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Factors That Influence Muscle Shear Modulus During Passive Stretch

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Abstract

Although elastography has been increasingly used for evaluating muscle shear modulus associated with age, sex, musculoskeletal, and neurological conditions, its physiological meaning is largely unknown. This knowledge gap may hinder data interpretation, limiting the potential of using elastography to gain insights into muscle biomechanics in health and disease. We derived a mathematical model from a widely-accepted Hill-type passive force-length relationship to gain insight about the physiological meaning of resting shear modulus of skeletal muscles under passive stretching, and validated the model by comparing against the *ex-vivo* animal data reported in our recent work (Koo et al, 2013). The model suggested that resting shear modulus of a slack muscle is a function of specific tension and parameters that govern the normalized passive muscle force-length relationship as well as the degree of muscle anisotropy. The model also suggested that although the slope of the linear shear modulus-passive force relationship is primarily related to muscle anatomical cross-sectional area (i.e. the smaller the muscle cross-sectional area, the more the increase in shear modulus to result in the same passive muscle force), it is also governed by the normalized passive muscle force-length relationship and the degree of muscle anisotropy. Taken together, although muscle shear modulus under passive stretching has a strong linear relationship with passive muscle force, its actual value appears to be affected by muscle's mechanical, material, and architectural properties. This should be taken into consideration when interpreting the muscle shear modulus values.

1. Introduction

Although elastography has been widely used for diagnosis of breast cancer, liver fibrosis and thyroid nodules, the musculoskeletal applications are just beginning to be realized. Non-invasive measurements of muscle mechanical properties may provide a quantitative assessment of muscle to assist with diagnosis of muscle disorders and in monitoring disease progression and treatment response. Recently, a state-of-the-art ultrasound elastography technology called Supersonic Shear Imaging (SSI) has been used for evaluating changes in skeletal muscle shear modulus (i.e. an index of stiffness) associated with aging (Eby et al., 2015), neuromuscular (Lacourpaille et al., 2015; Lacourpaille et al., 2014), and neurological (Lee et al., 2015) conditions. Briefly, SSI uses acoustic radiation force induced by a focused ultrasound beam to generate a remote vibration within a target muscle. This vibration results in propagation of transient shear waves in all directions. An ultrafast ultrasound imaging sequence is performed to acquire successive raw radio-frequency data along the principle axis of the ultrasound probe at a very high frame rate (up to 20 kHz). A one-dimensional cross correlation of successive radio-frequency signals is then used to calculate the shear wave propagation speed along the principle axis of the probe (V_s) using a time-of-flight estimation. Two-dimensional maps of the shear modulus (G) can then be directly deduced in real time (<15 ms) by the following relationship (Bercoff et al., 2004):

$$G = \rho V_s^2 \quad (1)$$

where ρ is the muscle density (1000 kg/m³).

Using SSI, our recent experimental works have demonstrated a strong linear relationship between shear modulus and muscle force during passive stretching (Koo et al., 2013) and isometric contraction (Bouillard et al., 2011; Bouillard et al., 2014). These linear relationships have provided the foundation of using muscle shear modulus measurements to estimate muscle

slack length (Koo et al., 2014; Maisetti et al., 2012), understand force sharing strategies during isometric contractions (Bouillard et al., 2014; Bouillard et al., 2012), and gain insight about motor adaptations during experimental muscle pain (Hug et al., 2014a; Hug et al., 2014b; Tucker et al., 2014). Emergence of these novel applications highlights the importance of understanding the physiological meaning of muscle shear modulus. However, to the author's knowledge, little is known about the mechanical, material, and architectural factors that influence the muscle shear modulus values. A better understanding of the physiological meaning of muscle shear modulus will help data interpretation and facilitate its use to gain insights into muscle biomechanics in health and disease.

This study aimed to provide a deeper understanding of the factors that influence muscle shear modulus measured during passive stretching. We derived a mathematical model from a widely-accepted Hill-type passive force-length relationship of skeletal muscle to define the mechanical, material, and architectural factors that could affect the muscle shear modulus values. Validity of the model was evaluated by comparing against the ex-vivo experimental data reported in our previous work (Koo et al., 2013).

