Video Article

Magnetic Resonance Elastography Methodology for the Evaluation of Tissue Engineered Construct Growth

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Abstract

Traditional mechanical testing often results in the destruction of the sample, and in the case of long term tissue engineered construct studies, the use of destructive assessment is not acceptable. A proposed alternative is the use of an imaging process called magnetic resonance elastography. Elastography is a nondestructive method for determining the engineered outcome by measuring local mechanical property values (i.e., complex shear modulus), which are essential markers for identifying the structure and functionality of a tissue. As a noninvasive means for evaluation, the monitoring of engineered constructs with imaging modalities such as magnetic resonance imaging (MRI) has seen increasing interest in the past decade[1]. For example, the magnetic resonance (MR) techniques of diffusion and relaxometry have been able to characterize the changes in chemical and physical properties during engineered tissue development[2]. The method proposed in the following protocol uses microscopic magnetic resonance elastography (μMRE) as a noninvasive MR based technique for measuring the mechanical properties of small soft tissues[3]. MRE is achieved by coupling a sonic mechanical actuator with the tissue of interest and recording the shear wave propagation with an MR scanner[4]. Recently, μMRE has been applied in tissue engineering to acquire essential growth information that is traditionally measured using destructive mechanical macroscopic techniques[5]. In the following procedure, elastography is achieved through the imaging of engineered constructs with a modified Hahn spin-echo sequence coupled with a mechanical actuator. As shown in Figure 1, the modified sequence synchronizes image acquisition with the transmission of external shear waves; subsequently, the motion is sensitized through the use of oscillating bipolar pairs. Following collection of images with positive and negative motion sensitization, complex division of the data produce a shear wave image. Then, the image is assessed using an inversion algorithm to generate a shear stiffness map[6]. The resulting measurements at each voxel have been shown to strongly correlate (R^2>0.9914) with data collected using dynamic mechanical analysis[7]. In this study, elastography is integrated into the tissue development process for monitoring human mesenchymal stem cell (*h*MSC) differentiation into adipogenic and osteogenic constructs as shown in Figure 2.

Video Link

The video component of this article can be found at http://www.jove.com/video/3618/

Protocol

1. Tissue Construct Preparation

The tissue construct preparation process consists of three main stages: expansion of cell population, seeding of cells onto a biomaterial scaffold, and differentiation through the use of chemical signaling molecules. The procedure for construct preparation is based on methods conducted by Dennis *et al*., Hong *et al*., and Marion and Mao[8,9,10].

1. After culture and expansion of the cell line, seed the human mesenchymal stem cells (*h*MSCs) onto a gelatin sponge (4 mm diameter, 3.5 mm thickness) at a density of $1x10^6$ cells/ml for bone and $3x10^6$ cells/ml for adipose formation.
2. For differentiation of *h*MSCs into adipose, apply the adipose induction media consisting of 1 μM dexamethasone, 0.5 μM isobutyl-methylxanthine, 10 μg/ml human recombinant-insulin, and 200 μM indomethacin in cell expansion medium once cells appear confluent on the scaffold. After three days, replace the media with 10 μg/ml of human recombinant-insulin in expansion media for 24 hours then return to induction media. Repeat the cycle three times and then exchange only in maintenance media every two days.
3. To induce osteogenesis, prepare osteogenic induction media by making a final concentration of 0.1 μM dexamethasone, 50 μM of L-ascorbic acid-2phosphate, and 10 mM β-glycerophosphate in cell expansion medium. Replace with fresh osteogenic media every two days.

2. Actuator Characterization

Characterization of the actuator is a vital step for the MRE experiment. MRE relies on the propagation of mechanical shear waves to assess local values of mechanical properties; therefore, these mechanical vibrations need to be generated and characterized within the tissue of interest using a piezoelectric actuator. An illustrated example of the characterization process is shown in Figure 3. The goal of this procedure is to optimize the motion of the actuator in order to generate harmless shear waves with significant amplitudes (~250 micron).

