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Application of Elastography for the Noninvasive Assessment of Biomechanics in Engineered Biomaterials and Tissues

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Abstract

The elastic properties of engineered biomaterials and tissues impact their post-implantation repair potential and structural integrity, and are critical to help regulate cell fate and gene expression. The measurement of properties (e.g., stiffness or shear modulus) can be attained using *elastography*, which exploits noninvasive imaging modalities to provide functional information of a material indicative of the regeneration state. In this review, we outline the current leading elastography methodologies available to characterize the properties of biomaterials and tissues suitable for repair and mechanobiology research. We describe methods utilizing magnetic resonance, ultrasound, and optical coherent elastography, highlighting their potential for longitudinal monitoring of implanted materials *in vivo*, in addition to spatiotemporal limits of each method for probing changes in cell-laden constructs. Micro-elastography methods now allow acquisitions at length scales approaching 5–100 μm in two and three dimensions. Many of the methods introduced in this review are therefore capable of longitudinal monitoring in biomaterials and tissues approaching the cellular scale. However, critical factors such as anisotropy, heterogeneity and viscoelasticity—inherent in many soft tissues—are often not fully described and therefore require further advancements and future developments.

Keywords

Magnetic resonance imaging (MRI); Ultrasound; Optical coherence microscopy; Tissue engineering and regenerative medicine; Elasticity; Young's modulus

INTRODUCTION

The stiffness of a tissue, or its ability to resist deformation when subjected to an applied force, is often indicative of the pathologic, regenerative or aging state in most organs in the body, including heart,¹³⁷ liver,¹⁶⁰ and brain.⁹³ A softer tissue will deform (or undergo strain) more compared to a stiff tissue for the same applied force (or stress). Tissue stiffness is largely defined by chemistry and associated micro–macro structure of the extracellular matrix (ECM), which is critical in the regulation of cell phenotype, gene expression, and differentiation,^{46,55,64,127,142} and abnormal stiffening of ECM associates with fibrosis and

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cancer.^{37,45} Therefore, the ability to spatiotemporally monitor ECM stiffness at the cellular level may allow early diagnosis of degenerating tissue, but also assist in fabrication and *in situ* monitoring of engineered constructs post implantation.

Elastography is a specialized imaging-based method available to spatially map strain fields and elastic properties, including stiffness or shear modulus, in materials. Elastography infers properties based on the known application of a force, *via* pushing on a material or inducing a mechanical wave, in addition to the one-, two-, or three-dimensional (1D, 2D, or 3D, respectively) measurement of the resulting deformation within the material. Elastography is usually classified by image modality (e.g., magnetic resonance imaging (MRI) vs. ultrasound) and excitation type (e.g., static vs. dynamic). The imaging modality and method of excitation are both important (Figs. 1, 2), and together they help to determine the spatial resolution and signal-to-noise ratio (SNR) of the method.

The primary objective of this review is to provide a concise overview of leading elastography methods applied to study the biomechanics of engineered biomaterials and tissues. The emergence of tissue engineering and regenerative medicine prompted our interest to describe applications of elastography in these fields, considering the likelihood that: (a) knowledge of mechanical properties for donor tissues into native recipient tissues requires close matching to ensure integration and long term survival, (b) design of biomaterial scaffolds requires careful assessment of functional responses to spatial patterning of structures tailored to mimic native tissues, and (c) promotion of guided cellular behavior requires noninvasive study of cell–matrix interactions in long-term culture conditions. Consequently, we placed special emphasis on investigating the spatiotemporal limits of elastography methods to noninvasively assess cell-laden constructs alone, as well as post-implantation constructs in small animals and humans. We also explored the possibility of elastography to carefully probe longitudinal changes in elastic properties *in vivo* as functional indicators of repair success.

The literature search involved finding studies that employed both experimental and clinical imaging modalities (e.g., ultrasound, MR, optical, laser) to quantify stiffness or deformation (e.g., elastic modulus, Young's modulus, shear modulus, strain, stiffness, displacement) in biomaterials or native tissues (hydrogel, biopolymer, scaffold, collagen, tissue, human, animal) within the PubMed and World of Science databases. Studies which employed only rheology, atomic force microscopy (AFM) and nanoindentation as the main testing method were excluded. While we sought to limit our search to only peer-reviewed articles, because some of most recent and innovative techniques were available only as conference proceedings, a small number of conference articles were included in this review as well. Due to space limit, Brillouin microscopy,¹³⁵ an exciting novel non-contact optical method that uses spectral shifts of scattered light to measure stiffness and viscosity, was not included in the current review.

THE VALUE OF ELASTOGRAPHY IN BIOMATERIALS AND TISSUES

The stiffness of biomaterials and tissues have been traditionally attained by standard mechanical testing, often involving the measurement of mechanical force when a

displacement or deformation is applied in tension or compression.^{2,60,72} These methods often only provide bulk properties that do not reveal underlying heterogeneous spatial distributions that are intrinsic to biological and replacement biomaterials. Various related methods to measure stiffness across length scales include indentation,^{84,120} rheometry,^{62,73} and AFM.^{19,36,56} The material characterization *via* these methods is well established, but unsuitable for longitudinal monitoring of biomaterials and tissue constructs because the testing methods are invasive and usually performed *ex situ*.

Elastography is a powerful noninvasive mechano-imaging technique that can provide spatial (pixel-by-pixel) maps of mechanical parameters and behavior throughout a material, including strain, elastic and shear moduli, or viscoelasticity.^{63,163} Because numerous diseases are linked to changes in mechanical properties, such as osteoarthritis,^{52,53,99,100} heart disease,^{9,10} and cancer,^{35,89,148,155} probing changes in stiffness and related parameters by elastography may enable diagnosis of early pathogenesis. The high sensitivity of elastography further allows the use of micro-to nano-scale excitation that is nondestructive and therefore causes minimal influence to both structure and resident cells in engineered constructs. Furthermore, elastography has the ability to provide a high resolution 2D or 3D mapping of mechano-functional capacity of the material which can be used to deduce spatial heterogeneity^{51,134} and anisotropy,^{103,129} which are not accessible through traditional mechanical testing methods.