2. Methods

Model Development

Numerous studies (see Partridge and Benton (1981) for review) have indicated that passive muscle force increases exponentially with respect to muscle fiber length (l_m). Hence, we started with a widely-accepted exponential relationship (Giat et al., 1994; Zajac, 1989; Schutte, 1992; Buchanan et al., 2004; Koo et al., 2002) to model the passive muscle force-length relationship of a skeletal muscle:

$$F_p(\tilde{l}) = k_1 \cdot PCSA \cdot \sigma_m [e^{k_2(\tilde{l}-1)} - 1] \quad (2)$$

where \tilde{l} is the normalized muscle fiber length (i.e. $\frac{l_m}{l_{m0}}$, l_{m0} is muscle slack length); $PCSA$ is physiological cross sectional area; σ_m is muscle specific tension (i.e. the maximum isometric force exerted by the muscle per unit of PCSA); and k_1 & k_2 are dimensionless constants that govern the shape of the normalized passive muscle force-length relationship.

Based on the PCSA definition of Alexander and Vernon (1975), the relationship between PCSA and anatomical cross-sectional area (ACSA) is given by:

$$ACSA = PCSA \cdot \cos\alpha \quad (3)$$

where α is the muscle fiber pennation angle.

Hence, muscle stress ($\frac{F_p(\tilde{l})\cos\alpha}{ACSA}$) along the long axis of a muscle can be written as:

$$\frac{F_p(\tilde{l})\cos\alpha}{ACSA} = k_1\sigma_m[e^{k_2(\tilde{l}-1)} - 1] \quad (4)$$

Differentiating Equation (4), we have:

$$\frac{d(\frac{F_p(\tilde{l})\cos\alpha}{ACSA})}{d(\tilde{l})} = k_1k_2\sigma_m[e^{k_2(\tilde{l}-1)}] \quad (5)$$

where $\frac{d(\frac{F_p(\tilde{l})\cos\alpha}{ACSA})}{d(\tilde{l})}$ is the Young's modulus (E) of a resting muscle at length \tilde{l} .

Substitute Equation (2) into Equation (5), we have a mathematical model that relates Young's modulus with the passive muscle force along the long axis of a muscle (i.e. $F = F_p\cos\alpha$):

$$E = \frac{k_2}{ACSA}F + k_1k_2\sigma_m \quad (6)$$

It is well recognized that skeletal muscles are anisotropic and hence, $E \approx 3G$ (where G stands for shear modulus) cannot be applied to muscles. However, recent ex-vivo data (Eby et al., 2013) demonstrated that shear modulus measured along the long axis of a muscle is strongly linearly

related to the Young's modulus measured using traditional material testing (the goodness-of-fit R^2 statistics ranged between 0.916 and 0.988 among 4 specimens). Hence, G and E can be related with each other using a linear relationship:

$$G = c_3 \cdot E + c_4 \quad (7)$$

where c_3 (dimensionless) and c_4 (in kPa) are the slope and y-intercept of the linear regression line. These parameters are governed by the degree of anisotropy of a muscle.

Consequently, the relationship between shear modulus (G) and passive muscle force along the long axis of a muscle (F) can be mathematically formulated as:

$$G = \frac{k_2 c_3}{ACSA} F + (k_1 k_2 c_3 \sigma_m + c_4) \quad (8)$$

Model Validation

Validity of the mathematical model derived above (Equation 8) was evaluated by comparing against the ex-vivo experimental data reported in our recent work (Koo et al., 2013). In Koo et al. (2013), we dissected 16 gastrocnemius pars externus (GE) and 16 tibialis anterior (TA) muscles from 10 fresh roaster chickens. We measured muscle mass (m) and the distance between the proximal and distal muscle-tendon junction (l), and used them to estimate anatomical cross-sectional area (ACSA): (i.e. $ACSA = m/\rho l$, where ρ is muscle density which was assumed to be 1000kg/m^3). We also quantified shear modulus of each muscle along its long axis at different passive loads (F) at a location that approximated its maximum thickness. All shear modulus measurements were acquired by an Aixplorer ultrasound system (Supersonic Imagine, France) using a SL15-4 transducer in SWE mode (preset: superficial MSK; SWE opt: penetration; persistence: medium; smoothing: 5; gain: 70%; and HD/Fr rate: balance).