1. Prior to the experiment, apply 0.5% agarose gel to enclose the construct in a 10 mm test tube. The temperature of the gel should be approximately 37 °C to minimize damage to the construct.
2. After allowing the agarose gel five minutes to set at room temperature, insert the tip of the piezoelectric bending motor into the surface of the gel.
3. Attach the tube containing the sample and the actuator to a rigid support, and orient the beam of the Laser Doppler Vibrometer toward the tip of the mechanical actuator. Adjust the positioning of the system to optimize the reflected signal, use reflective tape if necessary.
4. Based on the expected resonance frequency of the mechanical actuator, set the function generator to sweep the desired frequency range (i.e. 20 to 2000 Hz in this experiment) using an operating voltage of 20 Vpp with a white noise signal.
5. View the characterized spectrum on the Polytec Vibrosoft program to identify the resonance frequency of the system and set the program to FFT and velocity as the y-axis.
6. For displacement measurement, set the actuator to deliver a continuous sinusoid at the indicated resonance frequency using an operating voltage of 200 Vpp, and note the generated displacement being delivered to the surface of the gel. Set Vibrosoft to display the FFT with displacement as the y-axis.

3. Image Acquisition

1. After completing actuator characterization, place the sample and actuator in the center of the MRI scanner. For tissue construct experiments, use a small and more sensitive RF coil (i.e. 10 mm in this experiment) for the transmission and reception of the RF signal. (The procedure shown uses a 9.4 T vertical bore magnet equipped with triple axis gradients, 100 G/cm).
2. Acquire a scout image for identification of the construct location.
3. Set the parameters for the acquisition. A typical *in vitro* sagittal scan will have a repetition time of 1000 ms, echo time of 20-40 ms, slice thickness of 0.5-1.0 mm, and field of view of 12x10 mm^2 with a matrix size of 128x128 pixels.
4. For the elastography parameters, set the actuator frequency to the value determined by the Laser Doppler Vibrometer characterization. In the current study, one bipolar pair was needed with a gradient amplitude of 50 G/cm. Other parameters to adjust include the delay which should be set to zero milliseconds for the initial acquisition.
5. Change the function generator to burst mode and adjust the parameters of the function generator to match those in the elastography acquisition parameters including the frequency and number of cycles. Also, set the function generator to be externally triggered.
6. For a sagittal image, set the motion sensitization to be in the positive slice direction and start the scan. Following acquisition, check the image and change the sensitization to the negative slice direction.
7. Execute the MATLAB program that will perform the complex division for generation of the shear wave image.
8. Assess the image for the presence of shear waves and possible artifacts such as phase wrapping.
9. If no adjustments to the image are necessary, adjust parameter array size to eight equally spaced values ranging from zero seconds to a full period of the characterized resonance frequency.
10. Acquire a scan in both the positive and negative slice orientations.
11. Once the images are acquired, use a MATLAB program designed for generating the shear wave data from an array of images.

4. MRE Experiment Image Processing

1. The final step of MRE is to calculate the shear stiffness from the shear wave images. Placing the data into the MATLAB program that will assess the three dimensional dataset (2 spatial, 1 temporal).

Note: By assuming a planar shear wave, the equations of motion decouple allowing the estimation of the complex-valued shear modulus as a function of the displacement and its Laplacian. The algorithm approximates spatial second derivatives with finite difference and computes the shear modulus on a pixel-by-pixel basis. From this complex number, many mechanical parameters can be deduced such as the shear wave speed, wave attenuation, shear stiffness, shear elasticity, shear viscosity, etc. The algorithm also allows the selection of regions of interest for which the mean and standard deviation of each parameter is calculated.

2. The imaging parameters need to be specified at the beginning of the program. Additionally, the upper limit of the elastogram can be adjusted to optimize contrast in the sample.

Note: The program provides intermediate results (wave after low-pass filters, wave after directional filtering, temporal FFT, line profiles, etc.) that help the user estimate the faithfulness of the recovery.

3. Some parameters can be adjusted based on this information, such as the levels of low-pass filters, the temporal frequency of the motion, the direction of propagation of the wave, etc. The standard deviation of a parameter in a specific region of interest is also an indicator of the quality of the calculation.

