Novel Uses of Ultrasound to Assess Kidney Mechanical Properties

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Abstract

Ultrasound is a key imaging tool for the evaluation of the kidney. Over the last two decades, methods to measure the mechanical properties of soft tissues have been developed and used in clinical practice, though the use in the kidney has not been as widespread as for other applications. The mechanical properties of the kidney are determined by the structure and composition of the renal parenchyma as well as the perfusion characteristics. As pathological processes change these factors, the mechanical properties change and can be used for diagnostic purposes as well as monitoring treatment or disease progression. Ultrasound-based elastography methods for evaluating the mechanical properties of the kidney use focused ultrasound beams to perturb the kidney and then high frame rate ultrasound methods are used to measure the resulting motion. The motion is analyzed to estimate the mechanical properties. This review will describe the principles of these methods and discuss several seminal studies related to characterizing the kidney. Additionally, an overview of the clinical use of elastography methods in native and kidney allografts will be provided. Perspectives on future developments and uses of elastography technology along with other complementary ultrasound imaging modalities will be provided.
Introduction

Palpation has been an essential tool medical practice for centuries because of its utility in detecting abnormalities within an organ using the sense of touch. However, not all organs are superficial nor is the sense of touch sensitive enough to detect subtle changes in the mechanical properties of the tissue under investigation. The native kidney and, in some cases, the transplanted kidney are not easy to evaluate with palpation due to their depth from the skin surface.

Different imaging modalities such as x-ray computed tomography, magnetic resonance imaging, and ultrasound can provide information about the morphology and vasculature of the kidney\(^1\)\(^-\)\(^3\). Ultrasound, due to its low cost and widespread availability, is commonly used for imaging of the kidney\(^1\). Laboratory-based renal function tests can provide a glimpse into kidney health\(^4\),\(^5\). However, microscopic changes in the kidney due to processes such as inflammation and fibrosis can cause loss of nephron function and thereby decrease the overall glomerular filtration rate (GFR)\(^6\)\(^-\)\(^9\). The remaining nephrons can compensate through hyperfiltration, but as disease progresses the availability of healthy nephrons may be insufficient to compensate and hyperfiltration can also lead to further nephron loss and eventual kidney failure\(^10\).

These same morphological changes can cause an alteration in the proportion of the tissue constituents such as the increased presence of inflammatory cells and edema in the interstitium or replacement of parenchyma with increased collagen content from global glomerulosclerosis and interstitial fibrosis with tubular atrophy. Further, nephrons can hypertrophy with both glomerulomegaly and tubular hypertrophy. With new proportions of tissue constituents and changes in the tissue organization and structure, the mechanical properties at the microscopic and macroscopic scale levels will change.

In addition to changes in tissue composition, the structure may be altered. The measurement of mechanical properties of the kidney may benefit from distinguishing nephrons with glomeruli in the superficial cortex from those in the deep cortex that extend their loop of Henle into the medulla. Indeed, the structural pathology with different processes may vary by kidney depth (e.g., glomerulosclerosis of aging occurs primarily in the superficial cortex)\(^9\). While current imaging techniques can distinguish cortex from medulla, they are insufficient to resolve
the components (glomeruli and tubular segments) of individual nephrons. Additionally, the macroscopic structure of the kidney, i.e., the nephrons and vessels, are organized in a radial fashion. As a result, we can consider the anisotropic mechanical properties when evaluating nephrons because the directionality will be important in interpreting the result.

Another important factor to consider is the perfusion of the kidney. The kidney receives 20-25% of the cardiac output\textsuperscript{11}, and the pressure in the kidney can affect the mechanical properties measured in the renal parenchyma. As a result, pathology of the parenchyma and the perfusion conditions will both contribute to the mechanical stiffness of the kidney, and results must be interpreted with this mind. With some pathological processes, this may make interpretation of stiffness difficult. For example, arteriosclerosis may decrease kidney stiffness from decreased perfusion, whereas the associated glomerulosclerosis, tubular atrophy, and interstitial fibrosis with arteriosclerosis may increase kidney stiffness.

