Characterization of USPIO nanoparticles for non invasive monitoring of inflammation in tissue engineered tissue vascular graft using in vivo MRI

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INTRODUCTION:

Magnetic Resonance Imaging (MRI) is a non-invasive method potentially well suited for monitoring cells grafts to identify and map the fate of transplanted cells. Biodegradable scaffolds seeded with bone marrow mononuclear cells (BMCs) are the earliest tissue-engineered vascular grafts (TEVGs) used clinically as venous conduit for congenital heart disease [1]. In animal recipients, TEVGs transform into leaving blood vessels similar in morphology and function to the native veins to which there are interposed [2]. A very recent study shows that the neovessel formation occurs through a process of inflammatory vascular remodeling where the seeded BMCs enhance the recruitment of host monocytes to the scaffolds [3]. This study reports the use of ultra small superparamagnetic iron oxide (USPIO) nanoparticles to noninvasively monitor the inflammatory remodeling process of neovessel development through the tracking of USPIO-labeled macrophages by serial MRI.

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MATERIALS and METHODS:

Animal preparation: USPIO-labeled and unlabeled macrophages were seeded onto biodegradable polymer scaffolds. Female C.B-17 SCID/bg mice were anesthetized with 0.5-1.5% isoflorane and underwent surgical implantation of scaffold seeded with USPIO labeled or unlabeled macrophages as an interposition grafts in the inferior vena cavas (IVC). ***Cell Counting:*** Two separate sections of each scaffold explant were stained with H&E and Prussian blue and imaged at a magnification of ×400. The number of nuclei were counted in four regions of each section and averaged to determine total cellularity. The number of cells staining positive for Prussian blue were counted in four regions of each section and averaged to determine total number of cells containing USPIO nanoparticles. ***MRI:*** Mice were anesthetized with 1.5 % isoflorane and serially imaged immediately after the scaffolds implantation and on post-operative day 7 using a Bruker horizontal-bore system with custom-made volume coil. T2-weighted images were acquired using a rapid acquisition with relaxation enhancement spin-echo sequence (RARE). Imaging parameters were as follows: field of view = 28 mm, image matrix = 128 × 128, slice thickness = 1 mm, RARE factor = 4, effective echo time = 34 ms and recycle time = 4s. For T2 maps (MSME) sequence was used with the following parameters: Number of echoes=6 and echo spacing =17ms, TR/TE =4000/17 ms, field of view = 28 mm, image matrix = 128 × 128, slice thickness = 1 mm. the data were used generated T2 map by pixel-by pixel fitting using custom-written code using Matlab . T2 values were measured in the scaffold, liver, muscle and fat.

RESULTS and DISCUSSION:

Mice received TEVG implants that had been seeded with either USPIO-labeled or unlabeled macrophages were serially imaged to investigate the possibility of tracking the fate of the seeded cells noninvasively. The infrarenal TEVGs were easily detectable retroperitoneally in the IVCs on T2 weighted RARE images and T2 maps at time 0 (immediately after implantation) and after 1 week **(Fig 1-A)**. The TEVGs seeded with USPIO-labeled macrophages appear with sharper borders **(Fig 1-A)**. The T2 values **(Fig 1-B)** measured at time 0 (t=0) were significantly decreased in TEVGs with USPIO-labeled macrophages compared to the seeded unlabeled control scaffold. The T2 values measured at both: time 0 and 1 week were similar in the the TEVSs with USPIO–labeled macrophages and the control scaffold seeded with unlabeled macrophages. The T2 values in the muscle, liver and the fat were similar in both groups at time 0 and 1 week post implantation. The decrease in the T2 values in the TEVGs seeded with USPIO-macrophages confirms that the seeded macrophages were retained on the scaffold after surgical implantation in numbers greater than the MRI cellular limit of detection. After the MR imaging, the grafts were harvested for histology. The TEVGs were stained with Prussian blue and the number of cells staining positive for intracellular iron were counted at time 0 and one week after implantation. There was a significant decrease in the number of cells staining positive for iron oxide one week after implantation. While the number of cells with intracellular iron decreased over the 7 days post-implantation, the total cellularity of the TEVGs significantly increased. The decrease of the USPIO-labeled macrophage in the scaffold after one week may explain the increase of the T2 value measured in the scaffold. These results demonstrate that the seeded cells do not actually become incorporated into the neovessel, but instead are important for inciting an inflammatory remodeling process, through the recruitment of host cells, that is critical for the development of the neovessel. This study demonstrates the feasibility of applying MR imaging for evaluation of *in vivo* TEGV performance and helps understand the mechanisms underlying the transformation of TEVG into neovessel

Figure1

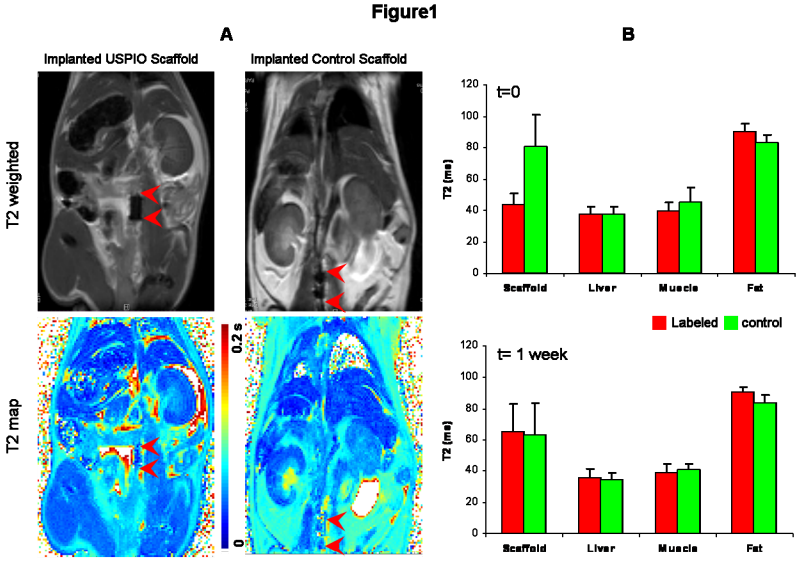

Figure1: (A) Representative T2 weighted slices (top row) and its corresponding T2 map (bottom row) illustrating the location of the implanted scaffolds (red arrows) . (B) Quantitative T2 values in the Implanted scaffolds, muscle, liver and fat immediately after the implantation of the scaffolds (t=0) (top row) and one week postimplantation (t= 1week) (bottom row).

References

[1 Isomatsu et al. (2006) *J Thorac Cardiovasc Surg 126:1958196,* [2] Shinoka et al (2005) *J Thorac CardiovascSurg 129:10641070*
[3] Roth (2010) *PNAS. 107:4669-74*