5. Representative Results

Figure 4 notes the change in mechanical properties throughout four weeks of osteogenic and adipogenic construct development. MRE was conducted at 730-820 Hz. While both seeded sponges started at approximately 3 kPa, osteogenic directed tissues resulted in a stiffness of 22

kPa; whereas, adipose directed tissues decreased in stiffness to 1 kPa. Furthermore, the osteogenic constructs showed a notable decrease in size in comparison from beginning to end of the study. Additional properties derived from elastography study are shown in Table 1.

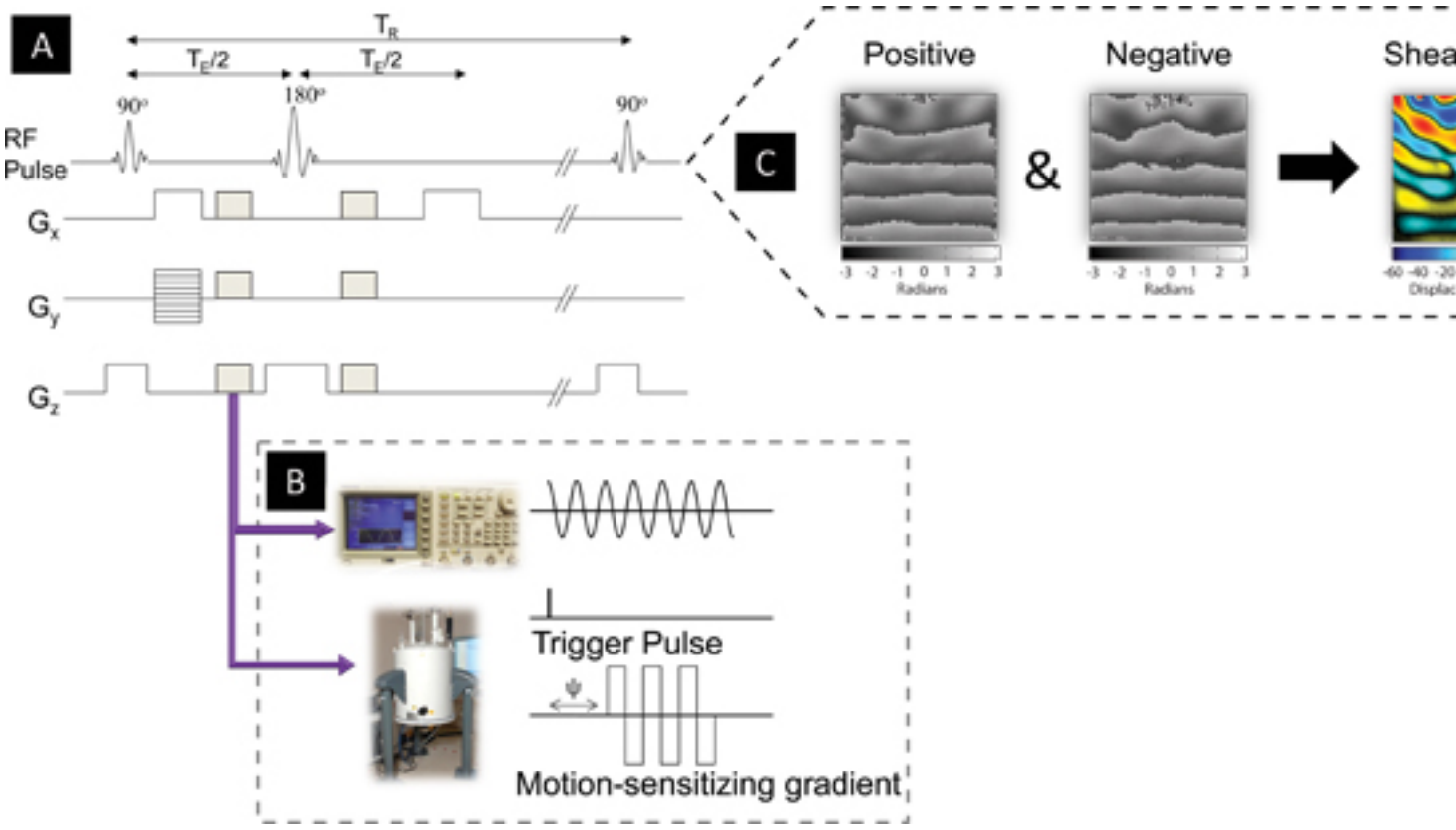

Figure 1. The image acquisition process for magnetic resonance elastography. During image acquisition, a pulse sequence (a) controls the synchronization (b) of the function generator with the bipolar gradients pulses of the MRI scanner. Following acquisition of bipolar gradients toggled in positive and negative orientations, (c) a shear wave image is produced using complex division.