While initially developed as a clinical modality, modern elastography methods now acquire data at spatial resolutions approaching 5–100 μm , allowing monitoring of material and ECM noninvasively at near cellular scales (Fig. 1). Currently, imaging systems employed in micro-elastography are based on both clinical and laboratory imaging modalities, including MRI (1.5 Tesla (T) to 14T),^{57,80,96,98,115,161} ultrasound (6–50 MHz)^{69,87,104,123,138,164} and OCT^{33,54,71,75,102,151} (Table 1).

MR BASED ELASTOGRAPHY

MRI is a noninvasive imaging modality capable of high spatial resolution (e.g., approaching 50 μm or better on dedicated research systems) with excellent contrast between neighboring soft tissues (e.g., bright cartilage adjacent to dark cortical bone) visualized in standard anatomical images. Due to the precise control of radiofrequency pulses and magnetic field gradients required for image acquisitions, MRI is further capable of defining oblique imaging planes to depict deep tissue structures. Furthermore, flexibility and continual development of custom pulse sequences enable direct measurement of physical parameters like displacement and shear wave velocity, relevant to elastography-based stiffness calculations (Table 2).

MR Elastography (MRE)

MRE exploits wave propagation through cyclic perturbation, allowing for a quantitative measurement of soft tissue mechanics at the micron level. Perturbations are provided through mechanical actuation, and coupled to MRI actions to capture the propagation of the shear waves through the material of interest. In a simplest sense, the speed of the shear wave propagation (c_s) through the material can be used to derive spatial shear modulus (G) and

density (ρ) of the material (Figs. 3, 4)^{94,117} by the relationship $c_s = (G/\rho)$. MRE was first demonstrated in 1995,⁹⁴ and it has since expanded to clinical applications at 1.5 T or 3T,^{25,26,80,83} and specialized applications at 9.4–11.7T.^{39,115,116,156,161} Traditionally, MRE employs either a plate or rod that vibrates transversely⁸³ or in torsion⁸² on the sample surface, and the use of a needle probe has also been examined at a lower excitation frequency (Fig. 2, Table 1).²⁶

MRE has evaluated biomaterials and engineered tissues, including the examination of *in vitro* and *in vivo* remodeling of human and mouse stem cells (hMSC, mMSC respectively) and chondrocytes in their respective matrices (e.g., agarose, collagen).^{39,115–117,156,161} A series of *in vitro* longitudinal studies were reported using a laboratory MRI system (11.7T) to map the spatiotemporal distribution of stiffness in tissue engineered constructs seeded with hMSCs and chondrocytes.^{116,117} The engineered scaffold with hMSC was simulated to induce either adipogenesis or osteogenesis, and elastography was performed weekly for up to 4 weeks using a rod actuator excitation (550, 580 Hz).^{116–118} High spatial resolution images (34 μm) were able to confirm the formation of islands of newly formed adipose and osteogenic tissues in the construct, but modulus elastograms were not reported. In another study,³⁹ a gelatin construct seeded with hMCS was stimulated to induce adipogenesis and osteogenesis. The study, while sharing many aspects of the experimental setup with previous studies,^{116–118} used a different inversion algorithm (termed algebraic inversion of the differential equation, or AIDE),¹¹⁰ and successfully obtained 2D shear modulus elastograms. The key difference seems to be a sample-specific tuning of excitation frequency to maximize the shear wave amplitude for each sample. This suggests that sample-specific frequency tuning is a critical factor in obtaining optimal shear wave generation. This additional step, however, may require extra time and could result in greater sample-to-sample variability.

The importance of the specific excitation method is increasingly being recognized for MRE. Recently, a novel concentric shear wave excitation method^{158,159} was used to study chondrocytes suspended in scaffolds,¹⁶¹ taking advantage of higher excitation frequency (5000 vs. 580 Hz^{116–118}) and generating circular shear waves with uniform amplitude across the cross sectional area of the sample (Fig. 4). The concentric shear waves were generated by axially vibrating the test tube containing a suspended scaffold, and an increase in construct stiffness (6.4–16.4 kPa) was observed over 3 weeks. However, spatial patterns of moduli were difficult to interpret, in part because of the small sample size (of 2–3 mm).

dualMRI

dualMRI (displacements under applied loading by MRI)^{27,101} is a 2D or 3D method that focuses on the measurement of displacement and strain obtained from stimulated echoes and phase-encoded data.⁷ Through the measurement of phase contrast, rather than magnitude-based tissue texture¹⁰⁷ or MRI tag lines,⁹⁸ dualMRI measures phase-related displacement at each pixel within the imaging volume while avoiding extrapolation from the region of interest. For dualMRI, phase contrast is obtained between a biomaterial or tissue in an undeformed (reference) and a deformed (current) state, which is typically applied *via* exogenous loading and computer-controlled pneumatic actuation.⁹⁷ By subtraction of the

phase data between the reference and deformed states, displacement and strain values can be calculated with precision on the order of 11 μm and 0.1%, respectively.²⁷

dualMRI has been applied *in vitro* and *in vivo* using clinical (3T) and laboratory MRI systems (7–14.1T) using various readout sequences, including fast spin echo (FSE),¹⁰¹ steady-state free precession²⁷ and single-shot fast spin-echo.²³ The system has been used to investigate strain behavior of explanted human and bovine cartilage,^{52,53,101} cartilage defects,⁹⁶ intact joints^{24,28,29} and intervertebral discs.^{22,24,51,68}

The value of acquiring strain at high spatial resolution (e.g., $100 \times 100 \mu\text{m}^2$) was demonstrated in a cartilage defect repair model. In this model, chondrocytes cultured in an agarose suspension for 35 days were inserted into a native osteochondral explant,⁹⁶ modelled after an allograft surgery. The combined specimen was compressed uniaxially, and dualMRI demonstrated increased strains in the soft agarose compared to surrounding cartilage. This demonstrated the potential of dualMRI to study stiffness mismatch under physiological loading conditions.

