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Passive Mechanical Characterization of Human Skeletal Muscle Rectus Femoris of Sri Lankan Test Subjects

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Abstract: Human Musculoskeletal System is involved in activities of daily living. The mechanical and numerical modeling of the skeletal muscles are important in sports medicine, orthopedics, forensic medicine and tissue transplant surgeries. This is the inaugural study of this nature in Sri Lanka and the objective of the study is to assess the mechanical properties of "Rectus Femoris" muscle. It was known that mechanical properties, including the toughness of skeletal muscle tissue, vary with the location of the sample along the muscle. Hence, the properties are highly inhomogeneous. Nevertheless, it was observed that the evolution of 'Damage' parameter of the samples is comparable in all samples. These observations are in contrast to traditional engineering materials used in design and manufacturing. It was also observed that properties vary with the sample of each cadaver. The main reasons for this inhomogeneity are due to vivid factors including medical condition, medicinal usage, nutrient level, physical exercises and genetics based factors of the soft tissue samples, etc. The study can be extended to other skeletal muscles to extract mechanical properties, and these properties are vital specifically in the selection of bio-compatible biomaterial to be used in Tissue Transplant Surgeries, and the data extracted are highly useful in Sport medicines, Orthopedics and Forensic Medicine and Tissue Transplant Surgeries.

Keywords: Uniaxial tensile test, Skeletal muscle issue, Rectus femoris, Soft tissue damage

1. Introduction

A fundamental difference within the broad domain of biological organisms, such as plants and animals, is the "Mobility". Within the animal kingdom, the aspect of "Mobility" could occur in various forms depending on the size of the animal species, ranging from micro (bacterial) to macro (human) level. Undoubtedly, soft biological tissues portray a momentous role for macro level species in "Mobility". In human body, there are three major types of muscles. They are skeletal cardiac and smooth muscles. Skeletal muscles are voluntary muscles where the movements are under voluntary control whereas the other two types are not. From a structural aspect, human skeletal muscle possesses a graded composite material system with multi-nucleated muscle fibres arranged into fascicles and eventually to muscles. These sub-constituents (muscle fibers, fascicles and whole muscle) are covered with endomysium, perimysium and epimysium (a network of collagen), respectively [4].

When it comes to human beings, skeletal muscle activity is vital for survival; from breathing to rapid activities such as sprinting, jumping and gait stability [3].

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Human mobility in regular life events and sporting endeavors would be greatly affected by virtue of convenient/prompt force generation within muscle fibers, skeleton, muscles, cartilage, tendons and other connective tissues that bind vital organs and complete structure.

Dysfunction and/or partial failure of the aforementioned counterparts are due to numerous diseases and accidents caused by motor vehicles or through sporting activities. This leads to surgical and non-surgical aids arising through the branch of surgery called "Orthopedics". Notwithstanding, for the treatment of certain modalities of carcinoma, replacement of the carcinogenic segment (biological material) with artificial bio-material is supposed to be one of the ideal methods to improve patient's quality of life during the postliminary stage of a surgery. Prior to replacement, selection of a bio material requires systematic characterization of the biological material. This will ensure that the potential bio material will mimic the biological material to be replaced under different physiological conditions inside the body. However, it was noticed that use of cadaveric samples in characterizing mechanical properties of soft tissues has never been performed in Sri Lanka and rarely noticed in international biomechanics research. In terms of literature data available worldwide, properties of soft tissue will be subjected to differ by many factors including the demographic area (genetics), age, sex and medical history of the cadaveric samples. Hence it is vital to assess these aspects in characterization of native soft tissues to distinguish available data in literature. In this preliminary study, uniaxial tensile tests were performed on cadaveric samples and mechanical properties of soft tissues were probed. Data extracted from uniaxial tensile tests were thoroughly analyzed through in-house non-linear optimization algorithm to extract important mechanical properties related to soft tissues.

