DYNAMIC UPTAKE OF A TARGETED Gd-CHELATE NANOEMULSION BY A $\alpha_v\beta_3$ EXPRESSING CELL LAYER FOLLOWED-UP WITH A MICRO-MRI SET-UP

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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. However, the efficiency of this approach is still limited due both to the low sensitivity of MRI and to the complexity of the binding and contrast mechanisms. In a previous work [1], a novel approach based on a dedicated micro-MRI set-up was proposed to investigate these issues with a cell monolayer model in a controlled microfluidic environment. Using this approach under physiological-like conditions, we demonstrate here the dynamic uptake of a RGD-targeted Gd-loaded emulsion by a HUVEC cell monolayer expressing the $\alpha_v\beta_3$ integrin, which is a promising target for molecular imaging due to its high level of expression during tumor angiogenesis.

MATERIAL AND METHODS

Cells: HUVEC cells were used due to their high level of expression of $\alpha_v\beta_3$ integrins. 280 000 of these cells were injected in 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 compact monolayer on the bottom of the channel.

MRI: Experiments were carried out on a 2.35 T scanner (Bruker, Germany), with warm air flowing in the gradient bore to maintain the cells temperature at 37°C. A birdcage coil (inner diameter: 7 cm) was used as a transmitter. For receiving the signal, a 6 mm diameter home-made Multiturn Transmission Line Resonator [2] was used and placed below the µ-slide, 180 µm away from the cell monolayer (figure 1). Orthogonality between the two coil axes was ensured to decouple the transmit and receive channels.

Very high resolution imaging was required to detect the cell layer since its thickness is about 10 µm. A 3D RF-spoiled FLASH sequence was applied with 12 µm resolution perpendicular to the cell layer (readout direction), an in-plane resolution of 200×400 µm^2, Flip Angle=25°, TR/TE=75/3.7ms, 12 kHz acquisition bandwidth and echo position of 5%. As the k-space was not fully acquired, the homodyne method introduced by Noll [3] was used to reconstruct the data to avoid distortions in the images. A 1D signal profile was extracted by projection of the 3D image matrix along planes parallel to the cell layer as previously described in [1].

Protocol: A flowing culture medium containing the emulsion at a concentration of 2.2 nM [Nanoparticles] was applied over the cell layer using a syringe pump and silicon tubing. The velocity of the flow inside the microfluidic channel was set to 0.15 mm/s, which is comparable to blood velocity in capillaries [4]. An image was acquired every 15 minutes with a scan duration of 2 min. Before each new image acquisition, the flow was stopped and the medium over the cell layer was flushed during 10 s with CA free medium in order to leave only the cell-bounded fraction of the emulsion. The operation with culture medium flowing, flushing and imaging was repeated over a total duration of 75 minutes. Two emulsions were investigated: a Gd-based nanoemulsion functionalized with RGD peptide binding the $\alpha_v\beta_3$ with a nanomolar affinity (Guerbet data) and a control one without the RGD peptide. For reproducibility assays, the protocol with each emulsion was applied three times using cells of passage 5.

RESULTS AND DISCUSSION

Figure 2 displays the 1D profiles obtained for the cells before and after a flow with the targeted emulsion has been applied for 75 minutes. A signal enhancement is well observed in the voxel containing the cells, due to the binding of the targeted emulsion on the $\alpha_v\beta_3$ receptors on the cells' surface. An enhancement is also seen on the adjacent voxels, due to the molecular motion of the relaxing water protons during the signal observation time [1]. Figure 3 displays the mean signal gain ($[S_{cell}(t)-S_0]/S_0$) observed in the cell layer as a function of time for either the targeted or the control emulsion, where $S_{cell}(t)$ is the signal of the cell layer at time t and S_0 is the signal of the cells before application of the emulsion. It indicates a 3-fold higher uptake of the targeted emulsion by the HUVEC cells when compared to the control emulsion. This can be attributed to the specific binding of RGD peptides to the $\alpha_v\beta_3$ surface receptors. Further experiments including competition assays are still needed to clearly assess the specificity of the bond between the emulsion and the $\alpha_v\beta_3$ integrins.

CONCLUSION

We developed a powerful tool and method to detect by dynamic micro-MRI the binding of a RGD-targeted emulsion on a living $\alpha_v\beta_3$ expressing cell monolayer in vitro. The setup is versatile and can be adapted for other CA and receptors with the possibility of varying the biological conditions and the CA flow dynamics. This in vitro molecular imaging model approach will be useful prior to in vivo experiments on animals, particularly to assess the specificity and characterize the binding kinetics of targeted CAs.

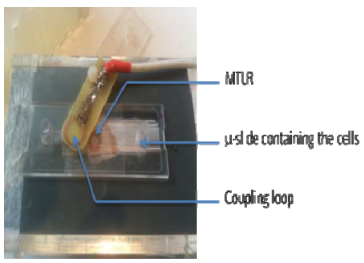

Figure 1 : Picture of the µ-slide I Luer containing the cells and the reception coil

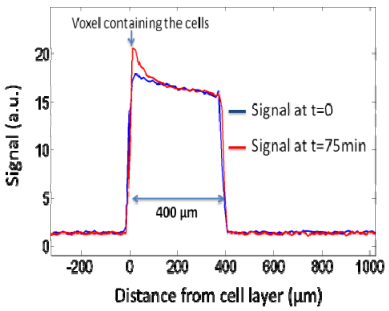

Figure 2: 1D signal for cells before (blue) and after 75 minutes (red) of the application of a nanoemulsion flow over the cell monolayer

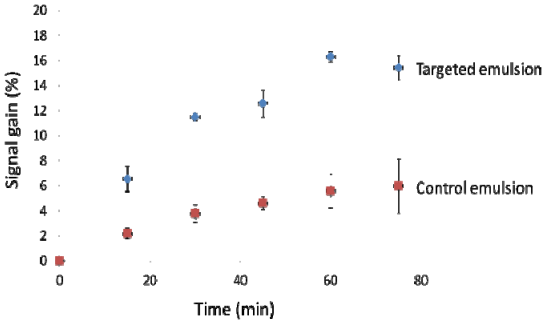

Figure 3: Signal gain as a function of the time over which the flow of emulsion is applied for both the targeted and control emulsion

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