Depth-dependent strain distributions were further explored in a subsequent study using a bilayer agarose gel construct with various densities (of 2 and 4%) (Fig. 5).⁵¹ The study aimed to describe how strain transfers in layered constructs with different stiffness, an important aspect in designing an engineered tissue construct for, and understanding the developmental process of, articular cartilage. Strain was consistently transferred to softer (2%) agarose gel layers, suggesting that chondrocytes in soft transplanted gels would experience larger strains compared to native tissues, which may be beneficial or detrimental to the healing response *in vivo*.

dualMRI in its current form does not provide absolute stiffness, or specific measures for Young's or shear moduli. A successful conversion from strain data to stiffness or stress¹⁶ requires finite element and optimization-based inverse modeling, which has been reported with some initial success.¹⁷ dualMRI acquires displacement and strain distributions directly in 2D or 3D, and so there is potential to describe stiffness heterogeneity and anisotropy with an appropriate choice of constitutive model. In contrast, MRE spatial resolution in the axis of the wave propagation is higher than that of the lateral axis (up to three times^{80,81}), and therefore measurement of anisotropy can be complex. Furthermore, due to the inherent high attenuation of shear waves in rigid materials, highly heterogeneous samples with a large stiffness mismatch may suffer artifacts.^{86,156}

ULTRASOUND ELASTOGRAPHY

In ultrasound elastography (UE), during imaging the material undergoes deformation with quasi-static compression or ultrasonic radiation force (dynamic UE; Fig. 2). In quasi-static UE, US signals are acquired using conventional B-mode ultrasound imaging, both before and after application of a small, applied quasi-static compression ($\sim 1\%$ strain), typically by loading the ultrasound transducer into the underlying tissue.¹⁴⁶ These compressive axial tissue displacements are then compared using cross-correlation (i.e., speckle tracking) to map strain throughout the 2D imaging plane to form an image of the strain tensor.^{112,141,147} In contrast to quasi-static UE, and similar to MRE, transient UE is based on imaging shear

waves, in which the propagation speed increases with tissue stiffness.⁴⁰ While UE techniques are available as clinical and as emerging methods,^{5,40,124,125,154} only a handful have been applied to the microscale characterization of tissue and cell-laden constructs. However, the newer ultrasound imaging units to perform elastography are becoming increasingly economical and simple to use, making UE increasingly attractive for tissue engineering *in vivo* and *in vitro*.

Quasi-static UE

While many groups have sought to adapt UE to produce quantitative outcomes (e.g., modulus), quasi-static UE remains a largely qualitative tool (see review by Varghese¹⁴⁶). The strain tensor distribution is dependent on the applied deformation (or stress distribution), and so cannot explicitly be related to a tissue's absolute elastic properties.^{132,153} Stresses applied to the tissue by the ultrasound transducer, and the resulting stress distribution within the tissue, are difficult or impossible to quantify. Thus, including materials of known properties within the 2D image plane, such as a reference material adjacent to the ultrasound transducer, have shown promise to improve the determination of stiffness and modulus.^{15,58,157} For example, under the equal stress assumption, both a reference material and the tissue would deform according to their modulus; by using the strain ratios from both materials, the Young's modulus may be calculated for the tissue. However, factors including strain dissipation underneath the ultrasound transducer, varying geometry,^{15,113,126} the potential distortion from speckle decorrelation in regions subjected to high strains,^{13,15,30} poorly defined physical boundary conditions *in vivo* (where most soft tissues are bounded by other slippery and deformable soft tissues), and the heterogeneity and hierarchy inherent in biological tissues prevents exact quantification of tissue properties. The physics underlying ultrasound of layered or non-homogeneous material volumes (including reference materials and/or tissues) is also complex. Moreover, many of these same complicating factors that prevent accurate calculation of properties also complicate UE strain assessment. A notable example exists where UE strain maps, encompassing deep image fields clearly show strain dissipation under a rounded transvaginal ultrasound transducer probe in a homogeneous tissue phantom.^{15,58} This strain profile largely matches that predicted by conventional contact mechanics and does not represent material properties, yet this phenomenon is easily misinterpreted as increasing stiffness, or modulus, with increasing distance from the transducer probe. Thus, similar to dualMRI, knowledge of the stress distribution within the tissue (or accurate determination of the boundary conditions) is critical to solve for the modulus using inverse methods (e.g., finite element models or iterative methods).¹⁴⁶ Quasi-static UE therefore provides only reasonable estimations of mechanical and material properties, but not absolute property measurements.^{15,58,157} Despite these challenges, UE has proved to be versatile and of reasonably high resolution for evaluation of strains in both tissues^{12,14,50,88,113,114,140,141} and biomaterials for tissue engineering.^{1,69,143,162}

High frequency (up to 60 MHz) transducers may be used to increase spatial resolution and provide reasonable strain measures at distances close to the ultrasound transducer.^{15,112,126} While the use of these transducers significantly decreases imaging penetration depth and inevitably restricts the examinable sample size to several millimeters, this feature may prove

beneficial for high-resolution evaluation of strain profiles within tissue-engineered constructs.

Early studies of UE for evaluating tissue engineering applications involved temporally changing the elasticity of a bilayer scaffold containing smooth muscle cells (SMC) on the top and a non-cellularized region in the bottom.¹ Following *in vivo* culture in rats, the scaffold was examined using a 50 MHz UE elastography. At the resulting 75 μm resolution, the denser cellularized region of the scaffold previously seeded with SMC showed a higher bulk strain (+4%) compared to that of lower non-cellularized region. These results suggested that the cellularized region possessed a lower stiffness. Moreover, the efficacy of quasi-static UE for imaging cell-seeded scaffolds was demonstrated. UE has subsequently been used to evaluate longitudinal changes in bio-degradable scaffolds implanted subcutaneously in live mice.⁶⁹ 2D strain patterns between day-0 and day-7 were observed: the 7-day scaffold showed a seven-fold increase in strain. The study, however, was limited to imaging within a 2D plane. Also, because a relatively low frequency (5 MHz) transducer was used, the resulting spatial resolution was only 250 μm . Yet these results also demonstrate the capability of UE as a tool for evaluation of ECM changes. More recently, a higher frequency (32 MHz) transducer enabled 40 μm resolution imaging during *in vivo* mouse studies,^{143,162} where extending the monitoring period to 12 weeks produced strain maps that were sensitive to scaffold degradation. However, a clear temporal trend was not established owing to the high amount of scaffold degradation and unpredictable recellularization. While these *in vivo* studies are limited in number, they serve to demonstrate the potential and clear technical challenges of micro-scale quasi-static UE for evaluating materials in tissue engineering.