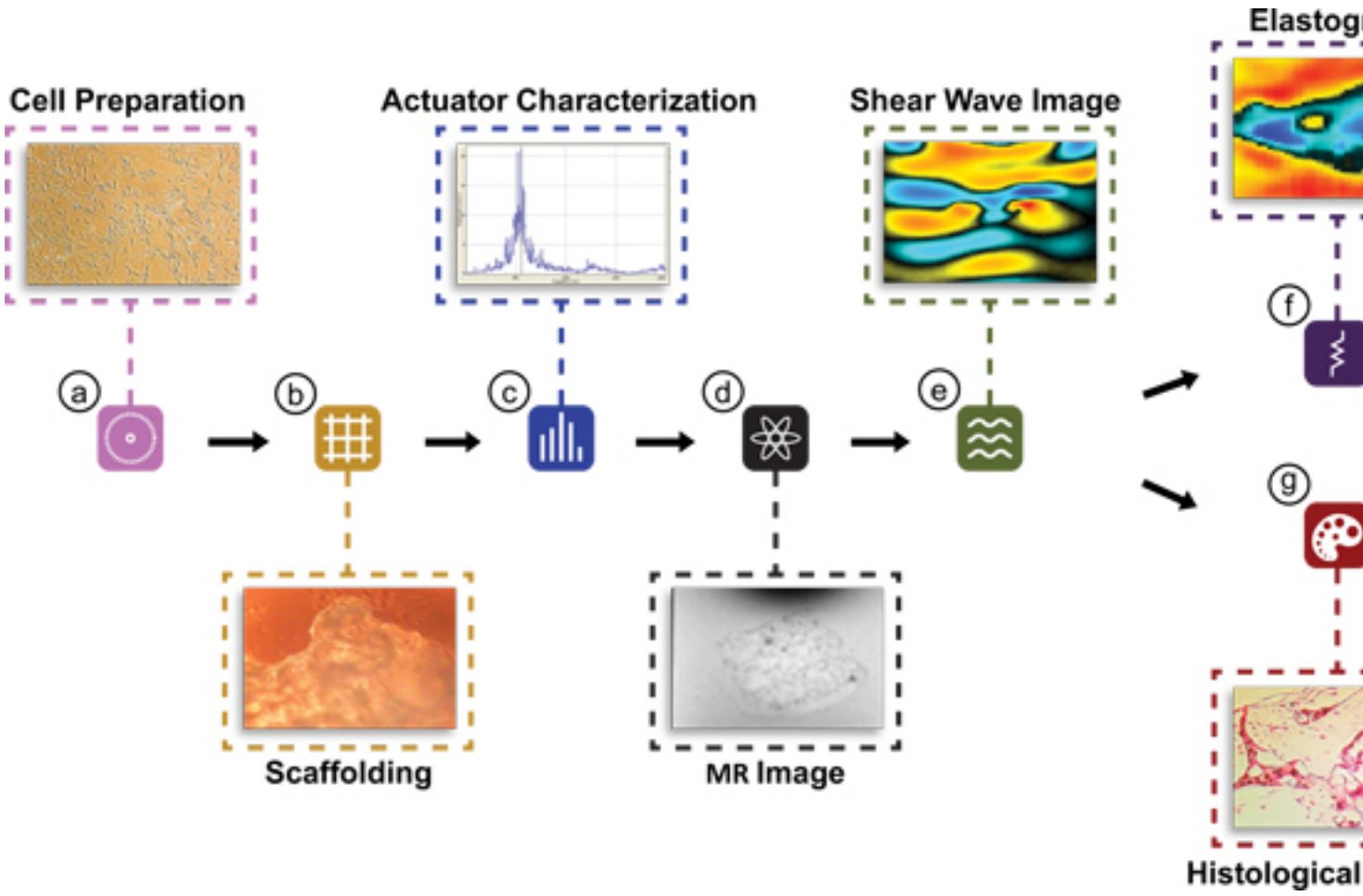

Figure 2. Flow diagram of the MRE process for tissue engineered constructs. First, cells (a) are first grown and expanded to the population size essential for the designed project. Then the cells are seeded (b) onto a biomaterial scaffold and chemical reagents are applied to signal differentiation. Scaffolds are characterized with MRE, whose first step (c) is the determination of the resonance frequency of the actuator coupled to the construct. Next, MRI images (d) are acquired to generate a shear wave image (e). Finally, an algorithm is applied to yield an elastogram (f) that maps the stiffness of the construct. Concurrently, constructs are sectioned for histological assessment (g) in order to validate differentiation.