Model validity was evaluated as follows:

(1) If Equation 8 is a valid model of the shear modulus-passive force relationship, we would expect a strong linear relationship between G and F in our ex-vivo experimental data. This was evaluated by fitting a least-squares linear regression line (See Equation 9 below) to the shear modulus-passive force data of each tested muscle and determined the goodness-of-fit of the data by calculating the coefficient of determination (R^2).

$$G = s \cdot F + G_o \quad (9)$$

where s is the slope of the G - F relationship, and G_o is the shear modulus of a resting muscle at its slack length.

(2) If Equation 8 is valid, s and G_o in Equation 9 should correspond to $\frac{k_2 c_3}{ACSA}$ and $k_1 k_2 c_3 \sigma_m + c_4$ respectively. Then we would expect: (a) a positive correlation between s and $1/ACSA$; and (b) no correlation between G_o and $ACSA$ in our experimental data. These were evaluated by computing Pearson's correlation coefficients (r) and subjected them to the test of significance. P values below 0.05 were considered significant.

(3) To verify the model is theoretically sound, we estimated s and G_o by substituting the nominal values of σ_m (225 kPa) (Powell et al., 1984), k_1 and k_2 ($k_1 = 0.129$, $k_2 = 4.525$) (Giat et al., 1994), and c_3 and c_4 ($c_3 = 0.1944$, $c_4 = -3.676$ kPa) (Eby et al., 2013) to Equation 8, and compared with those calculated from the experimental data. We expected that the estimated and measured parameters are at the same order of magnitude.

3. Results

As previously reported (Koo et al., 2013), ex-vivo animal data revealed a strong linear relationship between shear modulus (G) and passive muscle force along the long axis of a muscle (F) for all the 32 tested muscles (R^2 ranged between 0.971 and 0.999) (Fig. 1). Our data also

revealed a significant positive correlation between s and $1/ACSA$ ($r = 0.86$, $p < 0.001$) (Fig. 2) and no significant correlation between G_o and $ACSA$ ($r = -0.21$, $p = 0.25$) (Fig. 3). Using the nominal values suggested in the literature, the estimated G_o was 21.9 kPa, and the estimated s for TA and GE were 0.0071 and 0.0039 mm^{-2} respectively, which were at the same order of magnitude with those calculated from the experimental data (G_o : TA = 25.5 kPa, GE = 24.2 kPa; s : TA = 0.01665 mm^{-2} , GE = 0.00795 mm^{-2}). Coefficient of variation (CV) of s and G_o among the 32 tested muscles were 51.7% and 25.8% respectively.

4. Discussion

Starting with a widely-accepted Hill-type passive force-length relationship of skeletal muscle, we successfully derived a mathematical model that related shear modulus with passive muscle force along the long axis of a muscle. Although inconclusive, our ex-vivo animal data appears to support the validity of the model. First, we found a strong linear relationship between shear modulus and passive muscle force along the long axis of a muscle. Second, our experimental data revealed a significant positive correlation between s and $1/ACSA$ but no significant correlation between G_o and $ACSA$. Third, even without optimizing the model parameters, our estimated G_o and s (based on nominal values reported in the literature) were at the same order of magnitude with those calculated from the experimental data, indicating that the model is theoretically sound.

Lacourpaille et al. (2012) placed the ultrasound probe in the plane of muscle fascicles and measured *in vivo* G_o (i.e. shear modulus of a completely relaxed muscle at zero tension) on 9 human muscles using SSI. They found that their resting shear modulus values (in ascending order: medial gastrocnemius (MG), triceps brachii (TB), biceps brachii (BB), rectus femoris (RF), vastus lateralis (VL), brachioradialis (BR), adductor pollicis obliquus (APO), tibialis

