Dermatology Clinics & Research
DCR, 3(3): 174-179
www.scitcentral.com

ISSN: 2380-5609

Original Review: Open Access

Non-Invasive Viscoelastic Behavior of Human Skin and Decellularized Dermis Using Vibrational OCT

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Received October 24, 2017; Accepted October 27, 2017; Published October 29, 2017

Abstract

The need to measure the mechanical properties of skin, scar, tumors, extracellular matrices, and wound tissue has been a goal of researchers since the 1970s. A variety of methods have been used to evaluate the mechanical properties of tissues over the last 40 years including uniaxial and biaxial tensile testing, indentation and rotational tests, ultrasound elastography (UE), optical cohesion tomography (OCT), optical cohesion elastography (OCE), and vibrational analysis combined with OCT.
We have developed a test using vibrational OCT to image and measure the moduli of the components of skin and scar tissue non-invasively and non-destructively. Using images generated by OCT, maps of the modulus as a function of position can be generated that will be useful in marking the margins of scars, tumors as well as to evaluate the effects of cosmetic treatments to the skin.

Key Words: Collagen, Extracellular matrix, Optical coherence tomography, Vibrational analysis, Skin, Scar, Tumor, Tumor margins

INTRODUCTION

The need to measure the mechanical properties of skin, scar, tumors, extracellular matrices (ECMs) and wound tissue has been a goal of researchers since the 1970s. The pioneering work of Yamada [1] and Fung [2] illustrated how difficult this goal would be since the behavior of human ECM depends on strain-rate, direction of testing and is time-dependent [3]. A variety of methods have been used to evaluate the mechanical properties of skin over the last 40 years including uniaxial and biaxial tensile testing, indentation and rotational tests, ultrasound elastography (UE), optical cohesion tomography (OCT), optical cohesion elastography (OCE), and vibrational analysis combined with OCT [4-6]. Many of these techniques require the assumptions that the material is linearly elastic, Poisson's ratio is close to 0.5 and that viscoelasticity does not dramatically affect the resulting properties. However, skin is a non-linear material that is viscoelastic and has upward curvature to the stress-strain curve. This fact makes determination of the stiffness (tangent to the stress-strain curve and other mechanical properties very difficult to quantitatively analyze since the tangent to the stress-strain curve is constantly changing [3,5,6]. However, despite all of these problems, there is a need to be able to characterize the mechanical properties of skin, since this would give clinicians valuable information about pathological changes that occur during disease processes, the stage of diabetic skin ulcers and the efficacy of cosmetic treatments. In this paper, we will discuss the history of our efforts to simplify evaluation of the mechanical properties of skin and the development of a simple test to non-invasively and non-destructively determine the mechanical behavior of skin, tumors and scar tissue [3,5-7].

Measurement of the Modulus of Elastic Tissue and Collagen Fibers in Skin and Decellularized Dermis

Since the properties of skin are time-dependent and also depend on the rate of deformation of the sample, it is important to separate the elastic properties from the viscous properties [3]. The model used in most of our studies was

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Citation: Silver F H & Silver L L, (2017) Non-Invasive Viscoelastic Behavior of Human Skin and Decellularized Dermis Using Vibrational OCT, 3(3): 174-179.

decellularized dermis since the mechanical properties of this material are similar to normal skin and decellularized dermis can be freeze dried and stored at room temperature in a sterilized form [3,5-8]. In addition it is also composed primarily of collagen fibers with some elastic tissue still present simplifying interpretation of the mechanical properties [3,5-8].

The non-linearity of the stress-strain curves was dealt with by dividing the stress-strain curves into elastic and viscous components [3,5-7, 9,10] (**Figure 1**). The elastic component was measured as the stress at equilibrium in an incremental stress-strain experiment [3,10]. This test was conducted by the sequential addition of loading increments, followed by a relaxation period between each loading step [3,10] (**Figure 1).** The elastic stress-strain curve was broken into a linear low modulus region and a linear high modulus region [3, 5-7]. Equilibrium stress-strain curves were then used to calculate the elastic modulus as the tangent to the curve which turned out to be about 0.5 MPa and 15 MPa, respectively for the low and high strain regions [3,5,10]. The low strain region modulus was similar to that reported for elastic tissue and the high strain modulus was similar to that reported for collagen fibers [7,10]. The major problem with this approach was that the time required for the stress to reach equilibrium in the skin and in decellularized dermis was as long as 24 hrs and the test resulted in destruction of the material.

