Experimental Study of a Biomechanical Behaviour of Rat Patellar Tendon

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Abstract

Tendon is important structure of the human body, since it can sustain tensile loading. The primary function of this tissue is to stabilize the joints they attached to it during daily activities. As well as, tendon has viscoelastic properties that can determine their response to loading and restrict the potential of injuries. One of the major points that this paper works with is the study of the biomechanical behaviour of tendon in response to tensile loading to describe their biological behaviour. Also, conclude the mathematical expression that may illustrate the tendon behaviour. All of the experiments were made in Physiology laboratories / Medical College/ Al- Nahrain University on ten rats "Rattus Norvegicus" of [108- 360] gm weight for in- vitro tensile test. So that 20 specimens were dissected from the rat knees, for the patellar tendons which always hydrated to prevent the tissue dryness. The results of the study, shows the behaviour of the tendon in response to tensile loading with two techniques; the dead loads technique and the continuous loads technique. The stress- strain relationships were also evaluated, as well as, the modified superposition theory was applied at different strain levels to the patellar tendon. The obtained results show that the modified superposition theory gives good results that are partly similar to the experimental results. Also, the tendon shows longer initial pattern than that for the ligament due to the presence of higher elastin content in the tendon than in the ligament.

Key words:

Patellar tendon, Biomechanical behaviour, Viscoelastic properties, Modified superposition theory.

دراسة عملية للسلوك الميكانيكي الإحيائي لوتر الجرذ الرضفي

الخلاصة

الوتر هو نوع من الأنسجة الحيوية الموجودة في جسم الإنسان يصل العضلات بالعظام ويعتبر جزء مهم من تراكيب جسم الحيوانات الثديية، من حيث قابليته على تحمل قوى الشد. الوظيفة الرئيسية لهذا النسيج هي تثبيت المفاصل التي يتصل بها خلال قيام الإنسان بنشاطاته اليومية. كذلك يملك الوتر خواصا (viscoelastic) معتمدة على الزمن كمعظم الأنسجة الحية التي تبين استجابة الوتر لتأثير القوى وكيفية تجنب حدوث الإصابات. لهذا فان الهدف الرئيسي لهذا العمل هو دراسة الخواص الميكانيكية الإحيائية للوتر من حيث استجابة النسيج لقوى الشد وصياغة تعبير رياضي يوضح خصائص الوتر. أجريت جميع التجارب الملية في مختبرات قسم الفسلجة في كلية الطب/ جامعة النهرين باستخدام الحيوانات المختبرية (الجرذ) الذي يتراوح وزنه (108 -360)غم، حيث شرحت 20 عينة للأوتار الرضفية من مفاصل ركب الجرذان ورطبت باستمرار أثناء إجراء التجارب لمنع جفافها. نَتائِج الدراسةِ، بينت سلوكَ الوترِ استجابة لقوى الشد باستخدام تقنيتين؛ تقنية الأحمال الميتةِ وتقنيةِ الأحمال المستمرةِ. كذلك عِلاقات الإجهادِ المطاوعة قُيّمتْ أيضاً، بالإضافة إلى ذلك طبقت نظرية (modified superposition) بمستويات الإجهادِ المختلفةِ إلى نتائج الوترِ الرضفي. أظهرت النَتائِجَ المحسوبة نظريا بأنّ نظرية (modified superposition) تُعطي نَتائِجَ جيدةَ وهي مقاربة للنَتائِج المحسوبة عمليا.

