In silico simulation and in vitro evaluation of an elastomeric scaffold using ultrasonic shear wave imaging

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Abstract. Biodegradable elastomeric scaffolds for soft tissue repair represent a growing area of biomaterials research. Mechanical strength is one of the key factors to consider in the evaluation of candidate materials and the designs for tissue scaffolds. It is desirable to develop non-invasive evaluation methods of the mechanical property of scaffolds which would provide options for monitoring temporal mechanical property changes in situ. In this paper, we conduct in silico simulation and in vitro evaluation of an elastomeric scaffold using a novel ultrasonic shear wave imaging (USWI). The scaffold is fabricated from a biodegradable elastomer, poly(carbonate urethane) urea using salt leaching method. A numerical simulation is performed to test the robustness of the developed inversion algorithm for the elasticity map reconstruction which will be implemented in the phantom experiment. The generation and propagation of shear waves in a homogeneous tissue-mimicking medium with a circular scaffold inclusion is simulated and the elasticity map is well reconstructed. A PVA phantom experiment is performed to test the ability of USWI combined with the inversion algorithm to non-invasively characterize the mechanical property of a porous, biodegradable elastomeric scaffold. The elastic properties of the tested scaffold can be easily differentiated from the surrounding medium in the reconstructed image. The ability of the developed method to identify the edge of the scaffold and characterize the elasticity distribution is demonstrated. Preliminary results in this pilot study support the idea of applying the USWI based method for non-invasive elasticity characterization of tissue scaffolds.

1. Introduction
There is generally a growing interest in biodegradable elastomeric scaffolds for soft tissue repair in the biomaterials community. Such scaffold is designed to support cell adhesion and tissue in-growth until being replaced by the body’s own extracellular matrix (ECM). Despite the real remodeling process is complex, in general, the success of a scaffold relies largely on its biocompatibility, structural properties, as well as its mechanical properties. It is desirable to develop non-invasive methods for characterizing mechanical properties of scaffolds because it would obviate the need to destruct scaffolds and the mechanical property changes in the same sample can be monitored over time.

Ultrasound shear wave imaging (USWI) has been found to be a valuable non-invasive tool to investigate mechanical characteristics of biological tissue [1, 2]. USWI is a new method of obtaining
elasticity images by using acoustic radiation force to induce shear waves in tissue and measuring the propagation of the resulting shear waves in real time with high frame rate ultrasound imaging sequences [3]. Elasticity maps are recovered from the resulting spatiotemporal displacement field via inversion methods. The objective of this study is to demonstrate the ability of USWI to non-invasively characterize the mechanical properties of a tissue scaffold in vitro.

In previous work [4, 5], different kinds of biodegradable polyurethanes were developed which exhibited highly tunable mechanical and biodegradation properties for tissue engineering scaffolds. Among these, one kind is poly(carbonate urethane) urea (PCUU), a strong elastomeric, hydrophilic material which performs a good biocompatibility in vivo and shows favorable properties for soft tissue repair and regeneration [5].

In this paper, a cylindrical elastomeric PCUU scaffold was prepared and USWI was applied on the scaffold embedded in a stiffer background PVA phantom for elasticity characterization. An in silico simulation was performed prior to the in vitro phantom experiment to test the robustness of the inversion algorithm for elasticity map reconstruction. From preliminary in silico and in vitro evaluation results, USWI based method can be accepted as promising for non-invasive elasticity characterization of tissue scaffolds.

2. Materials and methods

2.1. In silico simulation of shear wave elasticity imaging

Assuming soft tissue is purely elastic, a two-dimensional numerical model of shear wave propagation through tissue with a circular elastomeric tissue scaffold inclusion was established by using a finite element software package (COMSOL). The Structural Mechanics Module was used for the present work. A rectangular thin excitation rod (54 mm × 0.5 mm) was created in the center, and a uniform plane shear wave was produced by oscillating the rod vertically with one cycle of a 300 Hz low frequency harmonic vibration. As the shear wave propagated sideways away from the center, the tissue medium was displaced along the axial direction.

The tissue medium with the following characteristics was simulated: a Young’s modulus of 30 kPa, density of $1.2 \times 10^3$ kg/m$^3$ and Poisson’s ratio of 0.49. To mimic the tissue scaffold, a circular inclusion of 10 mm diameter was created on the right. The circular region had a Young’s modulus of 1 kPa, density of 97.5 kg/m$^3$ and Poisson’s ratio of 0.49. We assumed that the boundary conditions were: free at the top of the tissue medium, the boundary of the excitation rod and the scaffold boundary; roller on other surfaces (bottom, left and right surfaces) [6, 7]. A triangular element mapping mesh (7045 elements) was used. Then a time-dependent analysis was performed. The simulation time was 15 ms and the time step was 0.1 ms. The simulation was run over 151 frames. The temporal and spatial profiles of shear wave propagation were recorded. The recorded time-varying axial displacements were processed in MATLAB. The output axial displacement data is a three-dimensional matrix (200×400×151), which indicates the data contains 151 frames and is divided into 200 and 400 units in the X and Y direction, respectively.