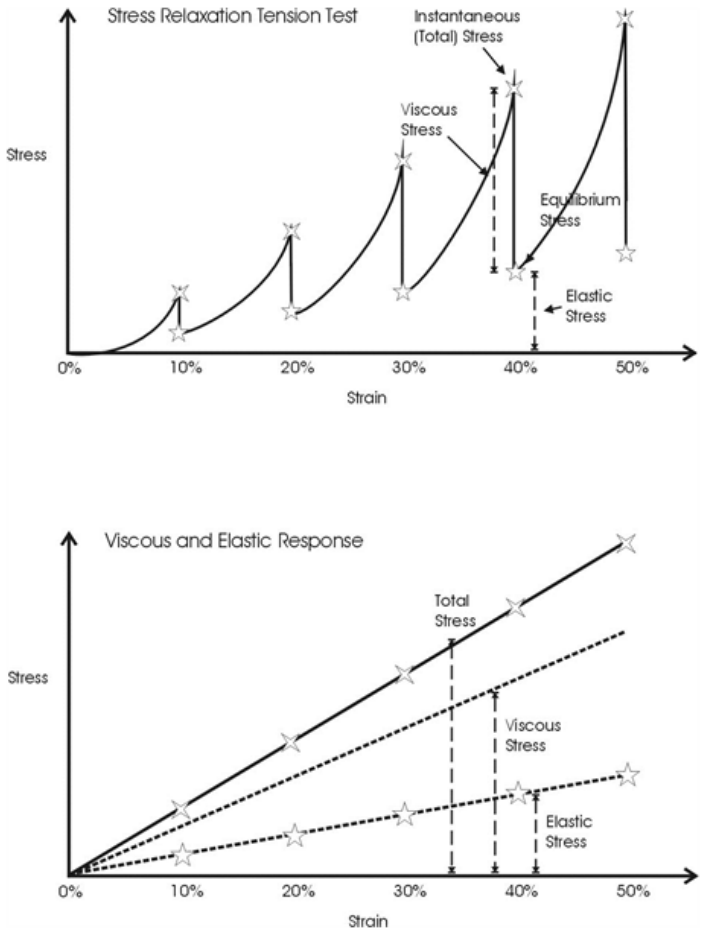

Figure 1. Diagrammatic representation of incremental stress-strain curves for skin and other extracellular matrices (ECMs) tested in tension. (Top) A strain increment is applied to the ECM and the initial stress is measured. The strain increment varies from about 2% for tendon to about 10% for skin. The stress is allowed to relax at room temperature until an equilibrium value is reached. The process is repeated until the sample fails. (Bottom) Plots of all the initial (total) and equilibrium stresses are made versus strain as well as plot of the total stress minus the equilibrium stress versus strain. The equilibrium stress versus strain curve is equivalent to the elastic stress-strain curve while the difference between the total and equilibrium stress is the viscous stress. This figure was adapted from Silver, 2006 (10).

The modulus of a number of ECMs was tested using this approach and the results indicated that the elastic modulus was independent of strain-rate for strain rates up to 10,000% per minute [10]. When the slope of the elastic stress-strain curve at high strains is divided by the collagen content of the tissue, the fraction of collagen aligned with the tensile direction, and the change in strain after the collagen fibers of the tissue are stretched in tension, the resultant modulus was calculated to be between 4.0 and 7.0 GPa, values similar to those reported for the stiffness of the collagen molecule [10,11]. The slope of the viscous stress-strain curve reflected the length of the collagen fibrils and the viscous sliding of collagen fibrils by each other during tensile deformation [10].

Results of theoretical modeling studies suggested that the high strain elastic modulus was consistent with stretching of the collagen triple helical regions devoid of proline and hydroxyproline [12]. Therefore, the stiffness or modulus at high strains reflects the resistance to stretching of the collagenous components of skin and is a useful parameter to evaluate skin properties and their changes during wound healing and disease processes. Results of studies on hypertrophic scar tissue have shown that the major difference between scar and normal skin mechanical properties lies in the inability of scar tissue to reorient under an applied load in the same manner as does normal skin when stretched in tension [13]. However, since this approach required long time intervals and destruction of the tissue it would not be considered useful in studying skin mechanics *in vivo*. Therefore, our efforts became focused on development of a non-invasive and non-destructive test to measure the mechanical properties of skin *in vivo*.