Introduction

Tendon has similar structure to the ligament. Tendons vary in form, and can be rounded cords, strap like bands or flattened ribbons. When healthy they appear brilliant white, and have a fibroelastic texture. ***Structurally***, tendon is composed of tenoblasts and tenocytes lying within a network of extracellular matrix (ECM). Tenoblasts are immature tendon cells. They are spindle-shaped, with numerous cytoplasmic organelles reflecting their high metabolic activity. As they age, tenoblasts become elongated and transform into tenocytes. These have a lower nucleus to cytoplasm ratio than tenoblasts, with decreased metabolic activity. Together, tenoblasts and tenocytes account for 90- 95% of the cellular elements of tendons. The remaining 5-10% of the cellular elements of tendons consists of chondrocytes at the bone attachment and insertion sites, synovial cells of the tendon sheath, and vascular cells, including capillary endothelial cells and smooth muscle cells of arterioles. Tenocytes synthesize collagen and all components of the ECM, and are also active in energy generation. Tenocytes and tenoblasts lie between the collagen fibres along the long axis of the tendon. The dry mass of human tendons is approximately 30% of the total tendon mass, with water accounting for the remaining 70%. Collagen type I accounts for 65-80%, and elastin accounts for approximately 2% of the dry mass of tendons as shown in fig (1) [P. Sharma and N. Maffulli, 2006]. Tightly packed tropocollagen molecules; approximately 1.5 nm in diameter, aggregate into groups of five, becoming microfibrils of approximately 3.5 nm in diameter. The microfibrils group in to subfibrils, which, in turn, aggregate to form fibrils. Fibrils are approximately 50-500 nm in diameter with a periodicity (spacing) of 64 nm. Fibres are an aggregation of fibrils and are 50-300 μ in diameter [Benno M. Nigg and Walter Herzog, 1999]. They are the smallest unit of collagen hierarchy that can be seen under light microscope. Fibers have an undulating crimp with a distance between amplitudes of about 50 μ. The crimp period can vary dramatically in different location within the ligament. Fibroblasts tend to align in rows between fiber bundles and are elongated along the long axis in the direction of normal tensile stress.

Protein also presents in ligament and tendon which is called Fibronectin; this protein contains about 5% carbohydrate. This protein is found in small quantities in the matrix, usually in association with several other matrix component and blood vessels. Fibronectin also interacts with portions of the cell surface that are known to attach to intracellular elements, possibly forming part of an important matrix-cell feedback mechanism [Benno M. Nigg and Walter Herzog, 1999].

Water makes up about two-thirds of the weight of normal ligaments; 70 to 80% of the remaining weight is made up by the fibrillar protein collagen [*Jeffrey A. Weiss and John C. Gardiner,2001*] Water can be associated with other ligament components in a variety of ways. It can be structurally bound to other matrix components. It can be bound to polar side chains, be so-called "transitional water" (loosely bound) or be freely associated with the interfibrillar gel. Most water in ligaments is freely bound or transitional. Although the exact function of water in ligaments is unknown, it appears to be crucial for at least three main reasons:

- Its interaction with the ground substance and particularly the proteoglycans influences the tissue's viscoelastic behavior.
- It seems to provide lubrication and facilitate inter-fascicular sliding.
- It carries nutrients to the fibroblasts and takes waste substances away.

A study on the effect of tissue hydration shows that the structural properties of human patellar tendon are more sensitive to time when the tissues are fully hydrated [Tammy L. Haut and Roger C. Haut, 1997]. ***Elastin*** is an elastic substance that is found in very small amounts in most skeletal tendons in fibular form. However, elastin fibers are about twice as common as collagen fibers. The role of elastin is probably related to recovering tendon length after stress is removed. Elastin "protects" collagen, at least at low strains [Benno M. Nigg and Walter Herzog, 1999].

Physical Properties

The physical properties of all soft connective tissues can be classified into two general categories: structural and material. The structural properties of a tendon are derived from the behaviour of a tendon-muscle complex. The material properties describe the material

irrespective of geometry. They are usually measured in the midsubstance of the tendon [Benno M. Nigg and Walter Herzog, 1999]. The load-deformation curve of all collagen-based tissues have a characteristic upwards concave shape In which the stiffness varies non-linearly with force (fig (2)). This curve consists of the regions as follows:

- Toe region: which is the initial portion of the curve, the tendon deform easily without much tensile force.
- The linear region: which is the second portion of the curve followed the toe region in which the load and deformation are approximately linearly related; the slope of this region is often used to represent the elastic stiffness of the tissue.
- Microfailure region: which is the third region of the curve followed the linear region in which sharp falls in tensile force are seen, presumably as a result of a sequential microfailure of collagen fibers.
- Failure region: which is the last region of the curve in which further loading causes the force reduces to zero as the tissue fails completely.