Overview of Ultrasound-based Elastography Methods

As previously mentioned, different imaging modalities can provide morphological and functional (e.g., perfusion) information, but they do not provide quantitative information about the mechanical properties of soft tissues. Over the last two decades multiple elastography approaches for measurement of the mechanical properties of different organs have been developed and subsequently commercialized for use in clinical practice\textsuperscript{12-14}.

There are two commonalities among these different elastography approaches, which include applying stress to the tissue and measuring the response to that stress\textsuperscript{15}. The applied stress can be external mechanical actuation, external compression, an internally-applied acoustic radiation force (ARF), or endogenous motion such as motion due to the cardiac pressure pulse. In this paper, we will focus primarily on the excitation (applying stress) using a focused ultrasound beam to generate an ARF in the renal tissue\textsuperscript{16}. The motion from that applied stress can be measured using sensitive high frame rate ultrasound measurements which can detect micron-level displacements, and the mechanical properties can be assessed.
Ultrasound array transducers can focus the ultrasound “push” beam to specific locations within the imaging field-of-view\textsuperscript{17, 18}. To generate a force that will perturb the tissue to the level that the motion can be measured, long tonebursts are used, on the order of 100-1000 $\mu$s, resulting in hundreds to thousands of ultrasound cycles\textsuperscript{16}. As a comparison point, pulses used for B-mode imaging or Doppler measurements may be on the order of 1-10 cycles or bursts of only a few microseconds.

The push is applied and the transfer of momentum of the ultrasound waves to the absorbing tissue causes the tissue to be pushed in the direction of the ultrasound propagation\textsuperscript{17, 19}. When the pushing toneburst ceases, the tissue may still undergo motion in the direction of the ultrasound, but will eventually start to return to its equilibrium position. The motion within the push beam region-of-excitation (ROE) can be analyzed to evaluate the tissue including the level of peak displacement and other features of the time-dependent behavior of the response\textsuperscript{17}. Multiple ARF pushes can be used in time to interrogate the response, such as in one method called viscoelastic response (VisR) imaging\textsuperscript{20, 21}. These motion features are determined by the elastic or viscoelastic properties of the tissue. Figure 1 shows the intensity produced by a curvilinear transducer and measurements of motion inside and outside the ROE.

An elastic material will deform immediately due to an applied force. After the force ceases, the material will return back to its original position. In a viscoelastic material, the material’s response to a force will have some delay or time dependence\textsuperscript{22}. Additionally, when the force is taken away, the material will take some time to relax back to its original position. An everyday example is the behavior of a spring mattress and a memory foam mattress. A spring mattress will respond quickly to a depressing force and return to its original position almost immediately when the force is removed, while the memory foam mattress will take some time to deform and return to its original shape.

In many works related to characterizing the viscoelastic mechanical properties of soft tissues, various rheological models have been used and are modeled as different configurations of springs (elastic component) and dashpots (viscous component)\textsuperscript{23}. With respect to the example above, a spring mattress may be modeled using only spring components, and the memory foam mattress may be modeled using springs and dashpots arranged in series or
in parallel. These different rheological models allow for simplified explanations of mechanical behavior of tissues. Common models include the Kelvin-Voigt, Maxwell, and Generalized Maxwell models\textsuperscript{20, 24-31}. The most appropriate model may vary amongst tissues and the time scale used for fitting the measured behavior.

