Acoustic properties of chitosan-GelMA composite hydrogel

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1. Introduction

Protein-carbohydrate composite hydrogels are used in biomedical applications as they compositionally mimic human tissues[1, 2]. While carbohydrates provide structural support, proteins offer functional support to living cells, making them valuable in 3D in vitro models, wound healing, and post-surgical wound dressings. The physical properties of these hydrogel scaffolds determine their effectiveness. Traditional methods of assessing mechanical properties, such as atomic force microscopy (AFM), rheometry, and nanoindentation, are often destructive and challenging to implement in real-time in living conditions[3, 4]. A non-destructive, real-time, and non-contact method for evaluating these properties acoustically is presented. Here we propose an ultrasound imaging technique and a signal processing method to assess the acoustic impedance and velocity of Gelatin Methacrylate (GelMA) and chitosan composite hydrogels using scanning acoustic microscopy (SAM), enabling deep tissue imaging complementing optical imaging[5].

2. Materials and method

A Polymethylpentene (TPX) substrate with silicon gasket wells (Grace Bio-Labs) was used for holding hydrogels (**Fig. 1**). GelMA-Chitosan composite hydrogel was formed by mixing 2% (w/v) chitosan powder in the 5% GelMA solution, followed by UV curing.

Fig. 1 Sample Preparation for SAM imaging.

A 30 MHz polymer spherically focused transducer (Olympus) was used for SAM imaging. The incident acoustic wave on the sample generated four distinct wave components which were used for analysis. We used a signal processing technique involving the maximal overlap discrete wavelet transform (MODWT)[6] for determining impedance, where the signals are first filtered and then decomposed to remove low-power components, isolating the essential components. To estimate the reflectance and impedance from the acoustic responses, we solved an inverse problem. The characterization frequencies of the transmitted signal, and reflected signals through the reference (media), and target (hydrogel) media are represented as S_0, S_r and S_t, respectively[6]. The relationships between these parameters and the sample impedance (Z) were governed by the following equations[7]:

$$S_t = S_0 \frac{Z_t - Z_s}{Z_t - Z_s} \quad \text{(i)}$$

$$S_r = S_0 \frac{Z_r - Z_s}{Z_r + Z_s} \quad \text{(ii)}$$

$$Z_t = Z_s \frac{1-(S_t/S_r)\cdot(Z_s-Z_r)/(Z_s+Z_r)}{1+(S_t/S_r)\cdot(Z_s-Z_r)/(Z_s+Z_r)} \quad \text{(iii)}$$

Where, S_r = 31.7647, Z_s = 12.8, Z_r = 3.3075.

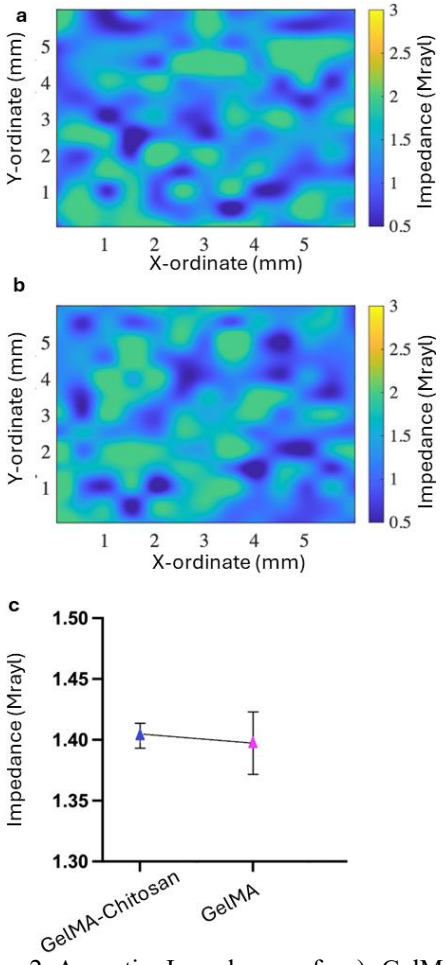

Fig. 2 Acoustic Impedance of- a) GelMA-chitosan, b) GelMA, and c) their comparison.

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4. Results and discussions

The reflected acoustic imaging response exhibits multiple peaks due to various interfaces, resulting in a time-frequency analysis problem. To address this, MODWT is applied, decomposing the signal into multiple time series with distinct wavelet

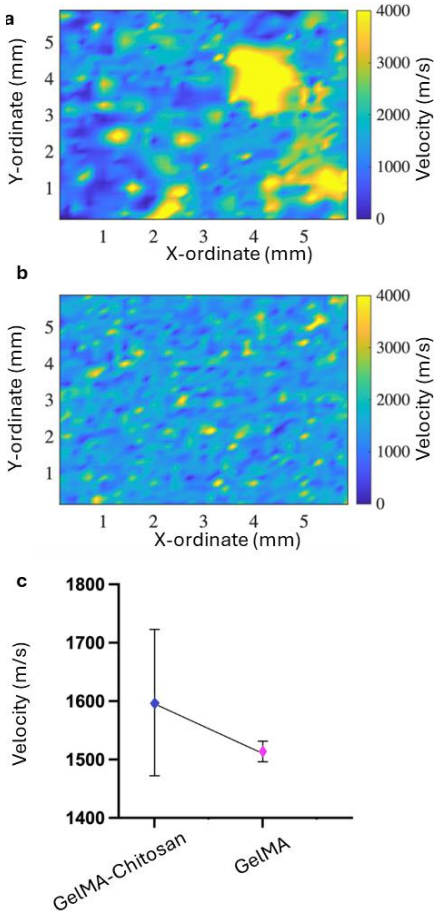

Fig. 3 Longitudinal velocity of - a) GelMA-chitosan, b) GelMA, and c) their comparison.

and scaling coefficients. These signals are reconstructed using inverse MODWT (iMODWT), and the power of each is calculated and normalized. The time series with the highest power is selected, and the FFT of its first 75 points is windowed to isolate key frequency components. This enhances the accuracy of acoustic impedance. The peak frequency in the power spectrum is then used to calculate acoustic impedance, followed by Kriging with a Gaussian variogram to estimate impedance across the domain using Latin hypercube sampling for initial point selection[8].

The average value of the acoustic impedance of the GelMA-chitosan hydrogel was 1.40 Mrayl, while its acoustic velocity was found to be 1600 m/s. Meanwhile, both the acoustic impedance and velocity values of the control sample (GelMA) was found to be lower than that of the composite hydrogel, at the average values 1.39 Mrayl and 1525 m/s respectively. **Fig. 2a** and **2b** show the distribution of the acoustic impedance at different points throughout the sample. Meanwhile the **Fig. 3a** and **3b** show the distribution of longitudinal acoustic velocity at different points throughout the sample in both composite hydrogel and control. This indicates the higher stiffness and non-uniform distribution of composite hydrogel as compared to the control.

5. Conclusion

We used SAM imaging and signal processing to evaluate the acoustic properties of soft, human-like tissue phantoms, which are designed to simulate various grades of body tissues. SAM provides impedance and velocity maps that shed light on tissue stiffness and material compositions, complementing current optical imaging methods[9]. This enables analyzing different soft tissue properties, aiding in better material design and biomedical applications.

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