As the force or displacement increases, however, the tendon stiffens, providing more resistance to deformation ensures efficient load transfer from muscles to bones [C. Ross Ethier and Carig A. Simmons, 2008].

Constitutive Equations

The elastic modulus was computed from the stress- strain curve, which represent the slope of the linear part of the curve [P. P. Provenzano, 2002].

$$E = \frac{\Delta\sigma}{\Delta\varepsilon} \quad (1)$$

Where, E is the elastic modulus. σ is the measured stress and ε is the measured strain, both can be calculated by [P. P. Provenzano, 2002],

$$\sigma = \frac{F}{A} \quad (2)$$

Where, F is the measured force and A is the calculated area [P. P. Provenzano, 2002].

$$\varepsilon = \frac{\Delta l}{l_o} \quad (3)$$

Where, Δl is the measured change in length and l_o is the original length.

The constitutive model that considered in this study is the nonlinear (modified) superposition model, as it is relatively simple to calculate and has been shown to fit the stress relaxation of connective tissues well. The general form of nonlinear superposition is given by [P. P. Provenzano,2002]:

$$\sigma(\varepsilon,t) = \int_0^t E(t-\tau,\varepsilon(\tau))\frac{d\varepsilon(\tau)}{d\tau}d\tau \quad (4)$$

The form of the relaxation function represented by nonseparable strain-dependent power law [P. P. Provenzano, 2002]:

$$E(\varepsilon,t) = A(\varepsilon)t^{B(\varepsilon)} \quad (5)$$

The function A (ε) represents the initial modulus (E_0) that represent a constant obtained from the stress–strain curve or isochronal curve describing the nonlinear elastic behavior. The function B (ε) describes the strain-dependent rate of stress relaxation and can take the form B (ε) =g (ε) n_0, where n_0 is some initial relaxation rate and g(ε) accounts for strain-dependent nonlinearity in relaxation rate. Substituting a Heaviside function, as described above, into eq. (4) results in [P. P. Provenzano, 2002]:

$$\sigma(\varepsilon,t) = E_0\varepsilon t^{g(\varepsilon)n_0} = \sigma_0 t^{g(\varepsilon)n_0} \quad (6)$$

Where, E_0 and σ_0 represent isochronal values of the tangent modulus and stress, respectively, and can be functions of strain to account for nonlinearities in the elastic response.

The rate of relaxation as a function of strain for tendon shows that the tendon relaxation rate increases with increasing strain as shown in fig (3)[Sarah E. Duenwald, 2010].

Material and Method

In the experimental work, a study was made in physiology laboratories of the Medical College /Al-Nahrain University on ten rats "*Rattus Norvegicus*", patellar tendon from rats of weights ranging (108-360) gm were used as an animal tissue model for ex-vivo testing. Each rat was anesthized using fluothane inhalation anesthetic drug in a secured closed container. Patellar tendon

Carefully excised from the rat knees and all of the extraneous tissues were removed with care not to disrupt the original and insertion sites as well as the tendon itself. Specimens were kept hydrated in a physiological normal saline solution. Twenty patellar tendons were used in the experimental work. After the preparation of the specimens, all of the instruments were connected together as in fig (4). In which, the tendon from the one end was fixed with the force transducer and the other end of the tendon was fixed with the displacement transducer and both transducer were connected to the polygraph.
There were two techniques in which the loads or weights were applied in order to initiate an elongation in the specimen. The first technique, in which, dead weights of totally 290gm were used. There was a gradual addition of 10gm in the weight in each step and the polygraph would trace the changes in the specimen tension and displacement in each addition of weight as shown in fig (5). The second technique, in which, a continuous loads or weights were used as shown in fig (6). In the first technique of the dead loads, the force polygraph plots the tension and the displacement polygraph plots the displacement generated in the specimen without time consideration. But, in the second technique of the continuous loads or weights, the force polygraph plots the tension of the specimen with time and the displacement polygraph plots the displacement with time. The length of the specimen, the diameter from different sides as well as the room temperature all were measured before the experiment was done. From the diameters, the area of the specimen was measured and assumed to be elliptical. During the experiment after the connection of the specimen with the transducer, the specimen should be hydrated continuously.

