MRI of Vascular Cells Labeled with SPIO-PLL Complexes for Heart Valve Tissue Engineering Studies

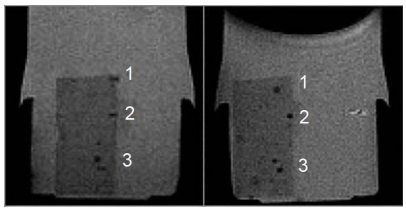
P. A. Schornack[1], and S. Ramaswamy[2]
[1]Radiology, University of Pittsburgh, Pittsburgh, PA, United States, [2]University of Pittsburgh

Noninvasive & nondestructive monitoring of the cellular function within the developing valvular tissue is a critical aspect of implant success. In-depth study on the longitudinal (temporal) position & migration patterns of cells during the tissue development process. This can be achieved through cellular MRI (cMRI) techniques such as with the labeling of cells with superparamagnetic iron oxide (SPIO) particles. The intimal & medial lining of the artery consists largely of vascular endothelial cells (VECs) & smooth muscle cells (VSMCs) respectively. Migration of these cell types to a potential tissue engineered heart valve (TEHV) implant is therefore of interest in understanding the valve remodeling process. In this regard, superparamagnetic (SPIO) iron oxide labeling & subsequent longitudinal cell visualization & tracking by magnetic resonance imaging (MRI) provides a method of studying this process. As a first step, here, we report on the labeling efficiency of human VECs & VSMCs using SPIO nanoparticles & the subsequent contrast that cellular MRI (cMRI) methods provide.

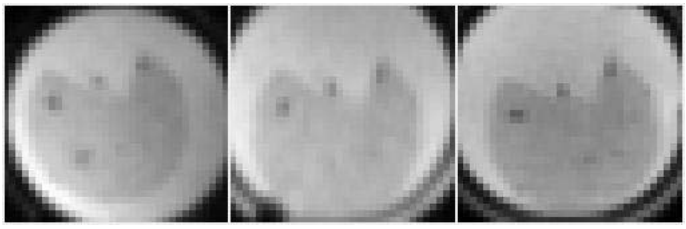
Methods.

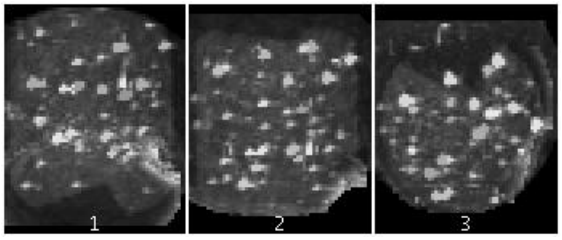
Human pulmonary artery smooth muscle & endothelial cells were cultured & expanded in supplemented medium/growth supplement. Cells were labelled with paramagnetic microspheres (fluorescence tagged (flash red) solid polymer of 0.86 μm diameter; 1.65 g/ml density with iron oxide core) at a concentration of ~ 36.5 x 105 microspheres/ml, with protamine sulfate, PS (varied from 0 to 9 μg/ml). The scaffold consisted of 4% Agar gel & a 50:50 blend of PGA□PLLA. SPIO particles were combined with different concentrations of the transfection agent (TA), protamine sulfate. VECs & VSMCs were allowed to incubate in the iron suspension overnight. Initial flow cytometric analysis on cell viability/apoptosis helped determine the best ratio of SPIO:TA to use. Subsequent MRI experiments were performed at 9.4 T to obtain T2 and T2* weighted images and maps. Cells were suspended in an Agar gel to coregister MRI slices with histological slices. A landmark notch on each gel ensured spatial accuracy of this co-registration.
Images were acquired on a 9.4 T Varian scanner. Spin echo images were acquired with 9 to 12 evenly spaced echo times between 10 & 120 ms with a repetition time of 2500 ms; these images permit calculation of T2 maps. Gradient echo images were acquired with multiple (10 to 14) echo times between 2.5 & 25 ms & a TR of 400 ms; these images permit calculation of T2* maps. The acquisition FOV was 30x30 mm^2 with a 128x128 matrix to contain the 3 sample tubes of the day 1 experimental time point, & 30x15 mm^2 with a 128x64 matrix for the 2 surviving samples of the subsequent time points on day 8 & 13. The multiple spin echo & gradient echo experiments were acquired with 10 interleaved 0.5 mm slices to cover nearly the entire sample of interest (ie, the gel containing iron oxide labeled cells).

Results & Discussion.



The images to the right show approximately the same region through the notch on the same sample acquired on day 1 and day 13 post-seeding of the gel scaffolds; these are T2w fast spin echo acquisitions with effective TE=72ms and TR=1500ms. The second figure on the right below shows the same slice on T2*w images with TE=6ms, TR=400ms, from the 3D collagen gel scaffolds at 1, 8, & 13 days after seeding.
These images demonstrate that seeded cells can be closely monitored longitudinally by MRI in 3D gel constructs & fibrous scaffolds for at least a 12 day duration. SPIO-labelled endothelial cells are considered to correspond to the regions of hypointensity on these T2*w MRI images. T2 and T2* maps were also obtained of these samples and show similar features as the T2*w images shown. Histological correlations studies are still needed & are currently underway. Both 2D & 3D gradient echo (T2* contrast) sequences clearly showed the static nature of these regions, which were spatially similar over 3 time points separate by 1 week.





The final figure on the right above shows 3 different views (rotated by approximately 60 degrees) of a 3D maximum intensity projection derived from the reciprical of 3D T2*w data with TE=6ms, TR=20ms. Hence, the bright spots correspond to regions of reduced signal on the T2*w Turboflash images, which would occur for regions of increased iron oxide content.

We intend to continue studies on HSMCs & HECs in mechanically conditioned environments (i.e., in a bioreactor). These effects also may play a role in retaining the cell source (e.g. stem cells) used for seeding TEHVs on the scaffolds. It is envisioned that SPIO-based cMRI can aid in protocol optimization (seeding, mechanical conditioning, etc) & in the long-term, provide insights on cell fate in TEHV construct & associated tissue regeneration processes.

Acknowledgements. 1]: AHA-Scientist Development Grant ID#0830061N 2] NIH R01 HL-68816 2] Use of MR facility at Dr. Seong-Gi Kim's laboratory at University of Pittsburgh