anterior (TA), and abductor digit minimi (ADM)) did not match with their muscle typologies (from the highest percentage of fast fibers to the highest percentage of slow fibers: TB, BR, VL, RF, BB, MG, ADM, TA, APO) (Johnson et al., 1973). These in-vivo human data indicated that G_o may be governed by multiple factors. Indeed, the model developed in this study suggested that G_o is a function of specific tension (σ_m) and parameters that govern the normalized passive force-length relationship (k_1 & k_2) as well as the degree of anisotropy (c_3 & c_4). It has been reported that specific tension of mammalian skeletal muscle from a variety of species generally ranged between 157 and 294 kPa (Close, 1972; Faulkner et al., 1980; Murphy and Beardsley, 1974). However, when the fiber length is normalized to a common sarcomere length (2.2 μm), except for the soleus muscle (a predominantly slow twitch muscle), a common value of 225 kPa has been reported across muscles with mixed fast and slow-twitch fibers (Powell et al., 1984). Hence, we do not expect a substantial effect of σ_m on G_o in muscles with mixed fibers. Conversely, the normalized passive force-length relationship has been shown to vary widely between different muscles and even between different specimens of the same muscle (Brown et al., 1996; Brown et al., 1999), indicating that k_1 and k_2 may have substantial effect on G_o . In addition, the degree of anisotropy may also have substantial effect on G_o because Eby et al. (2013) has showed that c_3 and c_4 could vary widely between specimens (c_3 ranged between 0.1539 and 0.2602, and c_4 between -9.1963 and 0.1928 among 4 swine specimens).

Our mathematical model also suggested that s (i.e. the rate of increase in shear modulus per unit passive muscle force) is proportional to k_2 and c_3 but inversely proportional to ACSA. Our ex-vivo animal data revealed that 74.2% of the variance of s can be accounted for by knowing ACSA. Further analysis revealed that after s was corrected by ACSA, the coefficient of variation of s among the 32 tested muscles reduced from 51.7% to 25.6%. These results indicated

that ACSA should be a primary governing factor of s . However, as discussed above, k_2 and c_3 could also vary widely between muscles.

It has been recognized that shear modulus depends very much on the angle between muscle fibers and transducer direction (Gennisson et al., 2003; Eby et al, 2013). Hence, we anticipate that G_o is related to pennation angle. However, this is not the case according to Equation 8. Failure of our mathematical model to capture this salient phenomenon may be attributed to the fact that the Hill-type muscle model assumes the passive component of a skeletal muscle as a homogeneous non-linear spring, which does not take into account of muscle anisotropy. Taken together, although resting shear modulus under passive stretching has a strong linear relationship with passive muscle force, it appears that both G_o and s are functions of mechanical, material, and architectural properties of skeletal muscles, and hence, shear modulus measurements should be interpreted with caution.

In summary, we developed a mathematical model to gain insights about the physiological meaning of muscle shear modulus measured by SSI. Validity of the model was verified by comparing against the *ex-vivo* experimental data reported in our previous work. Nonetheless, a separate study that quantifies not only G_o , s , ACSA, and α , but also c_1 , c_2 , k_1 , k_2 , and σ_m of the same muscle is needed to fully validate the model.

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Conflict of interest statement

The authors confirm that there is no conflict of interest in this manuscript.

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Figure Captions

Figure 1: Relationship between shear modulus (G) and passive muscle force along the long axis of a muscle (F) of a representative specimen. TA and GE stand for tibialis anterior and gastrocnemius pars externus respectively. Coefficients of determination (R^2) were 0.994 (left TA), 0.996 (right TA), 0.978 (left GE), and 0.992 (right GE) respectively. Although not shown here, strong linear relationship was observed for all the 32 tested muscles.

Figure 2: Relationship between rate of increase in shear modulus per unit passive force (s) and anatomical cross sectional area (ACSA). Given that all except one chicken were similar in weights and only TA and GE muscles were evaluated, the clustering appearance of the data was well-anticipated. Nonetheless, a significant positive correlation between s and $1/ACSA$ was noted ($p < 0.001$).

Figure 3: Relationship between shear modulus at its slack length (G_o) and anatomical cross sectional area (ACSA). No significant correlation between G_o and ACSA was noted ($p = 0.25$).

