

CHARACTERIZATION OF BIOENGINEERED TISSUES BY DIGITAL HOLOGRAPHIC VIBROMETRY AND MACHINE LEARNING

Colin Hiscox (1), Juanyong Li (2), Ziyang Gao (3), Dmitry Korkin (3), Cosme Furlong(1),
Kristen Billiar (2)

- (1) Mechanical Engineering Department, Worcester Polytechnic Institute, Worcester, MA, USA
(2) Biomedical Engineering Department, Worcester Polytechnic Institute, Worcester, MA, USA
(3) Department of Computer Science, Worcester Polytechnic Institute, Worcester, MA, USA

INTRODUCTION

Mechanical properties are critical and often overlooked factors defining the quality of bioengineered tissues. Current tests used to measure the physical properties of engineered tissues, such as uniaxial tensile tests and nanoindentation, tend to be invasive and destructive and thus not applicable for in-process quality control, requiring aseptic non-destructive assessment.

The goal of this work is to develop a noncontact nondestructive method to measure the properties of bioengineered tissues during production. We apply acoustic vibration to Apligraf living skin substitute and measure the modes and displacements of vibration of the entire surface of the tissue within its packaging. We then utilize finite element methods and supervised machine learning algorithms to relate the measured vibrations to the mechanical properties.

METHODS

Our methodology used three components to measure important information of the sample: optical coherence tomography (OCT), digital holographic vibrometry (DHV), and nanoindentation. All measurements and experiments were performed on samples of Apligraf generously donated by Organogenesis Inc. [1]

First a sample was measured with OCT (Telesto SD-OCT, Thorlabs). A b-scan taken at multiple points was used to estimate the thickness of each layer of the Apligraf nondestructively.

DHV, the primary tool, uses a speaker and a holographic sensor to induce and measure vibrations [2]. The sensor (Fig. 1) uses a 532 nm light to illuminate the sample while it is still in its packaging. In DHV, a subwoofer placed above the sample outputs single tone frequencies and sweeps between 70 Hz and 500 Hz. We identified vibrational modes of the 0th kind and imaged the sample at 12 equidistant points within a single vibration curve by modifying the phase of camera strobing for image acquisition. This gave a full field of view video with 12 frames that showed the nm-scale waveforms oscillating in the sample.

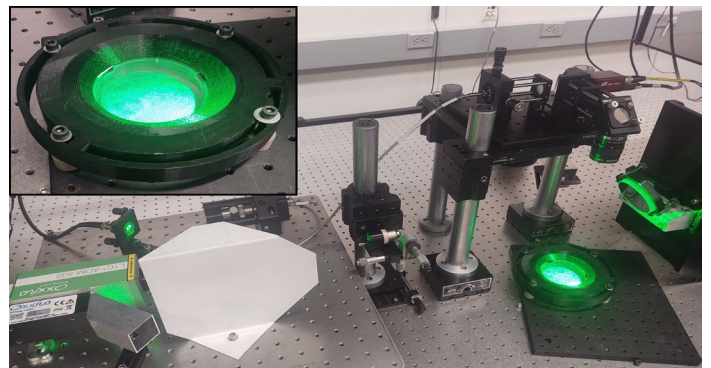


Figure 1: DHV sensor with inset of tissue during measurement [3]

Samples were also measured with nanoindentation at 4 multiple random points with a 51 μm -radii probe on a 3.45 N/m stiffness cantilever (Optics 11, Amsterdam, Netherlands). During indentation, the sample was covered by a lid with a small opening to minimize evaporation. The epidermis was measured then peeled back with a scalpel and forceps, and the tissue's dermis was measured the same way. This was used to validate the modulus of the epidermal and dermal layers to compare to simulated results and to algorithm predictions.

To demonstrate the utility of the method, two experiments were performed. In the first experiment, a single sample was measured with nanoindentation then with DHV. Four different speaker volumes were measured at each mode, measuring different pressures applied by the acoustic waves to the sample, to test for linearity of the tissue response.

For the second experiment, the sample was subjected to controlled drying to change the thickness and/or stiffness of the sample and measure the resulting change in frequency of vibrational modes over time. Due to the destructive nature of the drying, two different samples

from the same production batch were used for nanoindentation and DHV. Each sample was dried for 90 minutes in a 23 °C oven (Isotemp, Fisher Scientific) for controlled humidity and temperature, and measured at several time points: 0, 3, 6, 10, 15, 30, 60 and 90 minutes. Each sample was put back into the oven with an open lid after measurement until the next time interval is reached.

To integrate future machine learning algorithms into the methodology, we developed a finite element model (FEM) in Ansys APDL. The model includes three layers: the epidermis, dermis, and porous plastic membrane on which they sit. A modal analysis was performed in which the stiffness and thickness of the epidermal and dermal layers were modified across a range of expected values. This model output the frequencies of several modes for each combination of stiffness and thickness values.

RESULTS

The maximum positive displacement measured at the first four vibration modes for four different acoustic forces applied by the speaker show roughly linear relationships (Fig. 2) indicating that the tissue is within the linear region at these low displacements. For these analyses, the largest displacement is taken from the point in the 12-image set of measurements that reflects the top of the sinusoidal curve.