Development of Vibrational Optical Cohesion Tomography (OCT)

The concept of development of a test to measure the mechanical properties of skin came from the observation that when one vibrates a bowl of soft material like jello with a hard inclusion, the jello vibrates at a higher frequency than the inclusion. If one were able to measure the frequency of vibration of the jello and inclusion separately then the frequency of vibration of each material should be related to the material stiffness. It turned out that a technique termed OCT created images of a substrate by comparing a beam of light with part of the beam that is reflected off a substrate [14]. If one measures the displacement of each substrate at varying frequencies during vibration it is possible to calculate the modulus of each the material. **Figure 2** shows a plot of weighted displacement versus frequency for decellularized dermis showing one resonant frequency (frequency of maximum displacement) for collagen in decellularized human dermis.

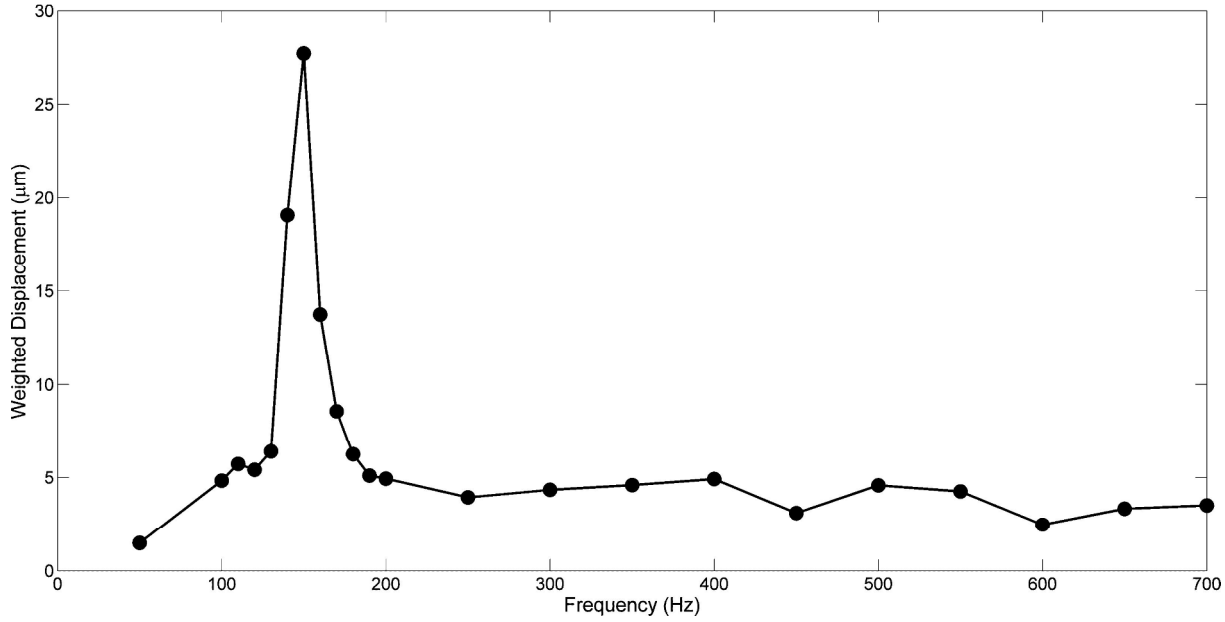

Figure 2. Weighted displacement versus frequency for decellularized dermis determined from vibrational OCT. The resonant frequency is determined from measurement of the maximum displacement of a sample vibrated between 0 and 700 Hz. Note the maximum displacement is measured and converted into a vibrational modulus using a calibration curve and equation (1). The modulus calculated from vibrational studies was calculated from the resonant frequency as described by Shah et al. (5, 6).

