A high T_1-relaxivity nanoparticle for in vivo MRI detection of kidney glomeruli

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INTRODUCTION - The goal of this work is to develop a non-invasive, MRI-based method for detecting kidney glomeruli in vivo. A single human kidney has between 200,000 and 2,000,000 nephrons, which are responsible for maintaining homeostasis by balancing blood ion and glucose concentrations and blood pH levels. Each nephron begins with a single glomerulus that serves as a size and charge selective filter for blood. Diabetes, obesity, and hypertension are strongly linked to a low number of glomeruli (Nglom) (1) and the volume of glomeruli (Vglom) has been linked to ~ 90% of chronic kidney diseases (CKD) (2). There are already excellent histological techniques for measuring Nglom and Vglom but these techniques require resection and destruction of the entire organ (3). Previous work has shown that Nglom and Vglom may measured ex vivo using intravenous injection of the MRI-visible cationized ferritin (CF) nanoparticle and magnetic resonance imaging (MRI) (4-6), though this technique is difficult to apply in vivo due to the signal darkening characteristics of the superparamagnetic CF and the dark blood background in the kidney glomerulus. A paramagnetic version of CF would make in vivo imaging of the kidney glomerulus a simpler task and lower the required dose of contrast agent to detect glomeruli. Recently, a high relaxivity, paramagnetic T_1 MRI contrast agent has been synthesized inside of the ferritin protein shell, thereby allowing for a brightened signal when imaged with T_1-weighted MRI (7). This is accomplished by doping the iron core of the ferritin protein with tungsten. This tungsten-iron loaded (WFe) ferritin nanoparticle is detectable with T_1-weighted MRI at concentrations as low as 25 nM. The goal of this work is to detect kidney glomeruli in vivo by cationizing WFe ferritin, injecting the cationized WFe ferritin (hereby referred to as paramagnetic cationized ferritin, or paraCF), and imaging the kidneys with T_1-weighted MRI. Such a tool may report on Nglom, Vglom, and total glomerular filtration surface area within a single voxel in vivo.

METHODS - *Loading the apoferritin core.* 48mM $FeCl_2$ was added at a rate of 12.5µl/min to a 2µM solution of apoferritin in an oxygen free environment using a syringe pump for a total of 140 minutes. 50 minutes into $FeCl_2$ pumping, 48mM Na_2WO_6 was added at a rate of 12.5µl/min using a syringe pump for a total of 40 minutes. A total of 1.75ml of $FeCl_2$ and 500µl of Na_2WO_6 was added to the protein solution. 200µl of 300mM sodium citrate was then added to the solution to chelate any remaining metal ions. The solution was the sonicated and centrifuged for 10 minutes at 957·*g* then dialyzed overnight against 3L of de-ionized water. Once dialyzed, the protein solution was filtered using 0.8µm and 0.2µm surfactant free cellulose acetate syringe filters to rid the solution of coarse non-specific metal oxides. A total tungsten and iron-loaded (WFe) ferritin protein concentration was obtained with a Coomassie Plus Bradford Assay Kit and inductively coupled plasma – optical emission spectroscopy (ICP-OES) was used to measure metal concentrations. *Cationization of WFe ferritin.* WFe ferritin was cationized by coating its protein surface with amine groups via a carbodiimide conjugation of N,N-Dimethyl-1,3-propanediamine (DMPA) to the carboxyl groups of the ferritin surface using 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as detailed by Danon, et al. (8). The resulting cationized WFe ferritin will hereby be referred to as paraCF. Once the cationization process was complete, the resulting paraCF solution was dialyzed against phosphate buffered saline (pH = 7.2) and concentrated to 11 mg paraCF per ml of PBS. *Animal preparation and imaging.* ParaCF was injected intravenously to two male sprague-dawley rats at a dose of 4.4 mg/100 g. Rats were imaged in vivo at 7T with a 2D T_1-weighted gradient echo MRI pulse sequence using a custom built, kidney-specific radio frequency coil (TE/TR = 10/54 ms, resolution = 117x117x400 µm), then sacrificed and fixed by transcardial perfusion of PBS followed by 10% neutral buffered formalin. Right kidneys were be prepared for immunofluorescence histology. *Immunofluorescence.* Kidneys were sectioned, washed, blocked, and incubated in a rabbit anti-horse ferritin primary antibody and an Alexa594 goat anti-rabbit secondary antibody. Sections were then incubated with DAPI for 15 min, mounted, and imaged on Zeiss 710 laser scanning confocal microscope. *Data analysis.* Signal changes in the renal cortex due to the accumulation of paraCF in glomeruli was quantified by normalizing the mean signal magnitude in the renal cortex of paraCF-inoculated and naive rats to the mean signal magnitude of the muscle surrounding the rat's spine.

