GENETICALLY FUNCTIONALIZED MAGNETOSOMES AS MRI CONTRAST AGENT SUITABLE FOR MOLECULAR IMAGING

Marianne Boucher[1], Nicolas Ginet[2], Françoise Geffroy[1], Sandra Préveral[2], Géraldine Adryanczyk-Perrier[2], Michel Pean[2], Christopher T Lefèvre[2], Daniel Garcia[2], David Pignol[2], and Sébastien Mériaux[1]

[1]UNIRS, CEA/DSV/I2BM/NeuroSpin, Saclay, France, [2]LBC, CEA/DSV/IBEB/SBVME, Saint-Paul-lez-Durance, France

Target audience: Researchers interested in molecular imaging applications, in contrast enhanced MRI with iron oxide-based nanoparticles, in functionalized contrast agent production and also in brain tumor diagnosis.

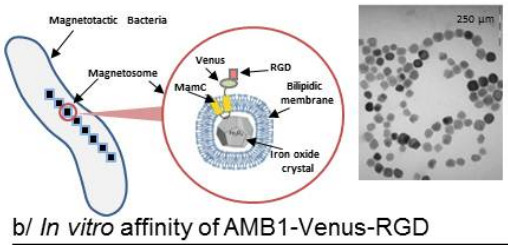
Purpose: Magnetosomes are iron oxide nanoparticles of interest for MR-based molecular imaging applications. These regular crystals of magnetite embedded in a lipid bilayer are produced by magnetotactic bacteria and can be functionalized for biomarkers targeting using genetic tools. These magnetosomes also present promising MR contrasting properties for T_2 and T_2^* imaging strategies [1]. Here, we intend to demonstrate the feasibility of harvesting RGD functionalized magnetosomes from genetically modified bacteria AMB-1. We then characterize the T_2-shortening abilities of these nanoparticles using transverse relaxivitiy measurement, and present an *in vivo* proof of their potential use as probe for contrast enhanced MRI.

Methods: Wild Type (WT) *Magnetospirillum magneticum* AMB-1 was transformed with a plasmid harboring the gene coding for Venus-RGD protein (or Venus, as control) fused with mamC, a gene encoding one of the most abundant proteins at magnetosome membrane. Magnetosomes (AMB1-Venus-RGD, AMB1-Venus or AMB1 WT) were purified after cultivation of these genetically modified or WT AMB-1 bacteria [2]. TEM image was acquired on a deposit of 10 µL of diluted AMB1 WT magnetosomes suspension on 200 mesh copper grids, covered by a formvar-carbon film and imaged with a TEM scanner at 80 kV (Zeiss EM9, Oberkochen, Germany). U87 cells, human glioblastoma cells known for overexpressing $\alpha_v\beta_3$ integrins which are targeted by RGD, were grown for 3 days after seeding [3]. Then the cells were incubated during 3h30 with media containing functionalized magnetosomes (AMB1-Venus / AMB1-Venus-RGD). For fluorescence microscopy, cells membrane was revealed with Texas Red wheat agglutinin and nucleus with DAPI. All MRI acquisitions were performed using an 11.7 T preclinical scanner (Bruker BioSpec, Ettlingen, Germany), with a dedicated volume coil for relaxivity measurement and a surface cryo-probe for *in* vivo imaging. MRI transverse relaxivity r_2 of AMB1 WT was measured using a T_2 mapping sequence (Multi-Slice Multi-Echoes, TE = 8 ms, 64 echoes, TR = 5000 ms, 4 averages, in-plane field-of-view = 3 x 3 cm^2, in-plane resolution = 250 x 250 μm^2, 6 slices of 1 mm thickness, total acquisition time 40 min) on dedicated phantoms made of 6 tubes containing different concentrations of contrast agent from 0.001 to 0.2 $mM_{[Fe]}$. All *in vivo* experiments were performed in accordance with the recommendations of French legislation and approved by a local ethical committee (ID 10-025). Male nude mice were implanted with U87 cells in the right brain hemisphere (2 µL, 60.10^6 cells/mL, 2 mm right compared to bregma and 3.5 mm deep) and were scanned between 14 and 16 days after cells implantation when the tumor has a diameter around 2 mm. T_2^*-weighted images were acquired pre- and post-intravenous injection of AMB1 WT (200 µL, 200 $\mu mol_{Fe}/kg$) with a FLASH sequence using the following parameters: TE/TR = 8/1600 ms, 1 average, in-plane field-of-view = 2.22 x 1.35 cm^2, in-plane resolution = 75 x 75 μm^2, 90 slices of 75 µm thickness, total acquisition time 5 min. In post-processing, a filtering method [4] was applied to reveal brain vasculature from hypointense voxels detected on FLASH images.