Recently, we have reported the use of vibrational analysis and OCT to characterize the mechanical behavior of decellularized dermis, pig skin, bovine cartilage and subchondral bone [5,6]. Our results indicate that Poisson's ratio for decellularized dermis ranges between 0.38 and 0.63,

values that are significantly different than 0.5 making the assumption that Poisson's ratio for skin and other collagenous tissues is 0.5 incorrect. This may lead to errors in calculating models dependent on an exact value of this ratio [4]. In addition, a relationship was shown to exist between the resonant frequency and the elastic modulus [5, 6]. The modulus measured using vibrational OCT and that determined from tensile incremental stress-strain curves for decellularized dermis and silicone rubber had a correlation coefficient in excess of 0.95 demonstrating that the modulus measured using vibrational OCT was very similar to the tensile modulus measured using incremental stress-strain curves [5,6]. The relationship between the modulus determined from vibrational and tensile testing is given by equation (1) where Ev and Et are the moduli determined from vibrational and tensile measurements in MPas.

$$Ev=1.026\ Et + 0.0046 \quad (1)$$

The measured value of the moduli did not depend on an assumed value of Poisson's ratio. Results of studies on decellularized dermis and silicone rubber at frequencies of between 50 and 1000 Hz suggested that the viscous component of the modulus measured at frequencies at or above the resonant frequency was 3%-4% [15]. Based on these results the modulus measured using vibrational OCT was considered to be an approximation of the "elastic modulus" and the viscous component was negligible [15]. While the modulus determined from vibrational OCT is an approximation of the elastic modulus it depends on the strain since not all the collagen fibers are recruited to bear loads at low strains [16] **(Figure 3).**

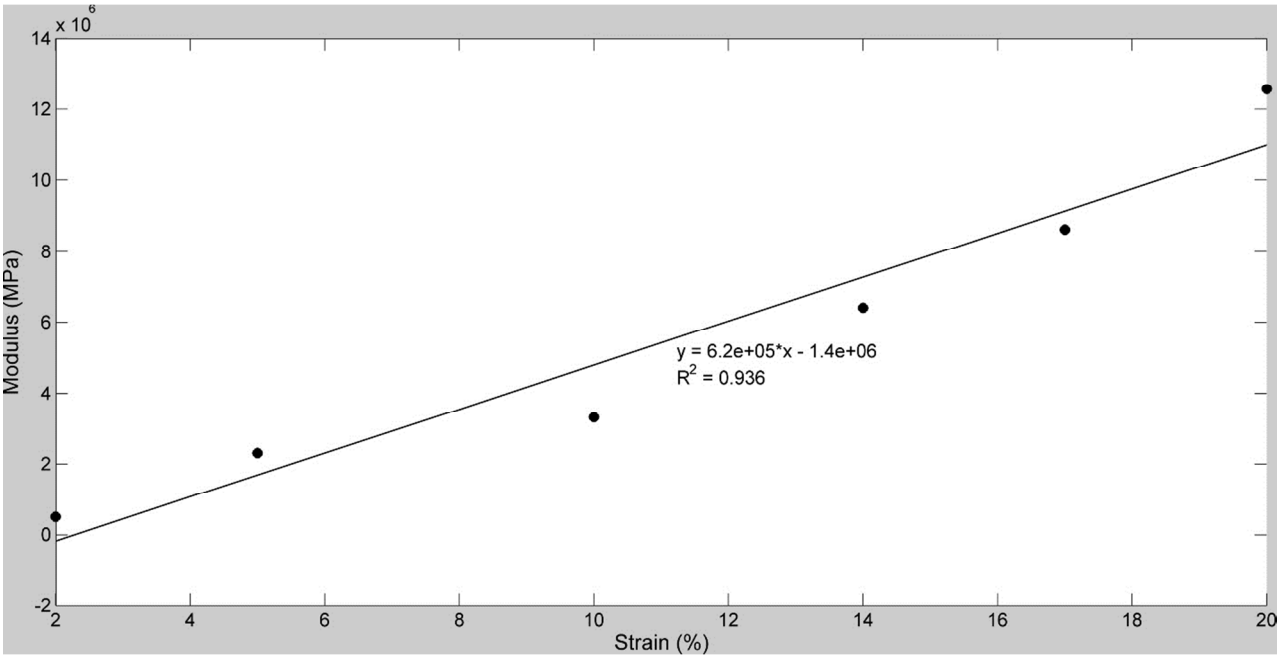

Figure 3. Plot of modulus determined from OCT and vibrational studies versus strain for decellularized dermis. Note the resonant frequency and modulus increases with strain due to the increased recruitment of collagen fibers with increasing strain during stretching.