2. Literature Survey

The forces generated by soft skeletal muscles can be additively decomposed into Active and Passive components according to Hill type muscle model. Physiologically, the passive response from the soft tissues arises due to the elastic spring like elements if stretched beyond its resting length. Active response is developed within the sarcomere, a sub unit in the muscle fiber. Characterization could be done either *in vivo* [13] or *in vitro* [14] techniques of human soft tissues, through appropriate deployment of live human subjects or surgical removal of the required soft tissue, respectively. Nevertheless, the passive response is measured through cadaveric samples and the active response is measured through twitches originating from the specific live muscle [15]. In literature, the passive response of the soft tissues mainly attained from animals [6], [7], [8], [9], plausibly due to adversity in attaining human cadaveric samples. The soft tissue will be loaded through different modes in the form of compression or indentation [10], [11] and more frequently in tensile mode [12]. Compression of soft tissue has been predominantly performed in two ways: confined and unconfined compression. In the confined case, the tissue is placed on a porous plate during compression while in the unconfined case, it is placed on a non-porous plate [16], [17]. In judgement on these studies, it is crucial to understand the mechanical behavior of soft tissues which is distinctively different from traditional engineering materials such as metals, ceramics and some industrial polymers. Human soft tissues are time dependent, inhomogeneous, non-linear and anisotropic, in nature. They are heterogeneous composite material though assumed to be uniform on the continuum scale. The specified approach is adequate in predicting mechanical properties of the overall tissue, whereas one may require to consider exclusive details of the tissue (fiber orientation, fiber composition and bi-phasic nature of the tissue) if leaping into smaller scale (micro level). For profound understanding at nano level sub structural components of each muscle fiber, such as actin filament and myosin heads in sarcomere unit should be considered in association with Sliding Filament Theory [1]. Nevertheless, force generation at nanoscopic level is mainly focused on active response rather than passive response of soft tissues [15]. Notwithstanding, young's modulus typically plays an important role in understanding tensile properties of a tissue through analyzing the linear portion of the stress strain curve.

Rectus femoris is a long muscle located in front /anterior compartment of the thigh. It is fusiform in shape. Rectus femoris flexes the hip along with the sartorius and iliopsoas and it extends the lower leg at the knee, working in conjunction with the other three quadriceps muscles [24]. During forceful exercise or trauma, the muscle can tear at either from proximal or distal end, leading to a debilitating injury and also severe pain [25].

Within the domain in Bio-Mechanics, there are few studies reported in relation to mechanical property measurement of rectus femoris muscle. A study was done by Enomotto et al. [26] comparing stiffness of 28 rectus femoris muscles between samples with Osgood – Schlatter Disease (OSD) and without OSD using shear wave elastography. Several years earlier, Agyapong-Badu et al. [27] measured ageing effect of muscle tone and mechanical properties of rectus femoris using healthy males and females with a novel hand held myometric device. Although specific studies adopt non-destructive methods (elastography related methods) in their evaluations of shear modulus, destructive methods in evaluating direct measurement of yield stress, Young's modulus and other basic parameters under passive conditions for rectus femoris muscle are seldom found, apart from the study done by Zhu et al. [28] with the use of mechanical testing machine but it was done for rectus femoris tendon. The outcome through this current study could be used to understand the macroscale mechanical properties of *rectus femoris* muscle tissues through adopting uniaxial tensile load and possibly adopting in clinical, surgical and related bio-material synthesis.