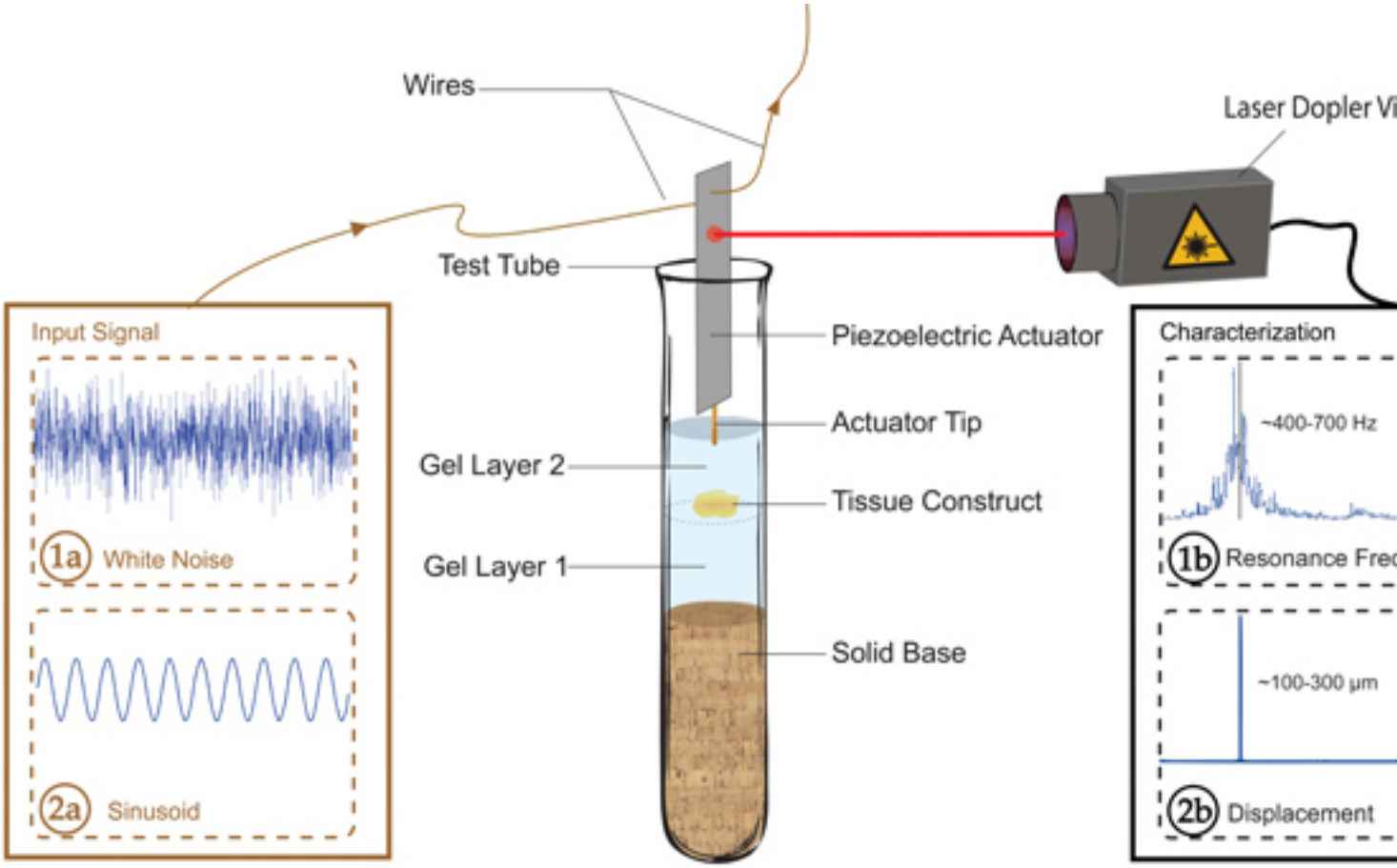

Figure 3. Actuator characterization procedure. The gelatin scaffold is enclosed by a 0.5% agarose gel. To characterize the motion being transferred into the sample a white noise is first sent into the system (1a) and the resulting motion is detected using a Laser Doppler Vibrometer (1b). Once the resonance frequency is determined, a continuous sinusoid signal at resonance (2a) is sent to determine the displacement (2b) transferred to the gelatin environment.