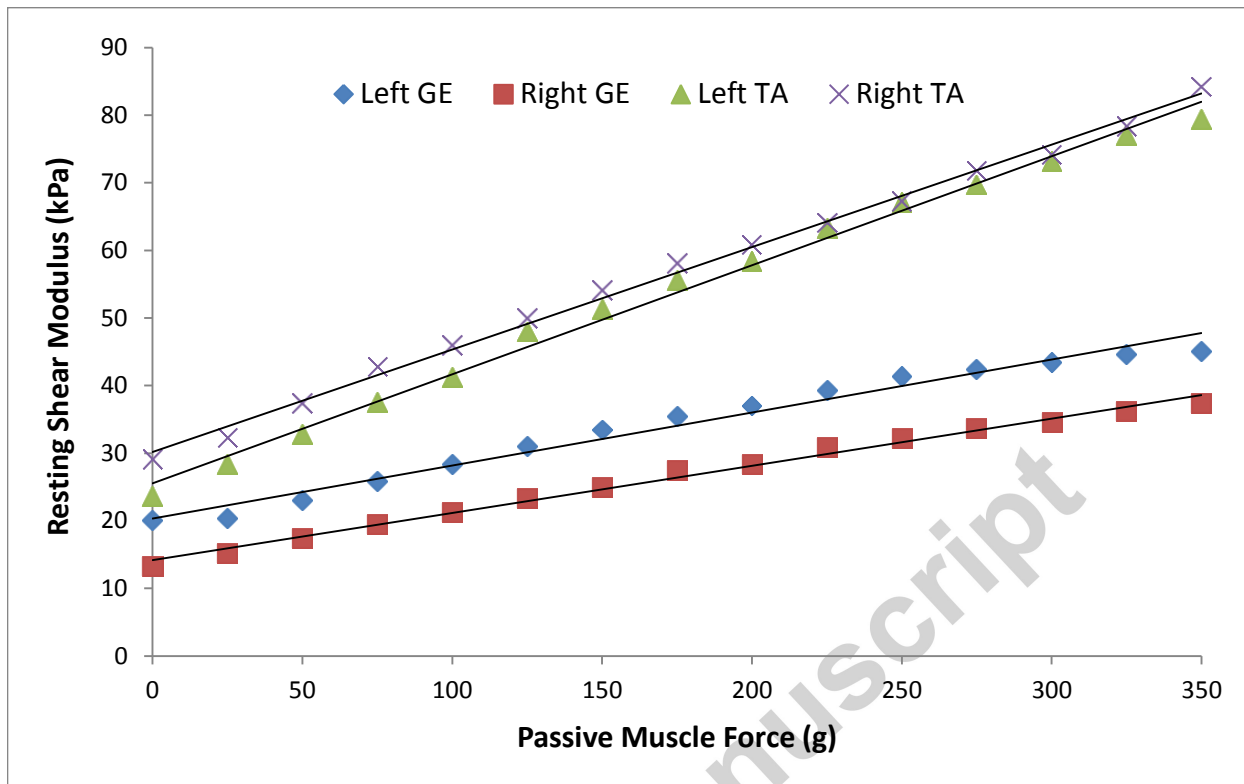


Fig. 1

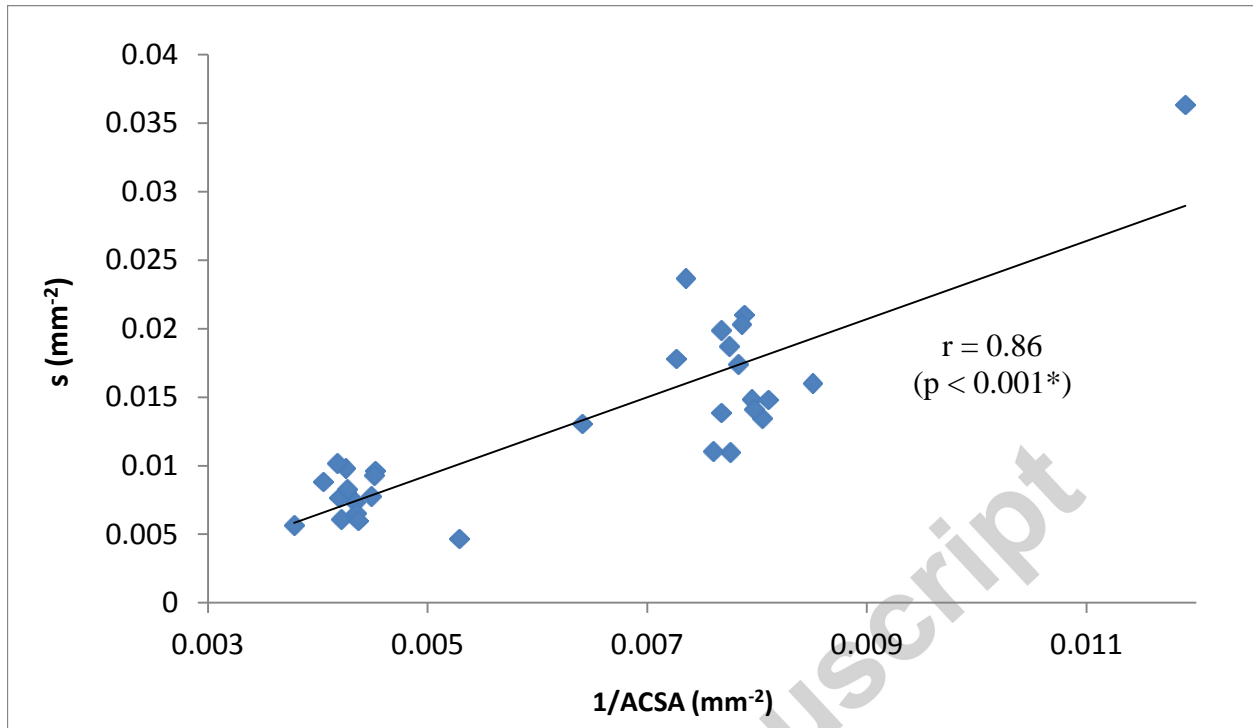