Results

The results could be divided in to three parts, the first part shows the force- deformation curves of dead loads technique and continuous loads technique. The second part shows the conversion of the force- deformation curves in to stress- strain curves. Then the last part contains the application of the modified superposition method to the experimental data. The results were analyzed by using the mathematical expressions in Microsoft Office Excel program. A gradual increase in displacement with the graduals adds on loading of 10 gm in each step for the rat patellar tendon shown in fig (7). Also, a gradual increases of tension with the graduals adds on of dead loads [10 gm for each step] of the rat patellar tendon shown in fig (8) and fig (9) shows gradual add on increases in tension and displacement with gradual loading [10gm for each step] of the rat patellar tendon, in which, an initial part of the curve contain a small part (0.4-0.9) mm called the "toe" region which followed by a more linear part (1-2.25) mm called linear region.

The average of all of the gradual increases of the tensions and displacements at gradual loading in all the experiments of the rat patellar tendons represented by smooth line which contain an initial "toe" region followed by a more linear region, the overall curve is shown in fig (10).

When continuous loads technique is used a continuous add on increases of displacement with the continuous increasing of weights which represented by smooth line for the rat patellar tendon is shown in fig (11). Fig (12) shows a gradually increased line represents the relationships between the continuous add on increases of tension with the continuous increasing of weights for the rat patellar tendon. Also, the continuous add on increases of the tension and displacement with continuous loading for the rat patellar tendon which represented by smooth line shows an initial "toe" region followed by amore linear region is shown in fig (13).

The average of all of the continuous increases of the tensions and displacements at continuous loading in all the experiments of the rat LCLs is shown in fig (14) this curve illustrate the "toe" region and the linear region.

The second part of the results shows the stress-strain curves for the rat medial collateral ligament. From the tension- deformation curve the stress-strain curve was plotted and also, it was necessary to determine the original length and the orthogonal diameters for each specimen along the experiments. From the measured diameters, the cross sectional area was calculated and assumed to be elliptical *[A=4/3(base length*height)]*. When rat patellar tendon is used, the original length (measured using ordinary ruler) of the rat patellar tendons specimens ranging from [5-6.5] mm and cross sectional area of [10.5- 11.4] mm^2 are used

to plot the stress- strain relationship. The average of all of the stress- strain curves for the rat patellar tendons in continuous loading shown in the fig (15) which represents a smooth line with two significant regions; the initial "toe" region and the linear region .

The third part of the results was the application of the modified superposition method. One of the major reasons that the modified superposition model would be preferred among the other models was that the variables of the theory could be clearly established from the experimental results without complications. This model was applied to the experimental data at different strain levels after the elastic modulus and the relaxation rate were determined from the stress- strain curves showed previously and the equation (6) was used. The elastic modulus was determined from the slope of the linear region of the stress- strain curve in fig (15) and shown to be equal to 48.5kPa also From the fig (3) the relaxation rate was shown to be equal to -0.045, -0.0875, -0.095, -0.12 and -0.15 for the corresponding strain levels of 2%, 3.6%, 4%, 5% and 6% respectively. For each strain level the theoretical results of the modified superposition theory and the experimental results were shown in the figures from (16) to (20) in which the experimental stress was denoted by stress e and theoretical stress produced from the application of the modified superposition theory was denoted by stress MS.

Discussion

Tendon consists of two major components; which is the collagen component and the elastin component. The collagen represents the major component that resisting the tensile stress, while the elastin represents the elastic component. In this study the modified superposition theory of eq. (6) is applied to the experimental results of the rat patellar tendon in the figures from (16) to (20) and two paths are distinguished:

- Non-parallel lines shown in the first sections of these curves between the experimental results and the theoretical results.
- Parallel lines shown in the second sections of these curves between both results.