The versatility of quasi-static UE has enabled many groups to evaluate a wide range of normal and pathological tissues for strain distribution, or to estimate Young's modulus, which may range over several orders of magnitude.¹⁴⁶ Quasi-static UE has been used to characterize tissues *in vivo* including skeletal muscle,^{12,21,34,157} breast tissue and tumors (where tumors are stiff inclusions in a softer background),^{21,50,140,141} tendon,^{32,43,92} and the porcine cornea,⁵⁹ as well as longitudinal strain monitoring in a cell-seeded collagen substrate⁸⁸ and in vascular scaffolds⁴⁴ *in vitro*. A 3D UE strain elastogram obtained from *in vitro* porcine cornea using a similar setup to Cohn's "elasticity microscopy"¹ revealed different strain patterns associated with axially heterogeneous micro-anatomical layers, but the elastogram was affected by a high level of noise. While the elastogram achieved high resolution ($23.9 \times 46.6 \mu\text{m}$), the scanning time was long at 23 min (vs. 1 min in other static UE studies) resulting from numerous cycles of loading/unloading that were required to map a 3D volume of the tissue.

One interesting application of quasi-static UE has applied non-contact mechanical excitation by pulsing water pressure through a cell-seeded tubular arterial scaffold in a bioreactor.⁷⁴ A different study employed a water jet to deform the surface of the sample.¹⁴⁹ In both cases, ultrasound transducers captured the movement within the sample to characterize 2D strain maps and, in the arterial scaffold a calculation of Young's modulus. These novel techniques enable UE to be performed on samples contained within a bioreactor and utilize natural, dynamic movements of the samples to produce the quasi-static deformation needed for elastography. In other *in vitro* and *in vivo* experiments, the undeformed and deformed states

are directed by subtle movements of the transducer or through respiration.¹¹¹ In a bioreactor setup, the use of a completely sealed system and controlled deformations (with a static ultrasound transducer placement) enables strain monitoring for extended durations. These experiments are promising despite moderate spatial resolution (100 μm) and the increasing speckle tracking error in regions furthest from the transducer.

While quasi-static UE has attractive features, research efforts have redirected towards alternative methods including acoustic radiation force (ARF)-based dynamic UE, such as ARF impulse (ARFI),¹⁰⁶ shear wave elastic imaging (SWEI),¹³³ and SSI (supersonic shear imaging).¹¹ Nevertheless, quasi-static UE remains attractive due in large part to the low cost of purchasing or building an ultrasound system, that can be made to perform UE by using a specialized transducer and adding appropriate software, as well as their ubiquitous presence in the clinical setting.

Acoustic Radiation Force UE

In the external static load methods described above, the ultrasound transducer is used only for imaging tissue displacement and strain, but it also can be used to induce a non-contact force. ARF is an ultrasound-based dissipative excitation method where a high-intensity acoustic pulse is used to remotely push a small region in the material.¹⁰⁵ The applied force is proportional to the acoustic intensity, and thus beam forming can be used to focus and steer the affected region. It thus offers a highly specific non-contact excitation, which can be manipulated to provide a wide variety of excitations: single- or multiple-push, as well as arranged in an array to provide specific types of deformation and even shear waves.⁴⁰

UE using ARF can be grouped as either static or elastic wave methods. The static loading method is achieved by using an ARFI to excite a focal region in the tissue, and B-mode images are captured in the vicinity during and after the excitation. Cross correlation speckle tracking is then used to map the displacement (i.e., ARFI imaging).^{88,90} Alternatively, the perturbation caused by ARF can generate elastic shear or surface (Lamb) waves from the recoil of the deformed region.¹²² The shear waves propagate much more slowly than longitudinal waves, and thus the displacement can be imaged with many frames as it traverses the field of view. By measuring both particle displacement and acceleration (i.e., the shear wave speed), the shear modulus (and thus Young's modulus) can be mapped by inverting the wave equation at each pixel location. When a single 'push' is used to generate a shear or surface wave, it is termed SWEI¹³³; whereas when there are multiple 'pushes' in a series with small offsets, then it is called supersonic shear imaging (SSI¹¹). SSI involves moving the focus of the radiation force zone at a velocity which is faster than the velocity of the shear wave, thus creating a Mach cone in a manner similar to a hypersonic aircraft. While there is a much wider variety of elastic wave imaging techniques based on ARF excitation (see Doherty *et al.*⁴⁰ for the review of ARF excitation methods), currently only SWEI¹³³ and SSI¹¹ have been employed for tissue engineering applications.

Static ARF UE

ARFI has been applied in a limited number of studies for examining stiffness changes in porcine and rabbit cornea following crosslinking treatments,^{138,145} as well as the *ex vivo*

human cornea.⁹⁰ However, static ARF UE methods tend to take long time (5–10 min) because the ARF focus must be steered point-by-point over the entire region of interest. Accompanying the ARF is dissipation of the acoustic wave's kinetic energy into heat, which may result in local temperature spikes and thermal safety concerns. For each ARF pulse generated, an increase of up to 0.15 °C may occur in the local tissue,¹⁰⁶ and the thermal expansion for this temperature rise can be considered negligible.¹²¹ On the other hand, a high resolution ARFI UE may involve many pulses that induce significant local heating in the tissue, which in turn may affect the normal cellular activities.

A dual confocal transducer setup was used to measure the crosslinking treatment of porcine cornea, where the outer transducer (10 MHz) was used to generate ARF pulses, while the inner transducer (50 MHz) was to use image deformation.¹³⁸ In this setup, a 2D strain maps of $177 \times 153 \mu\text{m}^2$ resolution with a field of view (FOV) of approximately $2 \times 4 \text{ mm}^2$ was achieved under 7 min (Fig. 6). To our knowledge, this work has achieved the most detailed 2D strain map using static ARF UE to date.