By virtue of the tissue being a mechanical continuum, when the tissue deforms and returns to its original position, it causes motion outside of the ROE and generates shear, or transverse, waves\textsuperscript{18, 19}. In this case, the particle displacement is parallel to the ultrasound propagation direction (z-direction, Fig. 1), but the wave propagates perpendicular to the direction of the ultrasound propagation. The shear wave velocity of the propagating wave is related to the elastic and viscoelastic properties of the tissue, by using specific equations, the shear or Young’s modulus can be estimated. Measurement of the shear wave velocity is encompassed by the general term of shear wave elastography (SWE). It should be noted that in the clinical implementations of SWE that the following relationships are used

$$E = 3\mu = 3\rho c^2$$  \hspace{1cm} (1)

where $E$ is the Young’s modulus, $\mu$ is the shear modulus, $\rho$ is the mass density assumed to be 1000 kg/m\textsuperscript{3}, and $c$ is the shear wave velocity (SWV). For this equation, the tissue is assumed to be linear, elastic, isotropic, homogeneous, and nearly incompressible (Poisson’s ratio, $\nu = 0.5$). To interpret between studies, Eq. (1) can be used to convert the measurements to consistent units\textsuperscript{32-35}. It should be noted that this conversion can be performed on individual data values, but for cut-off thresholds derived from receiver operating characteristic curve analysis, all values may need to be converted and the analysis performed again to obtain the proper threshold due to the nonlinear relationship in Eq. (1).

**Seminal Studies in Renal Elastography**

Acoustic radiation force-based methods have been used in many studies in the kidney to evaluate physiological and pathological states. We highlight a few early seminal studies in the kidney. Gennisson, et al., performed a fundamental study in a porcine model exploring the anisotropy of the \textit{in vivo} kidney as well as the
effects of renal artery flow, venous outflow, and urinary blockage on the mechanical properties\textsuperscript{36}. Shear wave measurements were made where the waves were propagating transverse to the renal structures in the middle of a longitudinal plane and parallel to the renal structures in one of the poles of the kidney as conceptualized in Fig. 2. The transverse, $\mu_{\perp}$, and parallel, $\mu_{\parallel}$, shear modulus changes as well as the anisotropic ratio, $AR = \mu_{\parallel}/\mu_{\perp}$, are listed in Table 1 for cases of the normal kidney, and when the renal vein or artery was occluded. The venous occlusion leads to a 4-5 fold increase in the modulus, whereas occlusion of the renal artery decreases the modulus. The anisotropic effects change markedly in the outer cortex and medulla with these variations in perfusion of the kidney.

Amador, et al., reported measurements in \textit{ex vivo} porcine kidneys to evaluate the role of anisotropy and other factors on the viscoelastic properties of the cortical tissue\textsuperscript{37}. The viscoelasticity was characterized using analysis of the shear wave motion in the frequency domain. The wave velocity at each frequency, phase velocity, is evaluated and the variation of the phase velocity, or dispersion, is fit to a parametric model involving springs and dashpots, which characterize the elastic and viscous components of the tissue, respectively. Using a Kelvin-Voigt rheological model, the mean shear elasticity (spring), $\mu_1$, and shear viscosity (dashpot), $\mu_2$, was 1.89 kPa and 1.61 Pa·s, averaged across four kidneys. It was found that the viscoelastic parameters were larger when the shear wave propagated parallel with the nephron structures compared to transversely (Fig. 2).

Amador, et al, explored an acute renal artery stenosis (RAS) model and evaluated the viscoelastic properties of the kidney as renal blood flow was reduced. As renal blood flow decreased, the shear elasticity and viscosity immediately decreased (100\% to 75\% flow) and then decreased slightly ($\mu_1$) or held constant ($\mu_2$)\textsuperscript{38}. Warner, et al., investigated porcine models of acute and chronic RAS with magnetic resonance elastography\textsuperscript{39}. In the acute RAS case, the modulus of the affected kidney decreased, while the contralateral kidney exhibited a higher modulus, and the mean arterial pressure and renal blood flow to the contralateral kidney increased in the pigs. In the chronic RAS study, the stenotic kidney had similar stiffness compared to control, but was lower than the contralateral kidney. Fibrosis was substantial in the stenotic kidney, but the authors concluded that renal
fibrosis may have been masked by the reduced perfusion of the stenotic kidney. Additional *ex vivo* and *in vivo* studies have addressed the aspects of increased and decreased renal internal pressure\(^{40-42}\).

