Introduction
Most assessments of the quality of tissue engineered (TE) products, for example histology or biomechanics, are either destructive or violate the sterile bioreactor environment. As a result, TE products are usually not evaluated prior to implantation, or destructive end-point tests must be performed on costly redundant samples. Additionally, conventional destructive mechanical evaluations (e.g., unconfined compression or indentation) take at least 4 hours per specimen and, therefore, are too time-consuming for large-scale evaluation. Consequently, non-destructive assessments are translationally impractical. Thus, there are unmet needs for non-destructive quality control (QC) assays. The purpose of this investigation was to determine the feasibility of using ultrasound (US) elastography to evaluate the mechanical properties of developing TE constructs in the sterile bioreactor environment.

Methods
The mesenchymal stem cells (MSCs) were obtained from healthy volunteer donors under the terms of an IRB-approved protocol. The cells were culture-expanded until the end of second passage and were vacuum-seeded onto 12 mm diameter by 1 mm thick collagen-chondroitin sulfate porous scaffolds. The scaffolds were grown in bioreactors, which were perfused continuously with a chondrogenic medium (Figure 1). Each week, over a three-week period, bioreactors, were removed from the incubator and placed in a device that we developed for US elastography (Figure 2). The sealed bioreactor is sandwiched between an US reflector (above) and an US transducer (Panametrics V208-rm, 20 Mhz) and load cell in series and placed in a device that we developed for US elastography (Figure 2).

Displacement of internal acoustic inhomogeneities due to the applied compression were found using a cross-correlation echo tracking method [1]. This method has been used for the ultrasound elastography of soft tissues [2, 3]. The normalized correlation coefficient of two series of discrete values \(X=x(0), x(1), \ldots, x(N-1)\) and \(Y=y(0), y(1), \ldots, y(N-1)\) can be written as

\[
R = \frac{\sum_{i=1}^{N-1} (x(i) - X) (y(i) - Y)}{\sqrt{\sum_{i=1}^{N-1} (x(i) - X)^2} \sqrt{\sum_{i=1}^{N-1} (y(i) - Y)^2}}
\]

where \(X\) is the mean of \(X\), and \(Y\) is the mean of \(Y\). If \(X\) and \(Y\) are exactly the same, then \(R=1\), and, if they uncorrelated, then \(R=0\). The cross-correlation was used to obtain time shifts between US signals at 0 µm compression \((\lambda)\) and at 50, 100 or 150 µm compression \((\lambda)\). An algorithm, written in MATLAB, used the normalized cross-correlation function \(\text{normxcorr2}\) to compute time shifts \((T)\) of the echoes in different regions through the thickness of a sample. The speed of sound \((c)\) was calculated based on the known applied compressive displacement and corresponding time shift in the reflector echo. The corresponding average displacement \((d)\) in each of four internal regions of the TE construct was then found from

\[
d = \frac{T}{2}
\]

These were compared with expected values for a homogeneous material.

Results
In TE constructs, multiple reflections from internal acoustic inhomogeneities were found (Figure 3), which is in sharp contrast to healthy native cartilage that is acoustically homogeneous. The displacement of internal reflections for three steps of compression were found (Table 1) and compared with the expected displacement for a homogeneous material. For example, in Region B under the second step, the actual displacement is much greater than the expected displacement for a homogeneous material \((\approx 70 \mu m)\), showing that this region is more compliant than surrounding regions.

Conclusions
By using US elastography to identify highly compliant immature regions within developing TE constructs they can be eliminated as candidates for implantation, thereby limiting the possibility of implanting inferior tissue.

Table 1. Displacement for each region under three steps of compression

<table>
<thead>
<tr>
<th>Region</th>
<th>Displacement (µm)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>12.03</td>
</tr>
<tr>
<td>B</td>
<td>48.45</td>
</tr>
<tr>
<td>C</td>
<td>98.50</td>
</tr>
<tr>
<td>D</td>
<td>148.43</td>
</tr>
</tbody>
</table>

Figure 2. Bioreactor in the apparatus used for ultrasound elastography. The TE construct is in the sealed bioreactor and is compressed between a micrometer driven reflector and ultrasonic transducer.

Figure 3. Ultrasound signals, showing a time shift, to the left, from zero compression (blue) to 100 µm compression (red).

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References

Contact: Joseph M. Mansour, Professor, Tel: +1 2163684190, E-mail: mansour@case.edu