3. Experimental Methodology

For the present study, ethical clearance was obtained from Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (No–43/20). Five samples were prepared from cadavers of the age 60 (Female) – 65 (Male). The complete soft tissue - "Rectus Femoris" was excised (In - Vitro) [19] from fresh postmortems and were transferred to Department of Mechanical Engineering, University of Moratuwa in normal saline at 4°C (in ice) within 2-3 hours to preserve the freshness. Each of the complete cadaver samples were split into separate samples based on the experimental requirement by surgical knife (Sample 4 – Cadaver – 1 and Samples 1, 2, 3, 5 from Cadaver -2). The length, width and thickness of each sample were measured (Table 1). Unlike general engineering materials, acquiring human soft tissue samples for characterization is arduous due to fitness of the sample for characterization and various ethical concerns. Uniaxial tension was adapted by materials testing machine Testomeric (M350-10AT) that has highest load capacity of 10 kN. The Rectus femoris superficial fibers were arranged in a bipenniform manner while the deep fibers were running straight down. In this experiment, the tension was applied along the long axis of the muscle. It was recognized that soft tissue samples would not be able to hold through conventional tensile grips. This was due to inherent low stiffness of the samples. Large clamping forces on soft tissue with serrated/pyramid grip surface would lead to irreversible damage / premature failure and, in turn, would lead to erroneous readings due to spurious localized stress concentrations. This was noted in similar studies in the literature [18]. A novel approach in holding the tissue firmly in the assembly was regulated through the use of surgical 'Straight Artery Forceps'. These forceps carry diamond marks on the grip surface, hence the tissue does not slip with tension. Consequently, the assembly of tissue with the forceps were attached to the tensile apparatus with the aid of in-built tensile grips. The arrangement is illustrated in Figure 1. In adopting uniaxial tensile testers in characterizing soft biological tissues, it is crucial to mask all mechanical parts of the equipment from fluids emanating from the tissue due to possible corrosion of the parts. In par with that caution, all the parts were masked accordingly for sustainable use of the machine in our future studies.

Table 1 - Dimensions of Soft Tissue Samples

Sample No	Sample Length (mm)	Cross Sectional Area (mm^2)	Cadaver Sample No
1	70	400	2
2	75	400	2
3	80	400	2
4	45	1575	1
5	70	400	2

Figure 1 - Soft Tissue Tension Setup with Straight Artery Forceps

Soft tissues could not be cut according to traditional tensile specimen geometry (dog – bone shape) due to challenges in preserving the geometry due to soft, and fragile nature of the sample. Displacement rate of the crosshead was set to be 25 mm/min; nonetheless, strain rates of the soft tissue samples were differed for certain samples by ~40% due to different lengths of the samples (Table 1). During specimen preparation, unlike for traditional samples, the length of the samples needs to be determined by the fitness of the sample to the experiment as the samples are extracted from cadavers. However, all the strain rates were maintained under quasi-static range (Table 2).

Table 2 - Strain Rates of the Samples under Uniaxial Tension

Sample No	Strain Rate (1/s)
1	0.00926
2	0.00556
3	0.00521
4	0.00926
5	0.00595

In literature, some authors have conducted uniaxial tension studies through "*pre-conditioning*" of the soft tissue through several loading and unloading cycles. Nonetheless, this procedure may not be appropriate for surgical and medical applications because mechanical responses during surgery are mainly due to virgin state rather than pre-conditioned state [19].

4. Shear Modulus of Soft Tissue

Based on the geometry of the rising segment of Force Vs Displacement curves, Sten – Knudsen et al. [23] proposed a methodology to ascertain the shear modulus of soft tissue based on the following expression.

$$F = 3\mu S_0 \alpha^{-1}\left(e^{\frac{\alpha d}{l_0}} - 1\right) \qquad \text{...(1)}$$

In Eq. (1) μ will resemble shear modulus for soft tissue as the muscles are considered incompressible and the Poisson's ratio is ~ 0.5. α is a dimensionless parameter called a curvature parameter. S_0, l_0 and d depict original cross sectional area of the muscle, initial length of the muscle and the displacement during the tensile test, respectively. μ and α were calculated for each curve using in house non-linear optimization algorithm.

5. Damage Assessment of Soft Tissue

Damage is the creation and growth of microvoids or microcracks in a solid material in which the medium is considered as continuous at a larger scale. Damage will decrease elasticity modulus, hardness, density and yield stress. In assessing damage of soft tissue under uniaxial loading, isotropic damage was considered. Damage variable (D) for isotropic scenario can be calculated by the following equation.

$$D = 1 - \frac{\bar{E}}{E} \qquad \text{... (2)}$$

In Eq. (2), E resembles Young's modulus of undamaged status of the material while $\bar{E}$ resembles actual Young's Modulus of a damaged material. In this effort, $\bar{E}$ was calculated as a secant modulus, by considering the reference at the origin of the elastic region (Figure 3). Undamaged Young's modulus (E) was considered through the assessment of the slope of elastic region of each curve with the aid of non-linear optimization algorithm.