**Clinical Studies with Renal Elastography**

Acoustic radiation force impulse (ARFI) imaging and VisR imaging utilize motion that is captured in the ROE for analysis. The ARFI method typically measures the peak displacement with an underlying assumption that stiffer tissue will deform less than softer tissue\(^ {17}\). The VisR method uses two ARF pushes applied in a precisely timed fashion at the same location to examine the time-dependent recovery of the tissue to evaluate viscoelastic properties of the tissue like the time constant (\(\tau = \mu_2/\mu_1\)) relative elasticity and viscosity, RE and RV, respectively\(^ {20, 21}\). The parameters were mapped in renal transplants and a region-of-interest (ROI) analysis was performed\(^ {13}\). The \(\tau\) ratios calculated from different ROIs could differentiate transplants with vascular disease, tubular/interstitial scarring, chronic allograft nephropathy and glomerulonephritis from control allografts.

Ultrasound SWE has been utilized in many different studies to evaluate different regions of the kidney for different diseases that affect the kidney. These evaluations are typically paired with measurements of kidney morphology (length, cortical thickness) from B-mode, Doppler measurements to determine resistive index. An example in a kidney allograft is shown in Fig. 3. We summarize some findings in native and transplant kidneys, though more thorough overviews may be found in the following review articles\(^ {44-49}\).

Native kidneys have been assessed in patients with chronic kidney disease (CKD) and diabetic kidney disease. In many of these studies, patients have been categorized using the estimate GFR (eGFR) measurements. There are conflicting findings with respect to the mechanical properties and the eGFR values. In some papers, the mechanical properties increased with progressive disease stages or decreased eGFR (i.e., negative correlation with eGFR)\(^ {50-56}\). However, other studies showed no correlation\(^ {57, 58}\) or positive correlation with eGFR\(^ {59, 60}\). The prevailing conclusion related to the negative correlation between mechanical properties and eGFR could be explained that more advanced CKD is associated with fibrosis, which can increase the stiffness of the
parenchymal tissue. The role of perfusion may also be a factor as the perfusion could be reduced with mild levels of fibrosis.

Asano, et al., studied patients with glomerulonephritis (N = 129), diabetic nephropathy (N = 107), and nephrosclerosis (N = 83). They found positive correlations between SWV and eGFR in each of the cohorts though the correlation coefficients ranged between r = 0.216-0.320 (p < 0.01). In the report by Leong, et al., 106 CKD patients were studied and compared with 203 control subjects. In this study, it was found the Young’s modulus increased in CKD patients compared to controls, having a moderate correlation coefficient of r = -0.576 (p < 0.0001).

More work has been reported in renal transplants and measurements are compared with Banff biopsy scores and renal function outcomes. Two early small studies compared SWV and Banff biopsy scores for measurements made in transplanted kidneys. The study by Syversveen, et al., examined 30 patients and did not find a significant correlation of SWV with fibrosis. Stock, et al., studied 18 patients and found positive correlations between SWV and level of fibrosis (Spearman correlation coefficient (SCC) = 0.47, p = 0.026). He, et al., reported higher values of SWV in dysfunctional allografts and a negative correlation between SWV and eGFR.

These various studies measuring elastic mechanical properties, either SWV or Young’s modulus, unfortunately demonstrate some inconsistencies and raise some questions related to how renal mechanical properties relate to renal structure and function in a comprehensive manner. Different studies found positive and no correlation with interstitial fibrosis. Several studies noted a negative correlation of SWV with eGFR. The dual roles of fibrosis and perfusion need to be studied in more detail to elucidate their contributions to the renal mechanical properties, for example studying kidneys with moderate or severe fibrosis compared to mild fibrosis.