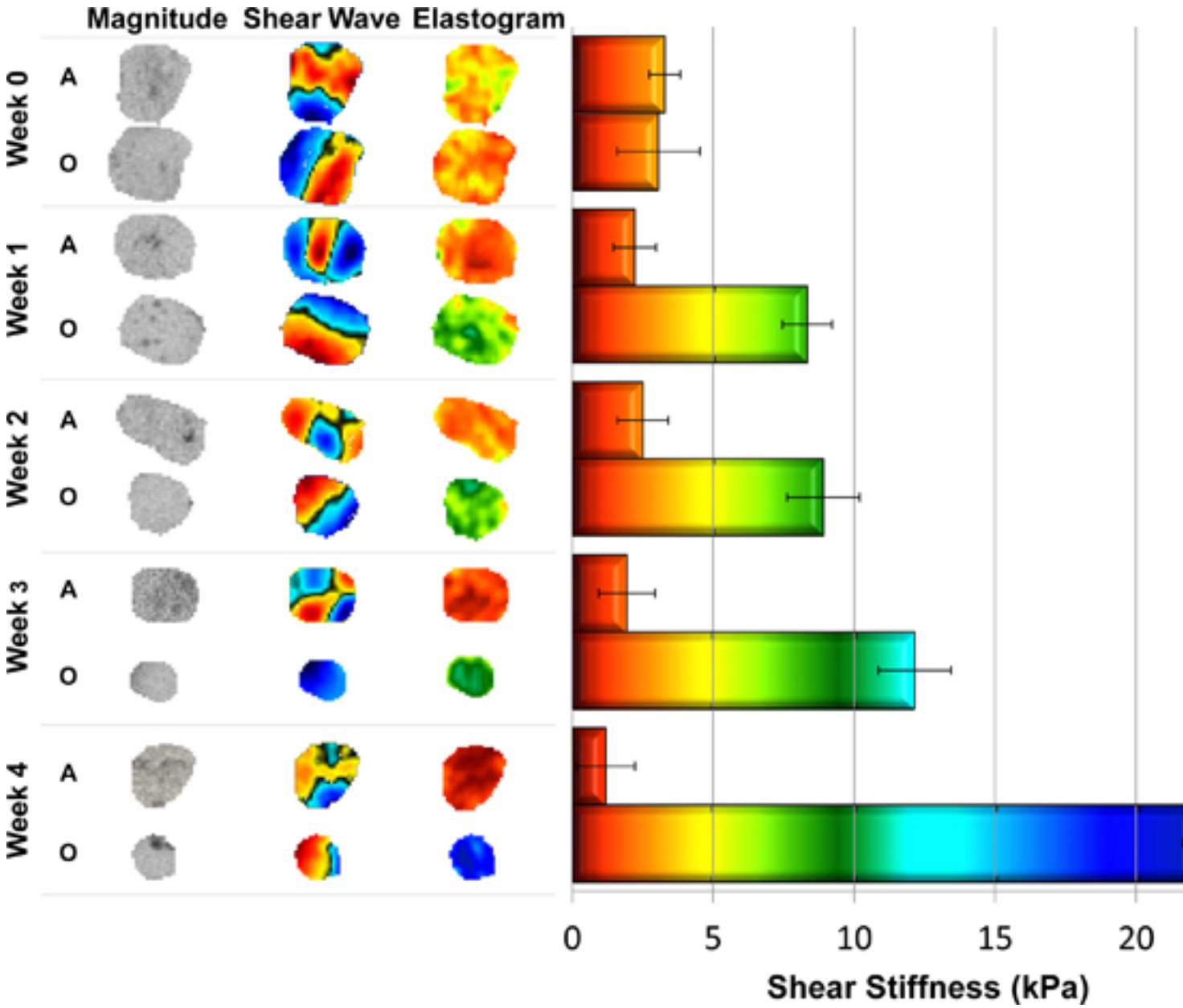

Figure 4. Construct development map over four week period. Adipogenic (A) and osteogenic (O) constructs are shown from left to right with corresponding magnitude and shear wave images, elastogram, and average shear stiffness. The colormap for the elastogram corresponds with the color scheme of the bar chart and error bars represent the standard deviation within each construct's region of interest.

		Week 0	Week 1	Week 2	Week 3	Week 4
Stiffness (kPa)	*Adipose*	3.28 ± 1.03	2.22 ± 0.99	2.51 ± 0.91	1.94 ± 0.76	1.19 ± 0.55
	Osteo	3.06 ± 0.45	8.34 ± 1.29	8.91 ± 1.28	12.15 ± 0.88	22.11 ± 1.46
Shear elasticity μ1 (kPa)	*Adipose*	2.51 ± 0.94	1.76 ± 0.69	1.55 ± 0.67	1.08 ± 0.46	0.68 ± 0.30
	Osteo	1.57 ± 0.35	4.37 ± 1.68	5.58 ± 1.48	8.22 ± 2.02	12.77 ± 3.17
Shear viscosity μ2 (Pa.s)	*Adipose*	0.43 ± 0.14	0.23 ± 0.14	0.17 ± 0.07	0.11 ± 0.08	0.10 ± 0.04
	Osteo	0.36 ± 0.07	0.74 ± 0.22	0.81 ± 0.20	0.71 ± 0.22	2.02 ± 0.37

Table 1. Mechanical properties of adipose and osteo constructs over a four week period of growth.

Discussion

In this procedure, the process of MRE for tissue engineered constructs is demonstrated from cell preparation to the generation of an elastogram. By applying a nondestructive mechanical assessment method to the tissue engineering pipeline, it is now possible to evaluate changes in engineered constructs throughout multiple stages of development. In addition, MRE complements other MR methods for monitoring tissue engineered constructs such as diffusion, magnetization transfer, and chemical shift analysis[1].

When performing MRE experiments, a few limitations should be noted. The assessment of *in vitro* specimens is a time sensitive study. Therefore, it is recommended that studies should last no more than one hour so that any potential damage to the tissue construct is minimized. Additionally, faithful recovery of the stiffness map can be compromised due to constructs being either too small or stiff [6]. One possible solution to this problem is to operate at higher frequency (> 2.5 kHz), as the wavelength is inversely proportional to the frequency. Piezoelectric stack actuators driven by high voltage amplifiers are able to deliver sufficient motion at such frequencies to produce a full shear wavelength in the sample. Another possible amendment to the protocol is to use faster sequences such as fast spin-echo and echo planar imaging[11, 12].

Beyond the possibilities of MRE for tissue engineered constructs *in vitro*, the next step of pre-clinical assessment is to evaluate the development of tissues implanted into a living system. The application of MRE to mouse studies would provide another opportunity to nondestructively evaluate the development the tissue constructs. Extension of elastography for treatment of bone or cartilage defects would potentially provide a better understanding of how to produce longer lasting functional implants for use in regenerative medicine. Magnetic resonance elastography has the potential to play an increasing role in the validation of engineered constructs both *in vitro* and *in vivo*.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgements

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