In an alternative approach, Mikula *et al.*⁹⁰ added a low-power laser unit opposite to the dual confocal transducer, which temporarily generated microbubbles within the matrix through a transparent window, thus enhancing the scattering properties of the matrix to ultrasound B-mode imaging. While preliminary, gelatin embedded human corneas were examined to demonstrate the technology and measure the Young's modulus. The method involves novel use of cavitation in UE, but the laser power is currently four times higher than the FDA regulation limit for clinical application.

ARFI UE also has been used to detect changes in stiffness from crosslinking treatments in cornea of live rabbits monitored over 4 weeks.¹⁴⁵ In this study, the right eye was treated while the other side served as an untreated control. 1D M-mode (motion mode) displacement was tracked in real time as an ARF beam was applied in the central region of the cornea. A significant difference in relative stiffness was found between the treated and control corneas. While the study was limited to 1D displacement, it marks a promising tool that has potential for noninvasive tissue engineering and *in vivo* application with high temporal resolution.

Dynamic ARF UE

Mauldin *et al.*⁸⁷ were the first to use ARF to obtain bulk Young's modulus of porcine muscle by use of SWEI,¹³³ an ARF-based method that measures a shear wave generated from the recoil following ARF deactivation. However, the resolution of the elastograms was relatively low (0.5–1 mm). Nevertheless, this novel approach could provide a valuable tool for investigating the stress relaxation behavior of tissue.

Both elasticity and viscosity of the cornea were explored by employing SWEI.¹⁶⁴ Instead of using shear waves,^{103,104} the dispersion of vibration in the cornea was modeled as a surface wave propagating as a Lamb wave on a viscoelastic substrate. This approach is applicable to characterization of biomaterial substrates with thickness of less than 1 mm. The propagation speed of the Lamb surface wave was measured by analyzing A-line scans performed at 2 kHz before and after crosslinking treatment of the porcine cornea *in vivo*. Shear elasticity

and viscosity were calculated based on the Voigt model,²⁰ resulting in the first successful viscosity measurement *in vivo*. Unfortunately, the method does not account for the effect of the complex geometry on the surface wave propagation.

While SWEI is simpler to implement, SSI has an advantage of improving temporal and spatial resolution, image contrast and SNR by compounding multiple shear waves of different Mach number (pushing in different directions) and coherent compounding of multiple plane waves (imaging views).^{11,91} One simply increases the relative speed of the focus with respect to the shear wave speed to generate Mach cones of different angles. The rapid frame rate (10,000 fps) of plane-wave imaging allows multiple imaging views (coherent compounding) of the shear wave as it propagates through the tissue. When imaging outside the Mach cone, the forcing function can be ignored, and one can simply invert the wave equation to generate a shear elastogram. Owing to this advantage of ultrafast shear wave imaging, SSI has been adapted in both thin (cornea)¹⁰⁴ and thick tissue (mouse)¹²³ characterizations.

SSI was used to excite and image wave propagation in the porcine cornea to measure changes in stiffness from crosslinking treatments *in vivo* (Fig. 7).¹⁰⁴ An “ultra-fast” plane wave imaging probe captured a series of images to track surface waves propagating along the corneal surface. Another novel aspect of this study was that the probe was rotated 360° to provide an *en face* elastogram (*in vitro*), which revealed the 3D heterogeneous distribution of stiffness in the tissue. However, owing to limitations with the ultra-high speed imaging acquisition, resolution was relatively low (200 μm), and because wave propagation in the thin membrane of the cornea (0.5 mm) was complex to model, the velocity of shear wave was reported rather than the absolute modulus.

Kim *et al.*¹²³ were first to use SSI to measure longitudinal stiffness changes in a scaffold subcutaneously embedded in live mice—a significant advance from previous free-hand compression studies.^{69,143,162} They again monitored degradation of polymer-based scaffolds ($10 \times 10 \times 1 \text{ mm}^3$) subcutaneously embedded in the abdomens of 36 rats for 12 weeks. The shear waves generated by SSI were able to create clear planar wave patterns with a column height of approximately 40 mm, and the resulting measurement of shear wave velocity was used to generate a 2D shear modulus elastogram. At 4-week intervals, partially degraded scaffolds were excised from the rats and mechanically tested. The study demonstrated that there is a linear correlation between the externally derived Young’s modulus and the *in vivo* SSI derived shear modulus. Currently this study stands as the only long-term dynamic UE *in vivo* study.

UE is a simple and effective elastography technique where many of the novel excitation methods have been developed and translated to other elastographic modalities (e.g., translation of ARFI, SSI, SWEI to optical coherence elastography (OCE)). While only ARFI, SWEI and SSI have been discussed, imaginative combinations of pulse generation techniques ultimately may provide further advancement of current tissue engineering-oriented UE. In particular, ARF UE offers non-contact remote-controlled tissue forcing and rapid acquisition of elasticity and even viscoelastic properties of tissue,¹⁶⁴ the latter of which is currently not easily achievable by other types of elastography techniques.

At the present time, the image quality, including resolution, is subpar compared to that of MRE, dualMRI or OCE. However, with a number of successful studies—high resolution strain elastograms,¹³⁸ viscoelasticity,^{88,164} *in vivo* measurements¹⁰⁴ and 2D stiffness elastograms *in vivo*¹²³—have come to a point that any of these techniques can be applied to monitoring of stiffness in biomaterials and tissue engineering constructs.