Fig. 2

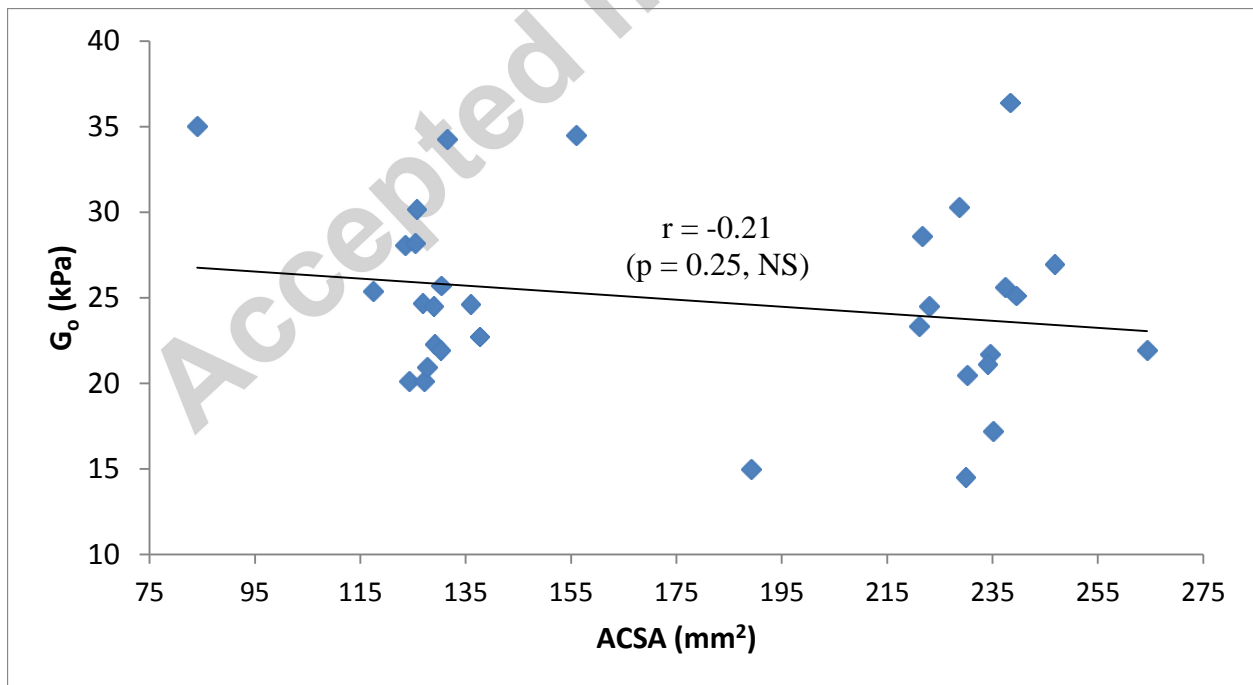


Fig. 3