6. Results and Discussion

6.1. Analysis of Stress Vs Stretch Ratio Curves and Young's Modulus of *Rectus Femoris* Soft Tissue

All the soft tissue samples were tensioned till the tissues were completely ruptured. Data retrieved in the form of force Vs displacement were converted to Eularian Stress Vs Stretch Ratio data and plotted as in Figure 2. Average curve was determined through, initially fitting a curve through non-linear regression for each stress –strain curve. This methodology is important in determining the average stress – stretch response as reading of forces is not normally obtained for a unique displacement value in each experiment. Fitness of the functions was assessed through R^2 values.

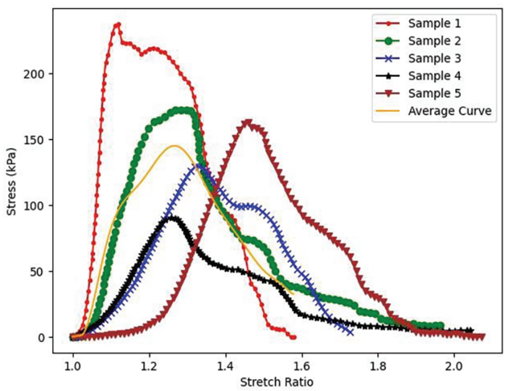

Figure 2 - Stress vs Stretch Ratio Curves for all the Soft Tissue Samples

To perceive the information in Figure 2, it is vital to understand different phases during the tensile loading regime. According to Holpzafel et al. [20] the loading regime can be divided into three phases as shown in Figure 3. All these three phases are realized from each curve of Figure 2. In average sense from tested values, the ultimate tensile strength of rectus femoris tissue for the selected age and gender is ~ 150 kPa; though the properties of rectus femoris muscle are scarcely found in the literature.

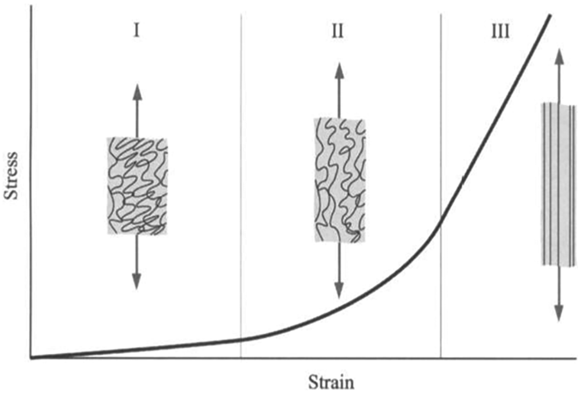

Figure 3 - Phases during the Initial Loading Regime [20]

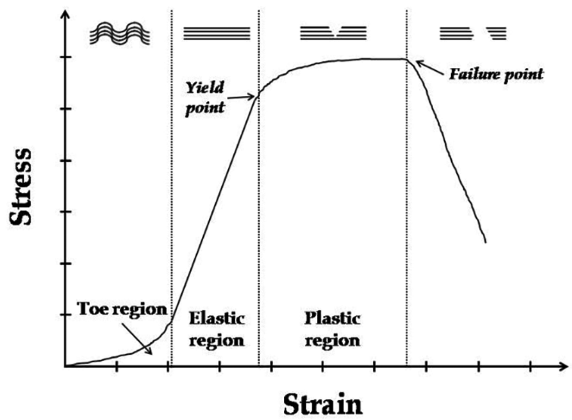

Figure 4 - Typical Stress vs Strain Curve for Tensile Tests of Skeletal Soft Tissue [21]

From Figure 2, with the focus on general appearance of the curves, it is evident that all possess a unique pattern at the earliest portion of each curve. This initial J – shaped portion of these curves is due to crimped collagen fibers being gradually elongated and later becoming straighter with larger stress response. It is also important to note that elastin fibers are mainly responsible for the stretching mechanism till the yield point (Figure 4). Elastin is a major protein which is a constituent of extra cellular matrix that exists as thin strands. For more details on elastin and collagen on soft tissues, readers are requested to follow Aziz et al. [22].

