

Targeting mesenchymal stem cells (MSC) using pulsed focused ultrasound: Implications for stem cell therapy

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Introduction: Stem cell therapy has shown promise in various diseases; however, the utility of this approach is limited by inefficient homing of cells to targeted pathology following intravascular injection. Focused ultrasound (FUS) exposures are presently used for ablating a variety of tumor types in cancer treatment. Alternatively, pulsed (p) FUS exposures can create non-destructive mechanical effects that enhance tissue permeability for increasing drug and gene delivery. In this study, pFUS to a kidney enhanced the homing and migration of superparamagnetic iron oxide nanoparticle (SPION) labeled MSCs following intravenous (IV) injection. The labeled MSC were detected by MRI and correlated with histology and microarray analysis for cytokine expression in the kidney.

Methods: Human MSCs were obtained from healthy donors and labeled with ferumoxides (FE), a SPION, and protamine (Pro). An image-guided FUS system was developed using a 1 MHz FUS transducer and a portable US system. In this study, nude mouse kidneys (n=48) served as a model tissue, in which unilateral treatment with pulsed (p) FUS to the kidney was performed and the contralateral kidney served as control. pFUS exposures consisted of 100 pulses in 6 contiguous regions (2 mm spacing), using a pulse width of 200 ms, duty cycle of 5%, and intensity of 4000 W/cm². 10⁶ FEPro-labeled MSCs were administered IV 1 hour post-pFUS followed by *in vivo* T2- and T2*-weighted MRI. Animals were euthanized on days 0, 1, 3, and 7 post-FUS and kidneys were collected for the following: *ex-vivo* MRI (7T), histology with Prussian blue (Pb), immunohistochemistry (IHC) for anti-human mitochondria marker (AHM) to detect MSCs, F4/80 for mouse macrophages (M), and molecular biology analysis was performed for tissue cytokine expression in mice treated only with pFUS and not receiving MSCs.

Results: *In vivo* and *ex vivo* MRI at 7T demonstrated hypointense voxels on T2*-weighted images in treated kidney on day 1 and 3 that were not detected in contralateral kidney (Fig. 1). Histological analysis revealed MSCs in peritubular regions and rarely in glomeruli in the pFUS treated kidney based on PB staining and IHC AHM stain on Day 1 and 3. IHC showed F4/80+ Pb-macrophages were rarely detected in peritubular or in glomeruli regions in treated kidney at day 1, 3 or 7. Transient minor mechanical/structural changes were observed in pFUS treated kidney. Microarray analysis of treated versus contralateral control kidney demonstrated significant (P<0.05) increases on days 0 and 1 post pFUS in pro-inflammatory cytokines (i.e., IL 1alpha, IL1beta, IL2, IL4, IL12, IL17, TNF alpha, and MCP-1) that would stimulate homing of MSC to targeted kidney.

Discussion: This study demonstrates that non-destructive pFUS exposures effectively enhanced the homing of FEPro-labeled MSC to the targeted kidney in animals receiving IV injection of cells based on MRI and IHC staining. pFUS to the kidney resulted in an increase in inflammatory cytokines that stimulated MSC migration to treated areas and early margination from the vasculature to peritubular regions. These results indicated that pFUS can be used to non-invasively enhance the homing of stem cells or genetically engineered cells to targeted regions in the treatment of disease.

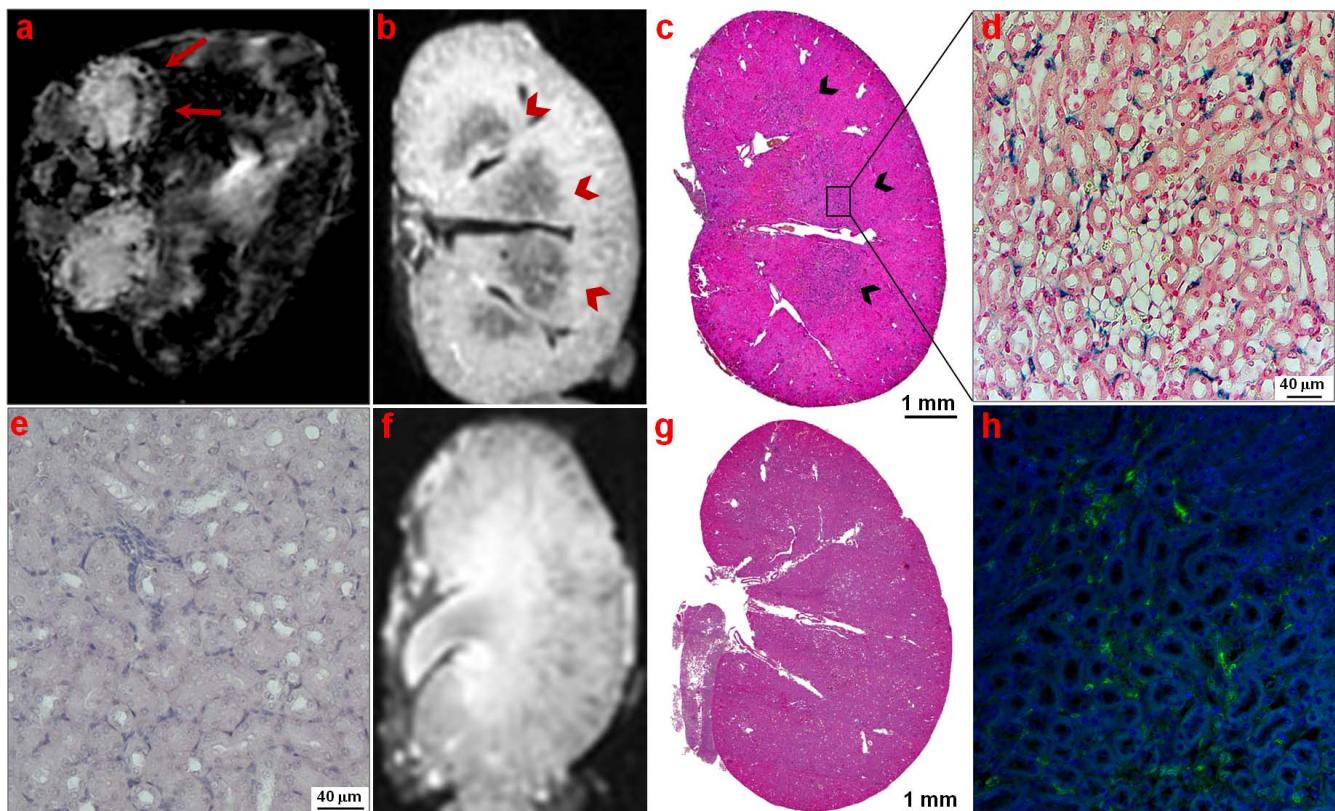


Fig. 1: **a** – *in vivo* T2* MRI of the treated mouse shows hypointense regions in the treated kidney (arrows); **b** – *ex vivo* T2* MRI of pFUS treated kidney shows hypointense areas (arrows) revealing presence of FEPro labeled MSCs; **c** – Pb staining of pFUS treated kidney shows the presence of MSCs (arrows); **d** – Pb positive MSCs visible in peritubular regions of the treated kidney; **e** – DAB enhanced IHC for macrophage marker F4/80 shows no or minimal presence of macrophages in treated kidney; **f** – T2* MRI of control kidney; **g** – Pb stain of the control kidney; **h** – AHM IHC (green) verifies the presence of the MSCs in the treated kidney.