Counting Total Number of Kidney Glomeruli Using MRI

S. C. Beeman[1], M. Henriksen[1], D. Frakes[1], and K. M. Bennett[1]
[1]Bioengineering, Arizona State University, Tempe, Arizona, United States

Introduction: The goal of this work was to measure the total number of glomeruli in the kidney. The kidney glomerulus is a major functional unit of the nephron, directly filtering components of the blood plasma both by size and charge. Changes in size and number of glomeruli have been correlated with development of both glomerular sclerosis (1) and hypertension (2). It is clear that non-invasive measurement of glomerular size and number would serve as a significant diagnostic tool since current techniques require resection and destruction of the entire kidney (3). Electron microscopists have long labeled anionic proteins and proteoglycans of the glomerular basement membrane (GBM) with cationic probes (4). Recently, intravenous injections of the iron binding protein ferritin, modified with cationic amine groups (5), have been used to detect individual glomeruli both *in vivo* and *ex vivo* (6). Here we show that an accurate count of individual glomeruli may be measured through intravenous injection of CF under T_2*-weighted imaging in conjunction with adaptive image thresholding techniques.

Methods: Synthesis of Cationic Ferritin: Cationic Ferritin (CF) was synthesized by conjugating horse spleen ferritin (Sigma Aldrich, St Louis) to N, *N* Dimethyl-1,3-propanediamine (DMPA) using 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), according to Danon et.al. (5). In Vitro Preparation/Imaging: A male Sprague-Dawley rat was given 3 bolus doses of 1 mg/100g of synthesized CF with 1.5 hours between injections. The control rat was given no contrast. The rats were sacrificed, the kidneys resected, and the capsules removed. The left kidney was placed in a 2% glutaraldehyde, 0.1M cacodylate buffered solution for imaging. The right kidney was prepared for histological counting as described below. The left CF contrasted and control kidneys were imaged in a Gadolinium-doped PBS solution on a Bruker 7T scanner using a 3D GRE sequence with TE/TR = 20/30.58 ms and a resolution of 54x54x54 μm. Histology: The right CF labeled kidney was cut into 1 mm^3 pieces immediately after resection and incubated in 5 ml of 6 N HCl for 1.5 hours. At 1.5 hours, the incubated tissue was crushed and strained through a 21-gauge needle until homogenous. The solution was then brought up to 30 ml with deionized water. Glomeruli in 1 ml of the solution were counted in a counting chamber (1 mm^2 scored 35 mm culture dish, Nunclon delta). The total number of glomeruli was calculated based on the average of 5 counts and the area over which they were counted. Post-processing: Glomeruli were segmented from the 3D MRI data using custom software written in Matlab (The Mathworks, Inc.). This software incorporated both a dynamic percentage-based threshold and a dynamic standard deviation-based threshold. Voxels below 12% and 2.5 standard deviations of the average voxel intensity over a 22-voxel interrogation window were classified as glomerular. The number of voxels classified as glomerular were summed, and this total was divided by an average glomerular size of 4.98 voxels (based on an average glomerular volume of $0.785x10^{-6}$ μm^3 (7)), yielding total renal glomerular count.

Results and Conclusions: The cortex of the kidney was punctate with hypointense spots, consistent with the distribution of individual glomeruli (Fig 1, left). Such spots of hypointensity were not observed in the control kidney (Fig 1, right). Dark spots in the kidney are consistent with previous observations of accumulation of CF in the GBM. This has been supported with immunohistochemistry and electron microscopy in previous work (6). Adaptive image thresholding and analysis of 3D data yielded a total glomerular count of 43,362 glomeruli in the imaged kidney. This is consistent with and lies within 10% of the histological count of 39,514 glomeruli in the contralateral kidney of the same rat. This is also consistent with glomerular counts on data collected at 11.7T (not shown). It has been reported in the literature that the total number of glomeruli in one kidney is within 10% of the contralateral (3), further supporting our counting method. We conclude that cationic MRI-visible contrast agents may be used to measure the number of glomeruli in a kidney *ex vivo* without complete destruction of the organ. This work, along with previous *in vivo* measurements of the GBM using MRI (6), suggests that accurate glomerular counting may be accomplished *in vivo*. Accurate, noninvasive glomerular counting may eventually be useful in the clinic as an assessment of hypertension and renal disease.

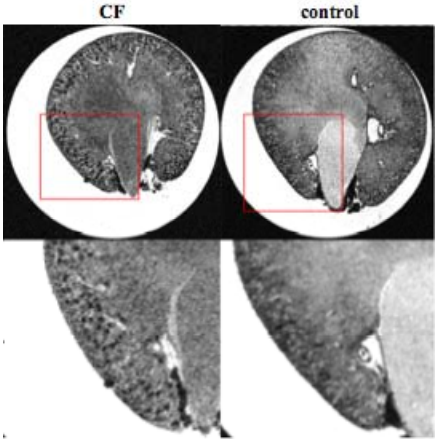

Figure 1: *Gradient echo MRI of fixed rat kidneys. Left shows the CF contrasted kidney and right shows the control. Hypointense spots are apparent throughout the cortex of the CF contrasted kidney, but not in the control.*

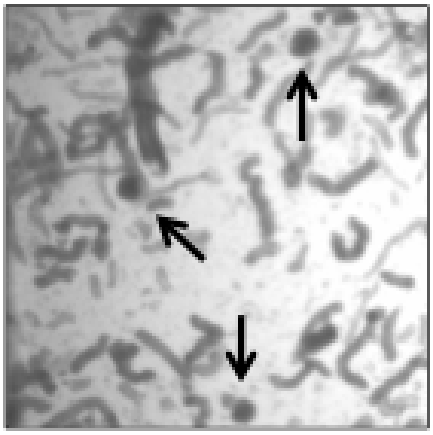

Figure 2: *4X light microscope image of glomeruli and tubules in solution in a 1 mm^2 scored counting chamber. The arrows indicate glomeruli for counting.*

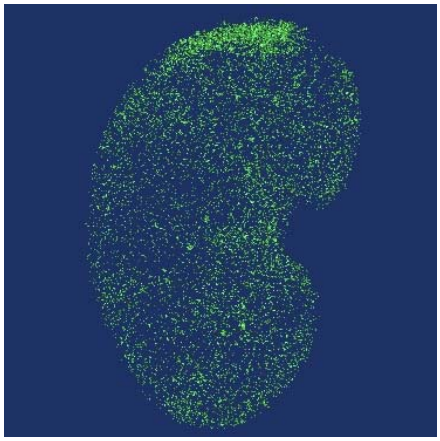

Figure 3: *Three-dimensional rendering of glomeruli segmented from MR imaging. Glomeruli are shown in green.*

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