Table 3 illustrates parameters of the stress – strain curve from our tensile experiment.

Table 3 - Key Parameters from Stress – Stretch Ratio Curves

Sample No	Yield Point (kPa)	Failure Point (kPa)	Plastic Range	Elastic Range
1	208.4	238.5	0.030	0.052
2	154.2	173.6	0.109	0.121
3	123.0	130.1	0.032	0.137
4	87.7	92.8	0.033	0.124
5	158.3	163.9	0.037	0.175

Within the elastic region of the curve, Young's modulus was calculated by fitting a straight line as shown in Figure 5.

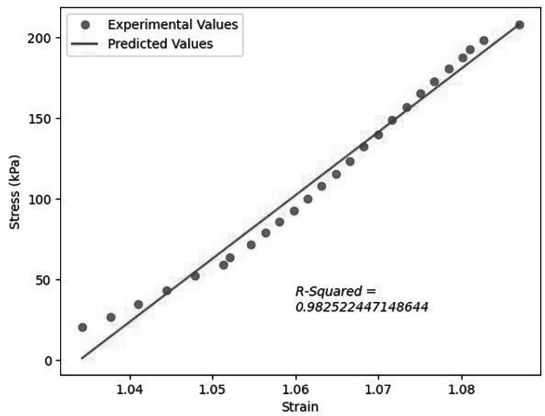

Figure 5 - Young's Modulus Calculation through Linear Regression Analysis

Young's modulus of values determined for all the curves are shown in Table 4.

Table 4 - Young's Modulus of Linear Regions from Stress – Stretch Ratio Curves

Sample No	Young's Modulus (MPa)	R^2 - Value
1	3.927	0.98
2	1.114	0.99
3	0.571	0.99
4	0.493	0.99
5	0.75	0.99

Specifically, in analyzing the data, it is wise to compare curves that have same strain rate and obtained from the same cadaver. Hence, samples 2, 3 and 5 can be comparatively studied. The data from Figure 2, and Tables 3 and 4, for the respective samples do not reveal a significant pattern with respect to the effect of collagen fiber content / elastin content etc. To obtain a clear understanding on the influence of these, further similar studies should be conducted for a specific age, gender and a strain rate. Nevertheless, it was reported by Korhonen et al. [21] that failure stress level depends on the amount of collagen fiber content for a given tissue volume. Correlating samples 1 and 4 (responses with same strain rate amid two different cadavers) with the data analyzed (Table 3, Table 4, Figure 2) illustrate feeble response from the cadaver 1. Notwithstanding, it was evident that properties witnessed from cadaver 2 change widely for each sample. Hence at this juncture, it is not possible to comment in comparative nature with cadaver 2 about the overall mechanical properties of the soft tissue of cadaver 1. This will also speculate that single soft tissue sample from a particular cadaver is inhomogeneous and the composition / constituents (and hence the properties) are highly dependent on the age, physical and chemical environmental factors, and genetic factors of the cadaver sample [18]. Hence difficult to present distinct / invariant values for mechanical properties of biological soft tissues. This is a distinct character in studying soft tissue mechanics and general engineering materials.

6.2. Toughness Analysis of *Rectus Femoris* Soft Tissue

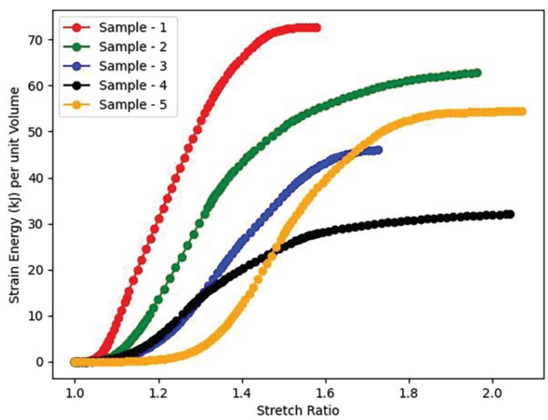

Figure 6 - Strain Energy Variation with Stretch Ratio for all Samples under Uniaxial Tension

Aligned with Figure 2, it is noticeable that the sample with highest failure point has highest cumulative strain energy (from Figure 6) and this descending trend between failure point and cumulative strain energy is followed notably by samples 1, 2, 5, 3 and 4, respectively. An important consideration in this aspect is that the level of failure point of each sample depends on following factors: structurally, by the amount of collagen fibers present in the tissue [21] and mechanically, by the applied strain rates [21]. The maximum value represented by each curve on Figure 6 represents "Toughness", the ability to deform and to absorb energy before fracture of the soft tissue. These values are tabulated as shown in Table 5.