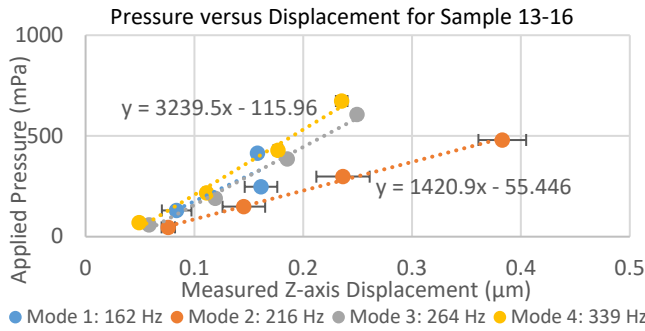


Figure 2: Amplitude of tissue displacement for multiple applied pressures during vibration [3]

In the second experiment, OCT measurements of thickness for both epidermal and dermal layers of the Apligraf tissue decreased with drying time (Fig. 3).

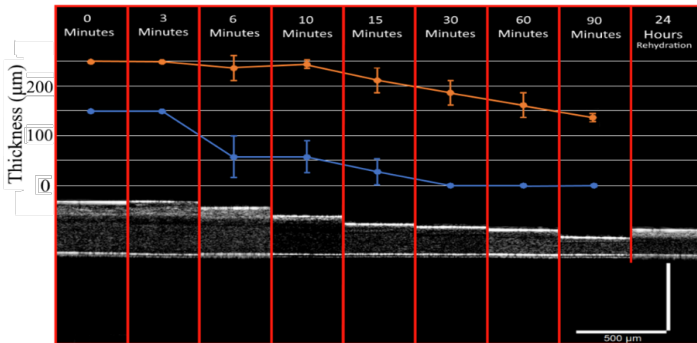


Figure 3: OCT measurement of tissue thickness during drying [3]

The frequencies at which each of the 1st four modes of vibration occur decrease monotonically with drying (Fig. 4). The modal FEA in which the layer thickness was decreased to match the measured data show the opposite trend in frequency of the fundamental mode (Fig. 5). This

result indicates that it is more likely that the increase on modulus with drying dominates the vibratory behavior.

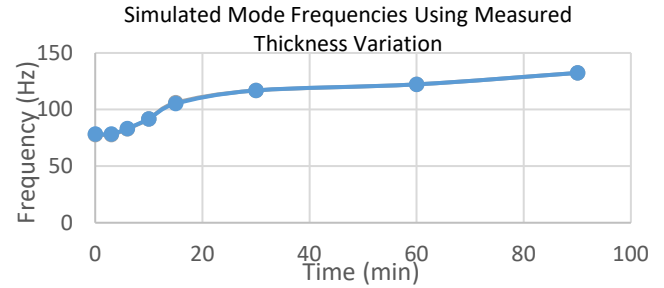


Figure 4: Expected frequency of vibration from FEM [3]

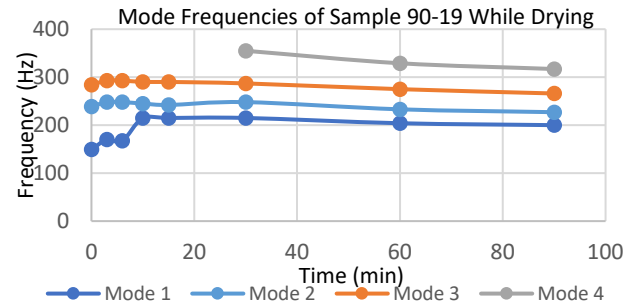


Figure 5: Frequency location for each mode during drying [3]

DISCUSSION

This study demonstrates the ability to measure the full-field surface displacements of engineered skin tissue nondestructively and aseptically within its packaging using stroboscopic holographic vibrometry. By utilizing multiple acoustic pressures, the linearity of the tissue behavior was confirmed. Drying the tissue demonstrated the ability of the system to detect changes in the physical properties, and the FE analysis indicates that the changes in modal frequencies are due to stiffening of the tissue. However, due to the complexity of the layered system which includes the agar and stiff membrane on which the tissue is grown, it is not currently possible to extract the precise mechanical properties of the Apligraf layers from vibrometry alone. Further, the interferometric images output from the holographic system are very large and complex and can take multiple hours to analyze. Machine learning models are being constructed to relate finite element results to experimentally measured results and to more quickly analyze the full-field surface displacement data to predict the mechanical properties.

Once completed, this non-invasive measurement technique and associated analysis method would likely take around 5 minutes allowing for in-line measurement during production. This QC assay has the potential to enable identification of faulty batches before the full growth time is reached, increasing efficiency of manufacturing.

ACKNOWLEDGEMENTS

This work was supported by the NSF (CMMI 1761432) and ARMI BiofabUSA (T0137). We would also like to thank Kate Faria and Organogenesis for providing the Apligraf tissue used in this research.

REFERENCES

- [1] Faria, K., *Personal Communications*, Organogenesis Inc., 2021
- [2] Kuppens, J. et al., Proc. SPIE 6293, Interferometry XII: Applications, 629309, 2006
- [3] Hiscox, C., MS Thesis, ME Department WPI, 2022