OPTICAL COHERENCE ELASTOGRAPHY

Optical coherence elastography,¹³⁶ is an emerging elastography technique that uses optical coherence tomography (OCT) as the main imaging modality. The excitation principles of OCE are similar to those used in UE—either static or dynamic—which can further be defined as contact or non-contact. OCE enables image acquisition at high spatial resolution (5–15 μm) and SNR,^{47,61} allowing displacement measurements approaching 10 nm,¹⁰⁸ although the imaging depth (2–3 mm) is limited.⁴⁹ OCE has been applied *in vivo* to characterize human skin,^{3,66} human cornea,^{8,41} and mouse cornea,^{77,85} and *ex vivo* to characterize cell-laden collagen matrix,^{33,71} breast tissues,^{4,67,79} arteries,^{128,130,131} blood clot,¹⁰⁹ liver,⁶ muscle,⁶ cornea,^{75,144,150,151} and cardiac tumor.¹⁵²

Static Displacement OCE

Displacement induced from uniaxial compression by a glass platen⁷¹ or a short deformation from a small volume (puff) of air^{8,41,78} can be used to produce deformation in the tissue. Traditionally, 2D strain is derived from displacement calculated from two consecutive images before and after compression. While the strain map is acquired at a high spatial resolution (3.4–11 μm), limitations include the inability to directly access Young's modulus due to an unknown stress distribution, and the potential high margin of error in strain analysis using speckle tracking (*via* cross correlation). Nevertheless, static compression based OCE has been employed to monitor fibroblast cellularization and the associated stiffness change in collagen matrix.⁷¹ The 2D strain matched the degree of cell proliferation and matrix deposition obtained from corresponding histological images. Interestingly, owing to low optical scattering of the matrix prior to cell proliferation, polystyrene microspheres were required in the co-culture; no displacement data could be obtained in matrix regions where microspheres were absent.

A translational application of OCE has been developed for the *in vivo* study of thin tissues such as cornea.^{8,41,78} Combined with fast scanning mode (at 100,000 fps), the local deformation of the cornea can be established. The advantage of this approach is the potential to monitor treatment in the context of mechanics, in this case through the application of Alphagan (brimonidine tartrate 0.2%, Allergan), which is used for ocular hypertension to reduce intraocular pressure in the eye. The study found four out of five subjects showed a measurable decrease in cornea deflection amplitude after the Alphagan treatment, indicating the possible clinical application of air puff OCE to characterize eye health. These studies have been extended to aging studies,⁷⁸ as well as the extension of time-displacement data to viscoelastic material descriptions, including the use of homogeneous isotropic Kelvin- Voigt materials,¹¹⁹ to reveal a strong positive correlation between the rate of relaxation and age.

Dynamic OCE

Dynamic OCE measures either wave amplitude or speed generated by various dynamic excitation methods. The amplitude of the wave is used to generate a 2D strain map, with higher stiffness regions corresponding to smaller strain than those from the regions of lower stiffness (assuming incompressibility and isotropy). The wave speed in soft tissue typically ranges from 1 to 10 m/s,⁴⁸ and fast scanning mode in OCT can track wave propagation to estimate velocity (Fig. 8). The application of dynamic OCE to engineered biomaterials and tissues has so far been limited, although OCE has been demonstrated to measure 2D⁶⁵ and 3D strain⁶⁶ maps *in vivo* (Fig. 9), and additionally in age-associated stiffening of cornea. Interestingly, instead of a mechanical actuator, ultrasound ARF has also been used to induced vibrations in chicken tendon embedded in collagenase and encased in an agarose gel, where a gradual decrease in stiffness from the degradation process could be monitored (over 90 min).⁵⁴ There is tremendous potential for OCE considering the superior spatial resolution (3–40 μm) to provide a highly localized biomechanical characterization at cellular level. This may prove especially important in longitudinal studies. Development of OCE is still ongoing, and challenges remain in the ability to determine moduli values from strain to fully characterize viscoelastic and anisotropic behavior.

OUTLOOK AND CONCLUSIONS

The current state of elastography techniques allow for noninvasive spatiotemporal monitoring of 2D and 3D stiffness in cell-laden constructs and tissues. While the ultra-high spatial resolution of OCE can reveal stiffness at the cellular level (3.5–25 μm), MRE and UE offer highly detailed spatial elastograms at tissue or near-cellular level (25–100 μm) with a larger FOV and the potential to probe deep tissue structures.

Through mechanical excitation, MRE provides a high quality stiffness elastogram. However, it is limited by the dependence on the shear waves, which rapidly attenuate and can limit some applications. Additionally, current MRE inversion algorithms are based on assumptions that the sample is homogeneous and isotropic (also see review by Doyley⁴²). It is therefore recommended that MRE is suitable for biomaterials or engineered constructs that are relatively large, isotropic and homogeneous. On the other hand, dualMRI is highly robust for displacement and strain calculations regardless of tissue size, heterogeneity or anisotropy,^{27,101} and is well suited for a wide variety of soft tissues and cellular constructs, regardless of the viscoelastic, anisotropic, heterogenous natures of the sample.

UE is a simplest form of elastography and is capable of providing 2D strain map and elastograms at moderately low cost with spatial resolution that can be superior to MRE or dualMRI. While traditional forms of UE employ quasi-static mechanical compression, more recent ARF-based excitation has opened the door for novel methods, such as SSI. In quasi-static mechanical and static ARF excitation, images of loaded and unloaded states are cross correlated to obtain strain maps, while dynamic ARF excitation generates shear waves that can be imaged to generate quantitative elastograms. UE suffers from many of the same attenuation issues as MRE and largely ignores the microscopic heterogeneous and anisotropic nature of the tissue in its stiffness calculations.

OCE offers image acquisition at an improved spatial resolution, approaching tenfold (to 3–40 μm) in comparison to other elastography techniques; thus OCE is capable of characterizing highly heterogeneous materials. Furthermore, the high sensitivity of OCE to nanoscopic displacement (10 nm¹⁰⁸) requires a minimal amplitude displacement in the tissue. This, in turn, allows use of a wider range of noninvasive microscopic excitation methods (e.g., magnetic nanoparticles,^{33,108} focused air puff,^{8,41,78,144,150,151} and laser⁷⁵) to measure real-time deformation. However, the major drawback of OCE is the reliance on light scattering of the sample, resulting in shallow image penetration (<3 mm), although there may be some potential to exploit tissue clearing methods.¹⁸

Currently, no elastography technique can obtain material properties that faithfully account for all aspects of viscoelasticity, heterogeneity, and anisotropy; and concessions are therefore made so that moduli can be deduced using simple models. Tissues have a multilevel and multi-dimensionally distinct structural architecture that gives rise to heterogeneous and anisotropic stiffness, and future elastography developments should aim to describe the properties that emerge from the underlying complexity and constituent interactions.