Table 5 - Toughness of Soft Tissue Samples

Sample No	Toughness (kJ / m^3)
1	72.75
2	62.88
3	46.05
4	32.16
5	54.43

6.3. Shear Modulus of *Rectus Femoris* Soft Tissue

Shear modulus of each soft tissue sample was calculated using Equation (1). These values in soft tissues are crucial in bio-mechanical studies in assessing resistant to shear loadings. Values shown in Table 6 were selected based on the fitness value obtained during the non-linear regression analysis.

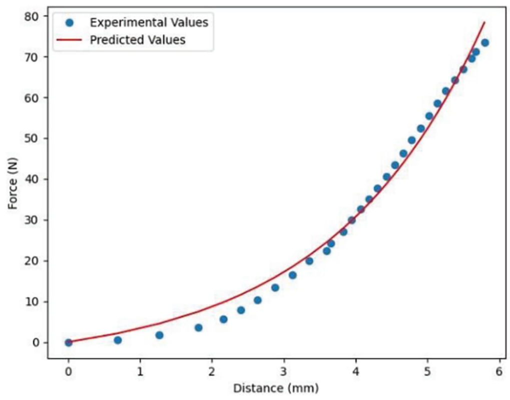

Figure 7 - Curve Fit between Prediction Vs Experimental Values in Determining Shear Modulus for Sample – 1

Table 6 - Fitting Parameters for Soft Tissue Samples for Equation 1

Sample No	Shear Modulus (MPa)	Curvature Parameter (α)
1	0.1522	32.79
2	0.2175	3.91
3	0.0564	3.92
4	0.0161	3.75
5	0.0125	7.27

Shear modulus values shown in Table 6 are predicted based on the curved portion of the Force Vs Displacement data obtained for each sample. Once more it is apparent that samples from same cadaver possess diverging values. This divergence is apparent due to variation of collagen, elastin and other sub-constituents of the soft tissue.

6.4. Assessment of Damage of *Rectus Femoris* Soft Tissue under Uniaxial Loading Condition

Degradation of soft tissues could occur biochemically in *in-vivo* status with age. From mechanical degradation perspective, it could occur if the soft tissue is loaded beyond the physiological limit at an ad-hoc event such as sporting or road accident; else it could happen under loading conditions during different surgical procedures. Assessment of '*Damage*' under these load conditions in *in – vitro* conditions would enable the clinicians to investigate the effect of different physiological factors on '*Damage*', hence could plan procedures accordingly. Initially, at each strain point secant modulus was calculated. Then the evolution of secant modulus estimated was used to calculate '*Damage*' variable (Figure 8).

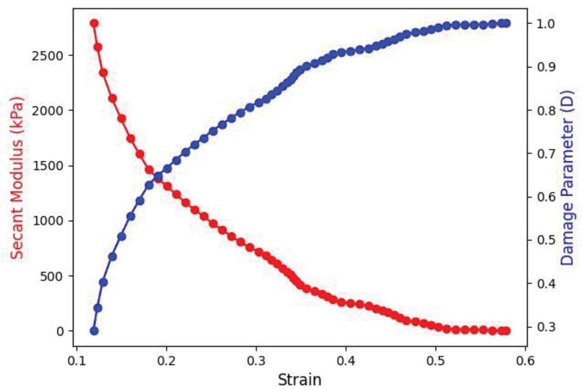

Figure 8 - Variation of Secant Modulus and Damage Variable with the Deformation for Soft Tissue Sample - 1