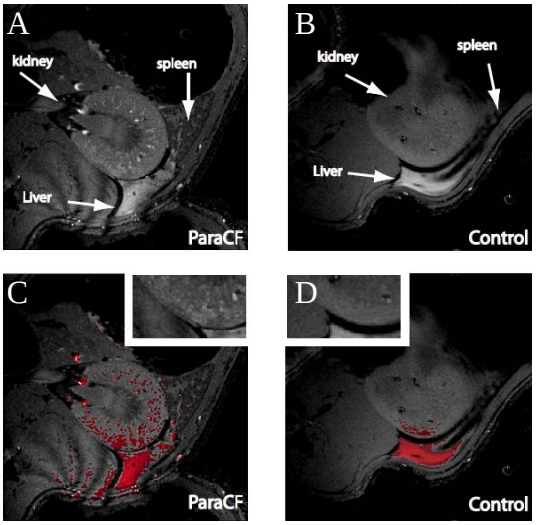

Fig. 1 – ParaCF labels kidney glomeruli and brightens the renal cortex when imaged with T_1-weighted MRI. Voxels with signal magnitude values 50% greater than those of muscle tissue are highlighted in red (C-D). ParaCF-inoculated kidneys show substantial signal enhancement in the renal cortex (A, C). Naïve control kidneys show no such signal enhancement in the renal cortex (B, D). Inset panels show the cortex of the kidneys.

RESULTS - T_1-weighted MRI revealed punctate signal enhancement in the renal cortex of paraCF-injected animals (Fig. 1). An ~45% increase in MRI signal magnitude was observed in the renal cortex of paraCF-injected rats (n=2) versus naive controls (n=2) (Average normalized signal magnitude values of paraCF-inoculated cortex = 1.6 versus 1.1 in naïve controls when the signal magnitude of the renal cortex is normalized against that of muscle). We calculate that paraCF accumulation in the glomeruli yields a 33% decrease in T_1 in the renal cortex. Immunofluorescence revealed a ribbon of red fluorescence in the glomerulus of paraCF-inoculated kidneys but not naive controls (Fig. 2).

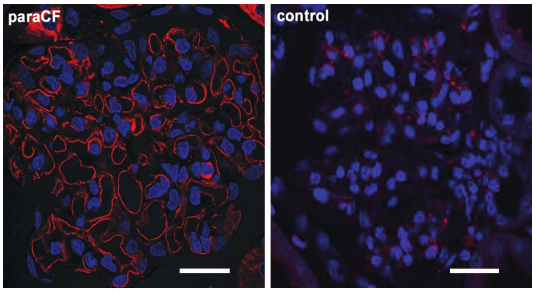

Fig. 2 - Confocal microscopy of a paraCF-labeled kidney glomerulus and a control glomerulus. ParaCF is labeled with a red fluorophore and cell nuclei are labeled with blue DAPI. A ribbon of red fluorescence was observed in the glomerulus in sections of paraCF-inoculated kidneys but not naive controls, indicating that paraCF is bound to the glomerulus. Scale bar = 20 µm.

DISCUSSION - In this work we have shown that paraCF can be used to detect glomeruli in vivo using T_1-weighted MRI and that its bright signal enhancement is easily visualized against the blood background of the kidney. The high r_1 relaxivity of paraCF may allow for the dosage necessary to detect glomeruli in vivo to be reduced as much as 20-fold compared to regular CF. Though regular CF has already been shown to have minimal effects on renal, hepatic, and immune function at MRI detectable doses (9), the ability to reduce the dosage of contrast agent will be beneficial to the translation of cationized ferritin-based agents from ex vivo applications to preclinical and clinical diagnostics. **Conclusions - This highly detectable, glomerulus-specific paramagnetic nanoparticle may be used to dynamically detect glomerular structure in vivo, raising the possibility of measuring Nglom, Vglom, and total glomerular filtration surface area in vivo.**

Refs: (1) Brenner et al. *Am J Hypertens* 1988 (2) Puelles et al. *Nephrol Dial Trans* 2012 (3) Bertram et al. *Cell and Tiss Res* 1992 (4) Beeman et al. *AJP Renal* 2011 (5) Bennett, et al. *MRM* 2008 (6) Heilmann et al. *Nephrol Dial Trans* 2012 (7) Clavijo Jordan, et al., *Proc 2012 WMIC* 2012 (8) Danon et al. *J Ultrastruct Res* 1972 (9) Beeman et al., *Magn Reson Med* 2012