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FIGURE 1.

Spatial resolution and scanning time for elastography techniques. Each point represents the spatial resolution and approximate scanning time reported in representative elastography studies (for specific citations, please see Table 1). MRE = MR elastography, UE = ultrasound elastography, OCE = OCT elastography.

FIGURE 2.

Typical excitation methods employed for micro-elastography. Popular excitation methods utilize mechanical means (A–G, L, M) or non-contact ultrasound (H–K), while other novel methods such as the use of magnetic field (M),³⁸ air puff (N)^{144,150} and laser (P)⁷⁶ have been demonstrated. Please refer to Table 1 for detailed descriptions and application of excitation methods and their respective studies. MRE = MR elastography, UE = ultrasound elastography, OCE = OCT elastography.

FIGURE 3.

MRE shear wave (top row) and elastogram (bottom row) of an 8-mm diameter bovine femoral cartilage performed at excitation frequency of 3×7 kHz. As the mechanical-excitation frequency is increased, higher number of shear waves can be fitted into the sample and therefore increasing the spatial resolution ($100 \times 100 \mu\text{m}^2$). Adapted from Lopez *et al.*⁸⁰

FIGURE 4.

MRE concentric shear wave (top row) and elastograms (bottom row) of chondrocyte construct measured over 3 weeks. A higher spatial resolution can be achieved by a novel concentric shear wave method than by traditional plate based excitation methods (field of view = $7 \times 7 \text{ mm}^2$; spatial resolution = $78 \times 78 \mu\text{m}^2$). Adapted from Yin *et al.*¹⁶¹

FIGURE 5.

dualMRI field data demonstrating strain transfer from the indenter (top) to softer regions of agarose gel constructs with different stiffness layer configurations (2–4% vs. 4–2% wt/vol). Transverse, axial, and shear strains are shown for each. The construct with higher stiffness layer at the top exhibits a stress shielding effect allowing more evenly spread stress distribution throughout the thickness; as such dualMRI is a highly informative elastography technique revealing complex internal strain in the tissue. Resolution = $100 \times 100 \mu\text{m}^2$. Adapted from Griebel *et al.*⁵¹

FIGURE 6.

Relative stiffness elastogram of a porcine cornea with localized sclerosis (centre of cornea) induced by formalin injection. Imaging was performed by ARFI (acoustic radiation force impulse) imaging method by using a dual confocal ultrasound transducer (ultrasound elastography). Resolution = $177 \times 153 \mu\text{m}$. Bar = 1 mm. Adapted from Shih *et al.*¹³⁸

FIGURE 7.

Ultrasound shear waves in porcine cornea. (a) Ultrasound shear wave elastogram of porcine cornea showing propagating shear wave induced by ARF (acoustic radiation force) ultrasound excitation applied at the white arrow. Ultrafast UE can capture rapidly propagating elastic wave in motion, which can be used to derive speed of elastic wave, and therefore Young's modulus. Lateral resolution = $200\ \mu\text{m}$. (b) En face group velocity (stiffness) elastogram of the cornea showing clear division of crosslink treated (CXL) and control regions. Bar = 2 mm. Adapted from Nguyen *et al.*¹⁰⁴

FIGURE 8.

OCE elastogram of rabbit cornea excited by an air puff showing dispersion of Lamb surface wave at $t = 0\text{--}4$ ms. The dispersion speed of surface wave can be used to deduce both elasticity and viscosity of thin material. Bar = 0.5 mm Adapted from Wang *et al.*¹⁵¹

FIGURE 9.

In vivo 3D OCE strain rate elastogram of dry (left) and wet (right) human skin showing anatomically associated stiffness distribution. Volume dimensions (xyz) = $2 \times 1 \times 1$ mm³. Lateral resolution = $40 \mu\text{m}$. Adapted from Kennedy *et al.*⁶⁶

Selection of tissue-engineering and biomaterial relevant micro-elastography techniques.

TABLE 1

Main excitation group	Excitation subgroup	Type	Characteristic parameter	Elastogram [§]	Res (μm)	Description of experiment (Monitoring duration)	Ref
Mechanical compression (contact)	Piezoelectric indenter ⁵⁷	MRE	1.5T	2D strain	300	Bovine cartilage digestion (15 h)	57
	Pneumatic indenter ⁹⁷	dualMRI	3T	2D strain	469	Human cartilage <i>in vivo</i>	23
			7T	2D or 3D strain	100	Bovine cartilage	97,98
			9.4T	2D strain	100	Cellularized scaffold (bovine chondrocyte) inserted into native osteochondral site	96
			14.1T	2D strain	100	Strain transfer characterization of multilayer agar phantom with different stiffness	51
	Transducer compression by a rig ⁶⁹	UE	5 MHz	2D strain	250	Scaffold degradation inside live mouse (7 days)	69
			20 MHz	1D strain	NA	Collagen scaffold + chondrocytes (10 days)	88
	Freehand compression ¹¹³	UE	32 MHz	2D strain	40	Scaffold degradation inside live rat (8 weeks)	143
			32 MHz	2D strain	40	Scaffold degradation inside live mouse (12 weeks)	162
	Slit-plate compression ¹	UE	50 MHz	2D strain	75	Cellularized scaffold with smooth muscle cells	1
Mechanical elastic wave (contact)	Glass cover compression ¹³⁶	OCE	53 MHz	3D strain	23.9	Porcine cornea	59
			TD-OCT	2D displacement	6	Development of cellularized (fibroblast) collagen scaffold (10 days)	70,71
	Acoustic ring actuator ⁶⁵	OCE	PhS-OCT	2D/3D strain	15.3/40	Human skin <i>in vivo</i> (dry vs. wet)	65,66
	Top down shear wave ⁹⁵	MRE	1.5T	2D shear modulus	120	Fetal bovine epiphysis	83
			11.7T	3D shear wave pattern	34	Scaffold + hMS → adipogenesis (2, 3 weeks)	116,117
			11.7T	1D shear modulus	109	Scaffold + hMSC → osteogenesis (4 weeks)	156
			1.5T	2D shear wave pattern	100	Bovine and shark cartilage	81
			1.5T	2D shear modulus	100	Bovine and shark cartilage + selective degradation of collagen and proteoglycan (16 h)	80