With this approach, one could analyse the evolution of '*Damage*' throughout its deformation. In this analysis, degradation of the stiffness of the soft tissue due to age factor was disregarded as sufficient data through uniaxial tensile tests for the *rectus femoris* tissue are not available for early ages for different genders. '*Damage*' level of virgin specimen (un-damaged) is considered to be zero while complete fracture of the material is resembled by '*Damage*' parameter of 1. From Figure 8 it is figured that the manner in which the *Damage* parameter increased while reduction of secant modulus or current stiffness of the soft tissue with deformation. This sequence was realized with all the soft tissue samples used. Figure 9 illustrates the variation of '*Damage*' variable of all soft tissues considered.

It is apparent from Figure 2 that mechanical degradation of the soft tissue initiated at distinct deformation stages. Hence, as witnessed in Figure 9, '*Damage*' variable begins to be visible at the corresponding strain / stretch level at which degradation outsets in Figure 2.

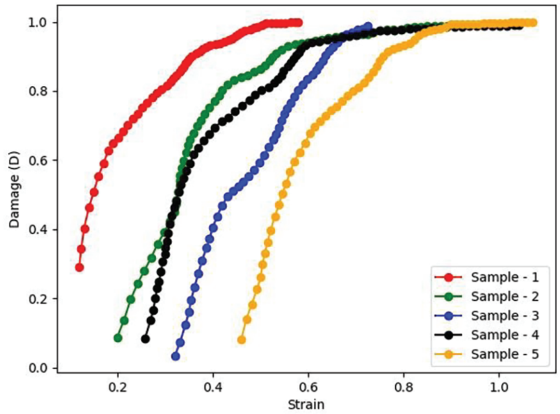

Figure 9 - Damage Evolution with the deformation of all Soft Tissue Samples under Uniaxial Loading Conditions

Figure 9 illustrates that sample 1 incur some *Damage* ~ 0.3, higher initial '*Damage*' status at low strain level compared to other samples. '*Damage*' initiation at a low strain level could be an inherent soft tissue property of sample 1. But higher initial damage at such low strain value is an issue due to low data points in between failure point and the immediate data point of sample – 1. More data points in between would earn a '*Damage*' evolution curve from zero.

7. Conclusions and Future Works

In-Vitro studies on mechanical characterization of soft tissues have not been conducted in Sri Lanka. The mechanical properties of soft tissues are crucial in design of bio-medical implants and soft tissue-mimicking bio materials. They are even crucial in certain surgical procedures to minimize the invasive damage through numerical simulation approaches. The presented knowledge is an initiative in Sri Lanka and shall proceed to numerical analysis with promising experimental data. One of the major challenges in soft tissue based experiment is the availability of convenient samples that would adhere to the standards of the test procedure. Unlike traditional engineering materials, biological soft tissues from cadaver should be considered for an experiment after obtaining ethical clearances. Besides these challenges, the obtained material properties are useful for surgeons / clinicians / physio therapists to study the degradation of the strength of the *rectus femoris* muscle tissue under the deformation region within physiological region of the deformation or above that limit. Subsequently, structure/ property relationship with the gender / medical history and genetic information of the soft tissue can be confirmed. Additional properties of the soft tissue that manifest viscoelastic behavior such as creep, stress relaxation and dynamic mechanical behavior can be studied from a different test procedure. This enumeration is designated as future studies. The study can be further widened through extraction of *rectus femoris* tissue from different age limits from both genders with different medical conditions.

It was found that mechanical properties of the same cadaver muscle tissue rectus femoris, are dissimilar for different samples considered from different locations of the same muscle. This makes soft tissues to be inhomogeneous in nature. Unlike in traditional engineering materials, mechanical properties of soft tissues should be accompanied by the specific location details of the soft tissue with the soft tissue sample size. Notwithstanding, it was observed that Damage evolution pattern for all the tissues are equivalent. The inhomogeneous nature of the human skeletal muscles tissues is an important factor to be considered in selecting compatible biomaterials to be used in Tissue Transplant Surgeries, and the data extracted are highly useful in Sport medicines, Orthopedics and Forensic Medicine and Tissue Transplant Surgeries.

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