained by the higher intracellular longitudinal relaxation rate [4]. Additional enhancements, up to +11 % and +20 % respectively with normal cells incubated with Dotarem and P866, are clearly related to the Gd content per cell as shown in Table 1. As expected, the P866 brings a brighter improvement due to both its higher specificity and r_1 relaxivity (at 2.35 T, r_1= 15.5 and 2.9 mM^{-1}.s^{-1} for P866 and Dotarem, respectively). Furthermore, folate deprivation in the culture medium leads to an even larger signal (+25 %): the increased number of available folate receptors on the folate deprived cell membranes corresponds to an elevated amount of bound and internalized P866. Finally, as depicted more accurately on Fig. 2, the signal enhancement is propagated far away from the voxel plane containing the cells. Although a truly quantitative model is still lacking to describe this phenomenon, it can be explained by the transportation of relaxing protons with water exchange through the cell membranes and molecular diffusion across the free medium [5]. In future investigations however, a separate transmit coil should be used to ensure an uniform excitation profile and get more quantitative measurements.

CONCLUSION

This study proposes a complete set up and method to detect a single cell layer and assess the specificity of a targeted CA in well controlled conditions. A theoretical model remains to be made to link the Gd content per cell to the signal enhancement according to the relaxivity and cellular distribution of the CA and to the water exchange rates. The proposed set-up will be used for control studies with non targeted CA with the same relaxivities as P866, or for more advanced specificity assessments, including competition with the endogenous ligand. It will also be extended to different molecular targets and other CA such as targeted iron oxide nanoparticles.

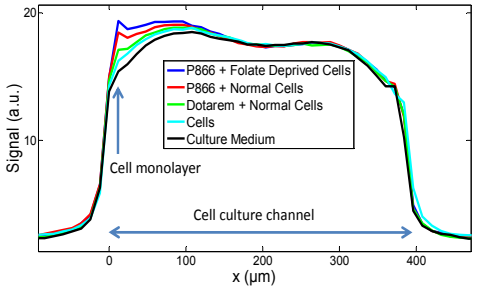

Figure 1: 1D signal for cells containing CA, cells without CA, and free medium alone.

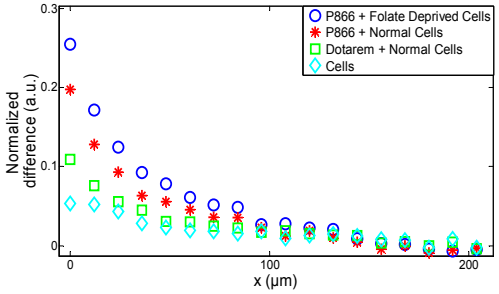

Figure 2: Normalized difference of the cell signals with reference to the free medium signal

Conditions of incubation	Relative signal enhancement (%)	Gd content per cell (fmol/cell)
P866 + Folate deprived cells	+25%	2.75
P866 + Normal cells	+20%	2.15
Dotarem + Normal cells	+11%	1.50
Cells	+5%	0

Table 1: Relative signal enhancement and Gd content per cells with the different incubation conditions

REFERENCES [1] : D. Voet et al, *Biochemistry*, 1990, p123, [2] : C. Corot et al, *Magn Reson Med*, 2008, 60: 1337, [3] : M. Woytasik et al, *Microsystem Technologies*, 2007, 13(11): 1575-1580, [4]: L. Zhao et al, *NMR Biomed*, 2008, 21:159-164, [5] : H.P. Huinink et al, *Magn Reson Med*, 2008, 59: 1282-1286