Another aspect of using SWE in renal allografts is that applied transducer force can alter the SWV measurements because the allograft is placed at a shallow depth. In normal scanning, applying significant
pressure by the transducer should be avoided\textsuperscript{45}, but if used intentionally, it could yield information about the nonlinear mechanical properties of the renal tissue\textsuperscript{75}.

**Current Challenges for Ultrasound Elastography Measurements**

While ultrasound-based methods have been used in transplant and native kidneys, ARF-based methods are limited to depths from the skin surface to about 7 cm. At increasing depth, ultrasound waves will attenuate and the ARF will be weak. Secondly, the ultrasound motion detection becomes more difficult due to lower signal-to-noise ratio (SNR) and poorer spatial resolution. The ultrasound amplitude used for the push pulse is limited due to regulatory limits such as those mandated for diagnostic ultrasound by the United States Food and Drug Administration\textsuperscript{76}. These limits are meant to prevent tissue damage due to thermal and mechanical bioeffects of ultrasound. Efforts are being directed towards examining the safety under conditions related to using ultrasound with higher values of acoustic output\textsuperscript{77-80}.

Another challenge is the effects of anisotropy on measurements of renal mechanical properties. The shape of the push beam and the direction of the propagating shear waves with respect to the directionality of the nephrons and vessels can have an effect on the consistency of the measurements. The measured responses with ARF beams of different shapes can help to discern the anisotropy of the tissue\textsuperscript{81}. For SWE measurements, the effects of anisotropy can be accounted for by making measurements in a consistent location such as in the middle of the cortex (away from the poles) in a longitudinal plane.

Another limitation of renal elastography research studies is that, typically, the mechanical properties are the primary focus, but the individual contributions to these properties from nephron size, nephron number, interstitial inflammation and edema, fibrosis, and blood flow characteristics are not provided. Methods that can achieve higher spatial resolution and extract more details about the macroscopic and microscopic features will be important to understand the state of the kidney. For example, in recent years, ultrasound methods have progressed to the point that microvessels can be observed without any contrast agents (Fig. 4) and super-resolution imaging of microvessels on the scale of tens to hundreds of microns is possible with the use of ultrasound microbubble
contrast agents. The efficacy of identifying specific diseases and chronic kidney disease severity may depend on more holistic characterization of the kidney.

**Future Opportunities for Renal Elastography Investigation**

Considering the current renal elastography literature, there is not complete consensus on mechanical property trends with different conditions. Consistency among different studies still need to be established such that these elastographic tools could be used and trusted more widely by nephrologists. Validation of mechanical properties against specific microstructural findings on histology and prognostic utility beyond common clinical tests (eGFR and proteinuria) are also necessary steps for establishing clinical utility. Reconciling the results from ultrasound-based methods implemented by different vendors is an important step as well as further exploring the role of evaluating the viscoelastic properties of the renal tissue.

While renal elastography has been explored in porcine and rabbit models, it may be useful in other preclinical models involving rats and mice. Research ultrasound devices such as those produced by Verasonics, Inc. and Sonovol, Inc. may make this possible. Integration of renal elastography ROE or shear wave measurements could provide more information on disease progression and the effects on the mechanical properties. Figure 5 shows results from a SonoVol Vega system for measuring SWV in a mouse using a high frequency ultrasound transducer. Measurements that integrate measurements of morphology, blood flow, and the mechanical properties could be quite revelatory to understanding pathophysiology.

As with many diseases, it is imperative but challenging to understand how changes of the tissue composition and structure alter the properties observed at the macroscopic scale. This can be done both with improved abilities to conduct preclinical and human studies as well as to model wave propagation in complex tissues from the microscopic scale to observe the effects at the macroscopic scale. Advances in blood flow imaging may also provide information to interpret how perfusion of the kidney affects mechanical property measurements.
Current Outlook

Ultrasound-based elastography techniques have allowed clinicians and scientists to obtain new insight into pathophysiology of multiple organs. Renal elastography has similar potential, but the kidney presents unique challenges that require special care to interpret measurements in light of the multiple factors that affect the measured mechanical properties within the kidney.