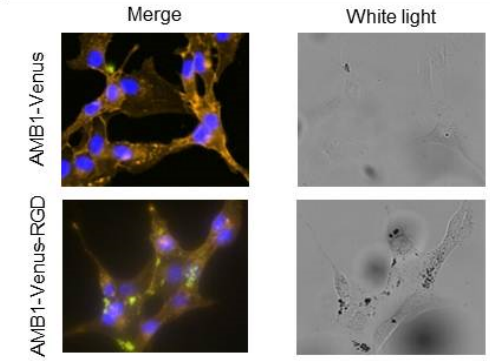
Results: TEM image showing extracted magnetosomes is used to evaluate their mean size: l = 45 nm (*Figure a*). Fluorescence images confirm that dark spots observed in white light (iron oxide core) are colocalized with YFP fluorescence of Venus protein (*Figure b*). In AMB1-Venus-RGD condition, such fluorescence pattern is observed inside a large number of U87 cells, whereas in AMB1-Venus condition, few aggregates are located outside cells. Transverse relaxivity of AMB1 WT has been measured at $r_2 = 240\ mM^{-1}s^{-1}$. An increase of the number of detected hypointense voxels corresponding to vessels is observed *in vivo* due to AMB1 WT magnetosomes circulating into blood stream after injection (*Figure c*).

Discussion: All AMB1 magnetosomes are made of a single crystal of iron oxide of a bigger size than usual SPIO [5], which may lead to enhanced T_2 contrasting properties [6]. Indeed measured transverse relaxivitiy is higher than the one of commercial SPIO [5]. *In vitro* fluorescence images highlight the affinity of RGD magnetosomes for U87 cells, confirming that the bio-produced RGD peptide at the magnetosomes surface is functional for $\alpha_v\beta_3$ targeting. Finally, *in vivo* experiment is consistent with relaxivity measurement, confirming that the sensitivity detection of AMB1 magnetosomes is suitable for contrast enhanced imaging applications.

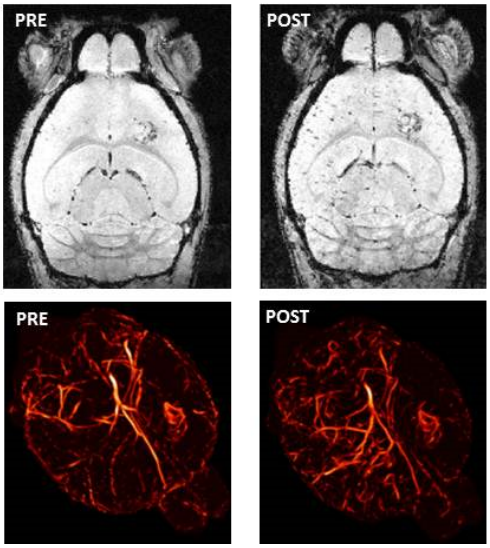
a/ From bacteria to bio-nanoplatform



b/ *In vitro* affinity of AMB1-Venus-RGD



c/ *In vivo* contrasting properties of AMB1 WT



Conclusion: AMB-1 magnetotactic bacteria can be genetically modified for expressing RGD peptide at magnetosomes membrane. The magnetosomes isolated from bacteria can then be used as MRI contrast agent with high contrasting properties, and seem also promising for molecular imaging applications, as suggested by these first *in vitro* and *in vivo* results.

References: [1] D. Faivre and D. Schüler, *Chem Rev*, 108(11), 2008; [2] N. Ginet et al., *PLoS One*, 6(6), 2011; [3] C. Giraudeau et al., *Angiogenesis*, 1(16), 2013; [4] A. F. Frangi et al., *Lecture Notes in Computer Science*, 1496, 1998; [5] C. Corot et al., *Adv Drug Deliv Rev*, 58(14), 2006; [6] Q. L. Vuong et al., *Adv Health Mat*, 1(4), 2012.