In order to reconstruct the elastic modulus of the tissue scaffold using the shear wave elasticity imaging method, inversion algorithms were developed using the finite difference method. The spatiotemporal displacement data were directly and locally inverted to obtain the shear modulus distribution map [8, 9]. This is achieved by calculating the ratio between temporal and spatial second derivatives of the longitudinal displacement $u_x(x, y, t)$. The shear modulus $\mu$ in the elastic medium was estimated by using the Helmholtz inversion algorithm:

$$\mu(x, y) = \rho \frac{1}{N} \sum_{n=1}^{N} \frac{\frac{\partial^2 u_x}{\partial t^2}}{\frac{\partial^2 u_y}{\partial t^2} + \frac{\partial^2 u_y}{\partial y^2}}_{t=nT},$$

(1)
where $\rho$ is density, $N$ is number of frames and $T$ is period of frame acquisition. For a nearly incompressible medium, the shear modulus is about one third of Young’s modulus. Therefore, using shear wave elasticity imaging combined with the inversion algorithm, the elastic properties of the elastomeric scaffold medium can be characterized.

### 2.2. Scaffold fabrication and phantom preparation

We present a tissue construct made of poly(carbonate urethane) urea (PCUU) which is a biodegradable elastomer for soft tissue repair. PCUU was synthesized from polyhexancarbonate (PHC, Mn=2000, Sigma) and diisocyanatobutane (BDI, Sigma) using putrescine (Sigma) for chain extension [4, 5]. The PHC:BDI:putrescine molar ratio was defined as 1:2:1. Salt leaching was used for scaffold fabrication. The synthesized polymer samples were completely dissolved in hexafluoroisopropanol (HFIP) to obtain a 20% (w/v) solution. The solution (1 mL) was blended with 4.5 g salt particles (100-150 $\mu$m) and the mixture was poured into a cylindrical glass mold. After complete solvent evaporation, the mixture was immersed in 30% ethanol solution for 2 days to remove the salt particles. Then the scaffold was placed in deionized water for 3 h to exchange the ethanol solution. After frozen at -80°C and lyophilized for 2 days, a porous cylindrical scaffold with a diameter of 10 mm was obtained. For in-vitro ultrasound evaluation, the PCUU scaffold was embedded in a phantom of 10% poly(vinyl alcohol) (PVA, Sigma (Aldrich) chemistry) mixed with 1.8% ultrasound scatterer ($\text{Al}_2\text{O}_3$, Atlantic Equipment Engineers). The scaffold was soaked in deionized water before being embedded to avoid bubbles.

### 2.3. In vitro evaluation of scaffold using USWI

Experiments were implemented on a Verasonics system using a L7-4 ultrasonic linear array (Philips) operating at 5 MHz. The L7-4 transducer is placed at the top of the phantom in stationary position to excite the medium in the phantom along its center axis at different depth consecutively, and receive ultrasonic signals. The cylindrical scaffold PCUU was placed horizontally in the phantom at some distance from the center source axis.

The ultrasound beam was focused along the internal vertical axis from 18 mm to 33 mm at 20 consecutive depths (0.75 mm increment) with a duration of 20 $\mu$s (100 cycles) each to generate displacement and initialize motion. The pulse repetition frequency (PRF) of the push pulses is 25 kHz. The successive pulses act as a line source that creates shear waves that mainly radiate in transverse directions in the medium. The induced motion is tracked by high frame rated pulses at 8000 Hz. The tracking sequence was repeated for 5 ms and 41 frames were obtained. The ultrasonic data were stored and interframe axial displacements were calculated from IQ data using the algorithm in Ref. [10]. The shear modulus distribution map was reconstructed from the filtered spatio-temporal displacement data using the inversion algorithm mentioned above. In generating the elasticity map, the Laplacian of Gaussian (LoG) operator was employed to extract the contour of the scaffold. The Gaussian kernel acts as a low pass filter to eliminate the high frequency noise of the image.

### 3. Results

Fig. 1 displays the shear wave propagation through tissue with a scaffold-mimicking softer inclusion of 10 mm diameter and resulting reconstructed elasticity map via Helmholtz inversion technique from in silico simulation. From Fig. 1(a), it is observed that the wave front bends backward when the shear wave propagates through the inclusion which indicates a decrease in wave speed within the inclusion. This is consistent with the shear wave velocity formula ($c = \sqrt{\mu/\rho}$). Fig. 1(b) exhibits a larger displacement in the inclusion compared to that in the bulk medium. Both evidences indicate that the inclusion is softer than the surrounding regions. Fig. 1(c) displays the reconstructed elasticity map from which the inclusion contour is well observed with insignificant artefacts generated at the backside of the inclusion. Values displayed in Fig. 1(c) are actually the ratio of shear modulus to density.
Figure 1. Shear wave propagation and elasticity map reconstruction from in silico simulation. (a) The axial displacement image at 8.7 ms. (b) The axial displacement image at 12.0 ms. (c) The reconstructed elasticity map. The scaling of the colorbars is in meters (a) (b) and m²/s² (c).

