Design of Implantable alginate MRI pH sensors for cell transplantation

Nikita Oskolkov[1,2], Xiaolei Song[1,2], Kannie W.Y. Chan[1,2], Jeff W.M. Bulte[1,2], and Michael T. McMahon[1,2]

[1]*The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins School of Medicine, Baltimore, Maryland, United States,* [2]*F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland, United States*

Target Audience: Researchers interested in MRI monitoring of cell transplantation

Purpose: Cell transplantation is an important strategy for treating otherwise incurable diseases, with numerous clinical trials now ongoing all over the world. Various biocompatible materials have been designed to support transplanted cells and protect them from the immune system. Our previous study showed that alginate-based hydrogels could be used for non-invasive monitoring of hepatocytes cell death *in vivo* using pH sensitive CEST magnetic resonance imaging (MRI) technique (Chan et al., 2013).

In this study we were interested in redesigning these for use on lower field scanners, through incorporating salicylic acid (SA)-based (Yang et al., 2014) pH-nanosensors into the biomaterial through dispersing SA-liposomes through alginate capsules. These SA agents display CEST contrast at high-frequency-offsets. In this abstract we show how these can be used for non-invasive MRI imaging of encapsulated cells.

Method: *LipoCEST* composed with DSPC:Chol: PEG2000-DSPE (47.5:47.5:5.0) were formed using a modified extended hydration method with the different sized salicylic acid derivatives CEST agents in the hydration solution. The concentration of labeled DSPE from 5-7 mole% while adjusting the DSPC and Cholesterol ratio downward in a manner similar to that we described previously (Liu G. et al., 2012). The liposomes are extruded to reduce their diameter using 400 and 100 nm cutoff membranes, with the final liposomes ~100-150 nm, measured using dynamic light scattering (DLS). Size exclusion chromatography using Sepharose G-50 matrix was performed to remove unincorporated contrast agent. The highest concentration that results in stable contrast will be incorporated into alginate capsules.

The synthesis of microcapsules is based on the method of Lim and Sun, (Lim et al., 1980). A suspension of at least 1.5% w/v ultrapurified sodium Protanal(R) HF alginate with ~ 15-30 nM SHY liposome concentrations is passed through the needle using a nanoinjector pump and the resulting droplets collected in a Ba^{2+} solution crosslinking the alginate to form stable capsules. After washing, additional layer-by-layer crosslinkings were done using polycationic peptide protamine sulfate (0.05% - 0.20%) and then 0.15% Keltone HVCR alginate (Fig. 1). *MRI imaging*: Capsules phantom CEST images were acquired on Bruker 11.7T scanners using a RARE sequence with a CW saturation pulse. Z-spectra were acquired by sweeping the frequency every 0.25 ppm from -10 to 10ppm. Parameters were B_1=3.6, 4.7, 5.9 μT, T_{sat}=3 s, TR/effective TE=6 s/17-19 ms, and matrix size=96x64. CEST was quantified as

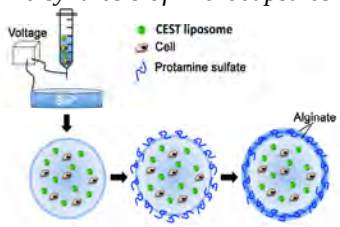

Fig. 1. Cartoon depicting the preparation of implantable alginate pH sensors

$$MTR_{asym} = 1 - S(+\Delta\omega)/S(-\Delta\omega).$$

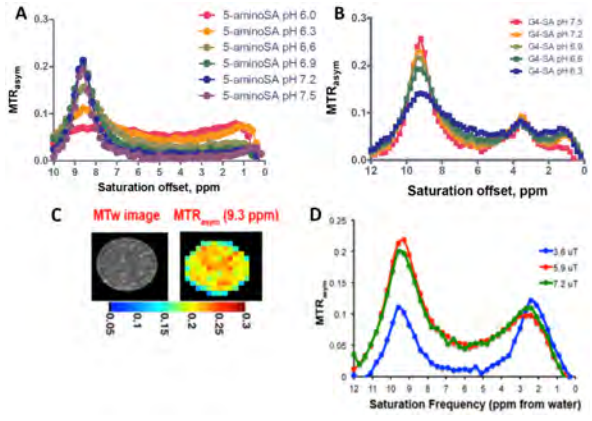

Fig. 2. MTR_{asym} of 5-aminosalicylic acid (A) and 5-aminomethyl-salicylic acid-dendrimer conjugate (B) at different pH; (C) MT_w and MTR_{asym} images at = 9.3 ppm for SA-based liposomal capsule; (D) MTR_{asym} at 11.7 T, T= 37 °C, t_{sat} = 4 s;

Results: The CEST contrast of small salicylic acid derivatives or macromolecular conjugates of salicylic acid derivative to PAMAM dendrimer (G4-SA) is pH-dependent (Fig. 2A, B). Both 5-amino and a macromolecular salicylic acid-dendrimer conjugates have appropriate pH sensitivity, displaying maximal contrast at pH 7.2 – 7.5, with the contrast significantly reduced by pH 6.0. This dependence is similar to found in L-arginine, which we have previously used for pH sensing. One additional benefit of the dendrimer, is the two types of exchangeable protons, OH (~ 9 ppm) and NH (~3 ppm), which display contrast and have a different dependence on pH allowing ratiometric pH measurements (Fig. 2B). As is shown in Fig. 2C, D these can be integrated into alginate capsules, which produce strong, frequency dependent contrast similar to the free compounds.

Conclusion: We prepared promising salicylic acid-based biomaterial, which produced high-frequency-offset CEST contrast, pH dependent and could be used for tracking of transplanted cells death. **Funding Support**: R01EB012590