Use of Vibrational OCT to Measure the Moduli of Skin *in Vivo*

Vibrational OCT has been used to image and measure the mechanical properties of skin and scar tissue *in vivo*. Results of *in vivo* vibrational OCT studies suggest that the modulus of normal skin is lower than that of scar tissue [16] and that of the margins of a healed scar have a resonant frequency different than that of normal skin (**Figure 4).** Recent results indicate that the margins of a scar can be mapped using vibrational OCT since the resonant frequencies and moduli seen at the interface reflect both the resonant frequency of normal skin and that of the scar tissue.

CONCLUSIONS

Using vibrational OCT the resonant frequency and moduli of the components of skin and scar tissue can be measured non-invasively and non-destructively. The numbers generated reflect to a first approximation the elastic moduli and do not depend on measurement of other parameters. The technique *in vitro* is calibrated using incremental tensile measurements and vibrational OCT results on the same sample. Using images generated by OCT, maps of the modulus as a function of position can be generated that will be useful in marking the margins of scars, tumors as well as to evaluate the effects of cosmetic treatments to the skin.

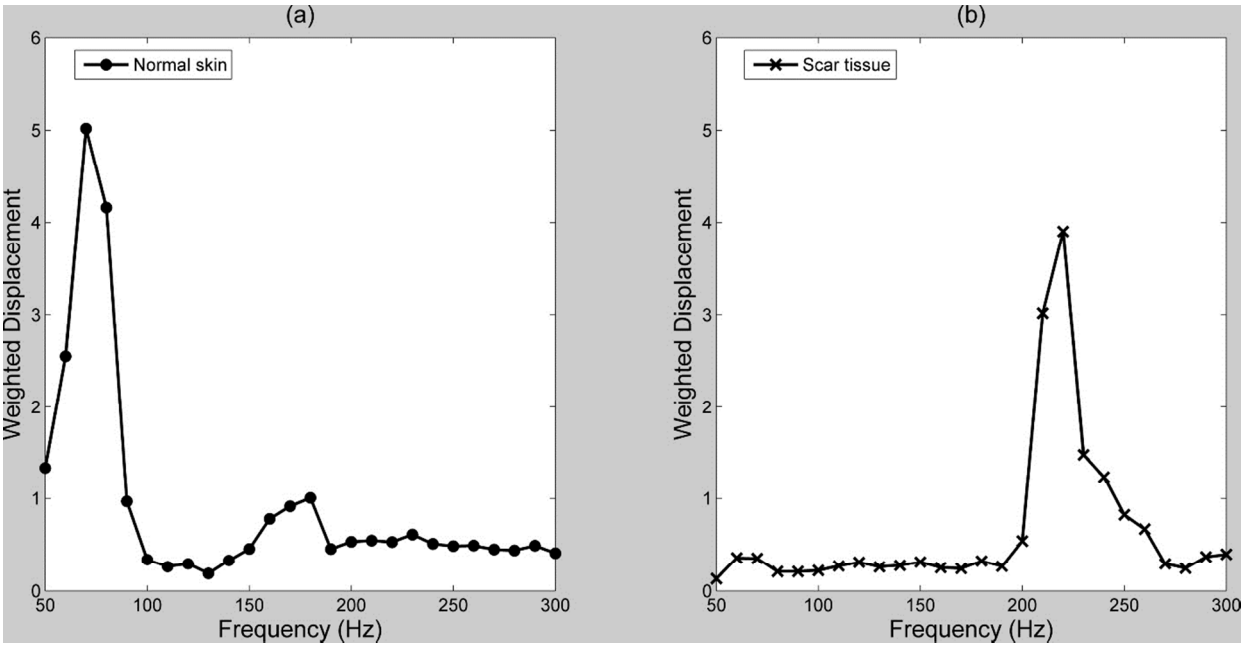

Figure 4. Vibrational OCT measurements on human skin and scar tissue. This figure shows the weighted displacement versus frequency for normal skin (left) and scar tissue (right). The resonant frequencies were 70Hz (normal skin) and 220 Hz (scar tissue). Note small peaks in normal skin at about 180 Hz and in scar at 70 Hz that represent collagen and elastic fiber contributions demonstrating the ability to measure the modulus of each component of a tissue. In scar tissue the elastic tissue contribution to the modulus is lower than that measured in normal skin while at low strains the collagen peak does not show up in normal skin [16]. The collagen peak appears in normal skin after the skin is stretched beyond the normal skin tension. The measurements shown in Figure 4 were made under normal skin tension.

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