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Table 1. Shear modulus measurements in pigs under different physiological conditions\textsuperscript{36}.

|                     | Transverse ($\mu \perp$), kPa | Parallel ($\mu ||$), kPa | Anisotropy Ratio ($\mu || / \mu \perp$) |
|---------------------|-------------------------------|--------------------------|----------------------------------------|
| **Normal**          |                               |                          |                                        |
| Outer Cortex        | 6.9 ± 1.4                     | 7.7 ± 2.3                | 1.12                                   |
| Inner Cortex        | 8.1 ± 1.9                     | 8.7 ± 2.5                | 1.07                                   |
| Medulla             | 6.6 ± 2.3                     | 7.7 ± 2.3                | 1.17                                   |
|                     |                               |                          |                                        |
| **Vein Occlusion**  |                               |                          |                                        |
| Outer Cortex        | 32.6 ± 10.7                   | 33.7 ± 20.6              | 1.03                                   |
| Inner Cortex        | 43.5 ± 15.1                   | 43.2 ± 16.8              | 0.99                                   |
| Medulla             | 36.6 ± 12.5                   | 47.9 ± 14.4              | 1.30                                   |
|                     |                               |                          |                                        |
| **Artery Occlusion**|                               |                          |                                        |
| Outer Cortex        | 3.4 ± 0.8                     | 4.6 ± 2.1                | 1.35                                   |
| Inner Cortex        | 4.6 ± 1.2                     | 5.1 ± 2.4                | 1.10                                   |
| Medulla             | 4.8 ± 1.2                     | 5.6 ± 2.9                | 1.17                                   |
**Figure Legends**

Figure 1. Uses of acoustic radiation force (ARF) to displace tissue and measurement in the region-of-excitation (ROE) with one acoustic radiation force impulse (ARFI) excitation or two ARF excitations for VisR. ARF excitations are shown by the orange rectangles. Shear waves are measured outside of the ROE at numerous locations starting at $x = 1.5$ mm and each curve is extracted in 2.0 mm steps. All data are from simulations described by methods in Palmeri, *et al* $^{28}$.

Figure 2. Direction of shear wave propagation with ARF applied in different locations demonstrated on porcine kidney cross-section. The shear waves propagate transverse to the tubules/vessels in the middle (yellow) of the longitudinal section. The shear waves propagate parallel to the tubules/vessels in the pole (white) of the longitudinal section.

Figure 3. Examples of B-mode, Doppler, and SWE images in a transplanted kidney in a 52 year old male patient five years post-transplant with a General Electric Logiq E9 scanner (General Electric Healthcare, Wauwatosa, WI). The SWE images shows the color-coded overlay for shear wave velocity. Data were acquired under a protocol approved by the Mayo Clinic Institutional Board with written informed consent.

Figure 4. Examples of B-mode image and microvasculature imaging in a native kidney in a healthy 31 year old male subject with a Verasonics Vantage research ultrasound scanner (Verasonics, Inc., Kirkland, WA). Vessels can be differentiated much better than those in the Doppler image in Fig. 3. Data were acquired under a protocol approved by the Mayo Clinic Institutional Board with written informed consent.

Figure 5. Long-axis view of kidney in a mouse using B-mode with overlaid SWV. B-Mode of a female C57BK6j mouse (5 mo) kidney is shown. The B-mode was acquired at 24 MHz and shear wave velocity overlay acquired with 17 MHz excitation and tracking pulses. Images acquired with the Vega robotic ultrasound system (SonoVol, Inc., Durham, NC, USA). Images courtesy of Dr. Michael F. Romero (Mayo Clinic) and Dr. Christopher Moore (SonoVol, Inc.). Data were acquired under a protocol approved by the Mayo Clinic Institutional Animal Care and Use Committee.
Figure 1

Region of Excitation (ROE)

ARFI

ViSR

Shear Waves (SW)