Main excitation group	Excitation subgroup	Type	Characteristic parameter	Elastogram [§]	Res (μm)	Description of experiment (Monitoring duration)	Ref
ARF compression (non-contact)	Circular shear wave ⁶¹ Needle/rod induced shear wave ²⁶	MRE	9.4T	2D shear modulus	NA	Scaffold + hMSC $\rightarrow$ adipogenesis & osteogenesis (4 weeks)	39
			9.4T	2D shear wave pattern	NA	Scaffold + mMSC embedded to live mouse (2 months)	115
			11.7T	2D shear modulus	78	Scaffold + chondrocyte (3 weeks)	161
	ARFI (Acoustic Radiation Force Impulse) ¹⁰⁶	MRE	1.5T	1D shear modulus	NA	Bovine gel phantom	26
		OCE	1.5T	2D shear wave pattern	NA	Muscle tissue in live rat and rabbit	25
ARF elastic wave (non-contact)	SWEI (shear wave elasticity imaging) ¹³³ ARF surface wave ³¹	OCE	PhS-OCT	2D shear modulus	5	Agar phantom	139
			6.15 MHz	2D Young's modulus	NA	Porcine muscle	87
			50 MHz	2D relative modulus	153	Porcine cornea + collagen crosslinking treatment	138
	Pulsating water pressure ⁷⁴	OCE	PhS-OCT	2D Young's modulus	8.9	Chicken tendon degradation (90 min)	54
		UE	15 MHz	2D shear wave velocity	200	In vivo porcine cornea + collagen crosslinking treatment	104
Miscellaneous	Magnetic nano-particles ³⁸	OCE	PhS-OCT	1D Lamb wave speed	NA	Bovine cornea + collagen crosslinking treatment	164
			PhS-OCT	1D Young's modulus	NA	Porcine gel phantom + microspheres	102
			5 MHz	2D shear modulus	NA	Scaffold degradation in live mice (12 weeks)	123
	Air puff ^{144,150}	OCE	21 MHz	2D displacement	100	Cellularized tubular scaffold with vascular fibroblasts & smooth muscle cells	44
			PhS-OCT	1D Young's modulus	NA	Collagen scaffold + hBEC (25.5 h)	33
Wire tapping ⁸⁵	Human cornea <i>in vivo</i> (+Alphagan treatment ⁸)	OCE	PhS-OCT	1D Young's modulus	NA	Rat liver + chemical fixation process (67 min)	108
			PhS-OCT	1D Lamb wave speed	NA	Rabbit cornea + collagen crosslinking treatment	144
			PhS-OCT	1D Lamb wave speed	9	Rabbit cornea	150,151
	Mouse cornea <i>in vivo</i> (age effect)	OCE	PhS-OCT	1D Wave deflection profile	3.4	Human cornea <i>in vivo</i> (+Alphagan treatment ⁸)	8,41
			PhS-OCT	1D Rate of relaxation/wave speed	11	Mouse cornea <i>in vivo</i> (age effect)	78,151
Wire tapping ⁸⁵	Mouse cornea <i>in vivo</i> (age effect)	OCE	PhS-OCT	1D Rayleigh wave speed	NA	Mouse cornea <i>in vivo</i> (age effect)	85

Main excitation group	Excitation subgroup	Type	Characteristic parameter	Elastogram [§]	Res (μm)	Description of experiment (Monitoring duration)	Ref
	Pulse laser ⁷⁶	OCE	PhS-OCT	1D Shear modulus	16	Primate cornea	75

MRE = MR elastography, UE = ultrasound elastography, OCE = OCT elastography (TD = time domain, PhS = phase sensitive), NA = not available. Characteristic parameter indicates either magnetic field strength for MRI, transducer frequency of US, or type of OCT.

[§] Here, 'Elastogram' refers generally to a mechanical readout, and not necessarily to the direct measurement elastic properties.

TABLE 2

Summary of four elastography methods identifying primary operating modes, advantages, and limitations.

	MRE	DualMRI	UE	OCE
Displacement/Strain	3D	3D ⁵¹	3D ⁵⁹	3D ⁶⁶
Stiffness or Modulus	3D ¹⁶¹	2D or 3D ¹⁶	2D ¹²³	2D ⁵⁴
Main advantage	3D stiffness elastogram can be obtained under ideal condition	Robust high precision strain elastogram	2D stiffness elastogram at modest cost	Highest resolution 2D stiffness elastogram with a minimal force requirement
Main limitation	Scaffold or tissue must be embedded in a gel	Steady-state must be reached	Elastogram quality is noisy	Shallow image penetration depth
Critical limitation for tissue engineering application	Shear wave in heterogeneous material is difficult to analyze	Cyclic load may affect or damage cells	Static load may affect or damage cells ARF may induce local heating	Tissue or tissue engineering material must not be translucent
Resolution range	34–120 μm	<100 μm	23–250 μm	3–40 μm
Ideal sample size (thickness) range	>10 mm [§]	>5 mm	>2 mm [§]	<2 mm ^{§§}
Scanning time (min)	10–60	10–60	0–25 (mostly real time in 2D)	0–25 (mostly real time in 2D)
Equipment Cost	High	High	Moderate	Moderate
Contact/Non-contact	Contact	Contact	Contact and non-contact	Contact and non-contact
Main contact excitation	Mechanical shear wave	Mechanical compression or shear	–	Vibrational actuator
Main non-contact excitation	–	–	ARF shear wave (SSI, SWEI)	Air puff
Monitoring 2D heterogeneity in stiffness	Limited	Allowed	Limited	Allowed
Monitoring 2D anisotropy in stiffness	Limited	Allowed	Limited	Limited
Monitoring 2D viscosity	Limited	Coupled with excitation frequency	Limited	Limited

[§] In MRE and UE, maximum sample size may be restricted by breakdown of propagating shear wave pattern.

^{§§} In OCE, due to excessive light scattering and absorption, beyond 2 mm in depth from the surface is ineffective.