Table 1 lists the inversion results by using elasticity modulus reconstruction algorithm compared to given values of $\mu/\rho$. The inversion results are expressed as mean ± standard deviation (SD). Despite of some variations as for spatial distributions, the mean inversion results are consistent with the given values with errors smaller than 1%. It is also found that the deviation from the mean value is larger for the inclusion.

|                  | Given $\mu/\rho$ (m²/s²) | Calculated $\mu/\rho$ (m²/s²) |
|------------------|--------------------------|-------------------------------|
|                  | Mean    | SD    | Mean    | SD    |
| Inclusion        | 3.42    | 0.68  | 3.44    | 0.68  |
| Bulk medium      | 8.33    | 0.29  | 8.39    | 0.29  |

Fig. 2 displays the B-mode image, shear wave propagation and reconstructed elasticity map from in vitro experiment using a cylindrical PCUU scaffold embedded in PVA phantom. Despite that the edge of the PCUU scaffold is not sharply defined in the reconstructed image due to the limited SNR, the PCUU scaffold can be detected from the background medium, and elasticity contrast can be clearly seen.

Figure 2. The B-mode image, shear wave propagation and elasticity map reconstruction from in vitro experiments using a cylindrical PCUU scaffold embedded in PVA phantom. (a) The B-mode image. (b) The axial displacement image at 2.75 ms. (c) The reconstructed elasticity map.
4. Discussion

In this study, USWI was investigated for characterizing mechanical properties of a PCUU scaffold using a commercial ultrasound scanner and a developed elasticity map reconstruction algorithm. Numerical simulation served to validate the inversion algorithm. Despite of some variations as for spatial distributions in the scaffold-mimicking inclusion, the mean inversion results in the reconstructed elasticity map compare well with the given values for both the inclusion and the bulk medium. Scaffolds used for soft tissue repair must have inhomogeneous elasticity pattern during tissue remodeling process, and the inhomogeneous elasticity pattern must be different from one sample to the other, therefore the mean value of the elasticity distributions can be a good signature of the mechanical characteristics of the inclusion.

In comparison with the numerical simulation, the image processing technique applied in the phantom experiment helped reduce boundary artefacts in the elasticity map of shear wave imaging and the elasticity distribution was smoothed. It can be seen from the B-mode image that the cross-section of the PCUU scaffold has a porous structure, which made the elasticity distribution of the PCUU scaffold in the elasticity map having a unique feature different from the simulated inclusion. In the elasticity map, the scaffold was visualized without definite boundaries; however the scaffold can be clearly differentiated from the tissue-mimicking background with a good contrast of elastic properties based on USWI acquisition.

There are several advantages for the USWI technique: It is relatively inexpensive, non-ionizing, portable, effective and reproducible; USWI enables the non-invasive evaluation of tissue stiffness even deep inside the body. Despite it is too early to know whether USWI could ultimately prove useful in monitoring scaffold performance in human, USWI integrated ultrasound scanners are already commercially available for human use.

Our study had some limitations. First, only one sample was studied. Different samples may have different mechanical boundary conditions. More experiments are necessary to validate the reproducibility of our results. Second, despite the viscoelastic properties were expected from the porous internal structure of the scaffolds, only pure elastic properties were examined. Third, although the developed method above characterized the mechanical properties of the inclusion and the scaffold, further investigation on absolute elastic modulus estimate is needed. The stiffness and the density of the surrounding tissue may be a good reference for the scaffold evaluation, since they are expected to remain near constant during the remodeling process. Last but not least, Helmholtz inversion of the wave propagation field was applied in the paper for elasticity evaluation. However, novel elasticity reconstruction and imaging processing technique needs to be developed to achieve more accurate elasticity characterization of the scaffolds and to optimize the scaffold visualization.

Although our study is preliminary and further investigations would be needed, the initial results are highly encouraging. So far, the work devoted to the exploration and understanding of the potential of USWI in characterizing candidate materials for tissue engineering applications seems to be very limited. This study provided some insight on understanding the potency of USWI in characterizing mechanical properties of a degradable polyurethane elastomer in vitro for soft tissue repair uses.

5. Conclusions

The ability of USWI as a non-invasive evaluation tool for characterizing the mechanical properties of a biodegradable elastomeric scaffold was demonstrated in vitro. An in silico simulation was performed to simulate the generation and propagation of shear waves in a homogeneous tissue-mimicking medium with a circular scaffold inclusion and the elasticity map was well reconstructed with the developed inversion algorithm. In the phantom experiment, the elastic properties of the tested PCUU scaffold can be easily differentiated from the surrounding medium in the reconstructed image. The mechanical property contrast observed by USWI for PCUU and tissue-mimicking background, suggests possible extensions of USWI to a wide range of materials. Preliminary results in this pilot study support the idea of applying the USWI based method for non-invasive elasticity characterization of tissue scaffolds.
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