From the observation of the rat patellar tendon it is clear that the second path has behaviour similar to polymers [P. P. Provenzano, 2002], which indicates that their structure at such strains will have a viscoelastic properties. While the first path of the curves may give an impression that it behaves more elastic the behaviour, which is not similar to polymers. This dual behaviour of the biological tissue could be explained by the following:

- In biological tissues, the combination of more than one material will give a behaviour that represents both materials. Since the tendon consist of collagen and elastin component, so that the collagen components are assumed to response to the initial state of tension, then before the elastin component are fully stretched, the collagen component will continue their stretching until the crimp is removed and the whole tissue is stretched. This speculated by the results shown in fig (15) of the rat patellar tendon, respectively. These figures illustrate a first portion that is called "toe region" which shows an elongation with little tension generated in these tissues; as the elongation increased a much tension are generated in the tendon and ligament which are shown in the second portion of the curves that is called the "linear region", which visualize the behaviour of the tensile component (collagen).
- The other explanation that describes the behavioural changes in the tendon assumes that these changes are related to their structural architectures [Nordin and Frankel, 2004], which depend on the assumption that both collagen and elastin will show their behaviour collectively.

The tendon microarchitecture visualized under light microscope showed that the proteoglycans which are arranged in the form of hinges are attached to the collagen fibers as shown in fig (21). These hinges may explain in part the initial stretching of the collagen fibers in the “toe” region of the stress- strain curves. So that when the load is applied, the tension is first generated in the collagen fibers until the coiling of the collagen fibers are removed and the fiber are stretched. After that, the tension is transmitted to the proteoglycans which have coiled arrangement, are slightly stretched and this tension is then transmitted to another collagen fiber and so on. So that, the collagen fibers are related to resisting higher tensile force more than the Elastin fibers.

Finally, the experimental results are typically succeeded to fit the applied model [modified superposition model] and show the biomechanical behaviour of the tendon.

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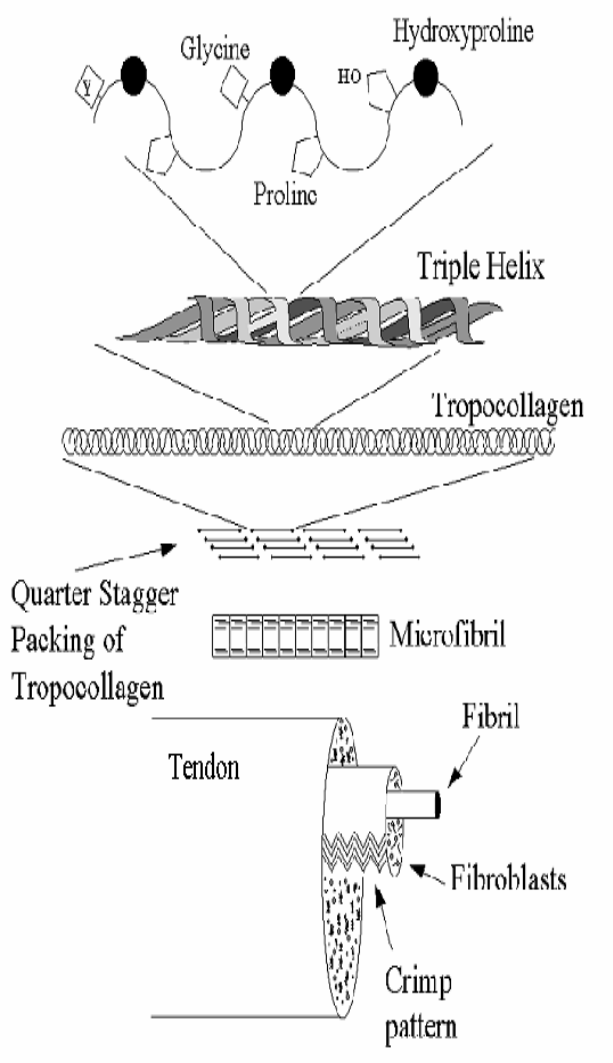

Figure (1) Schematic illustration depicting the hierarchical structure of collagen tendon midsubstance [K. Michelle quapp 1997]

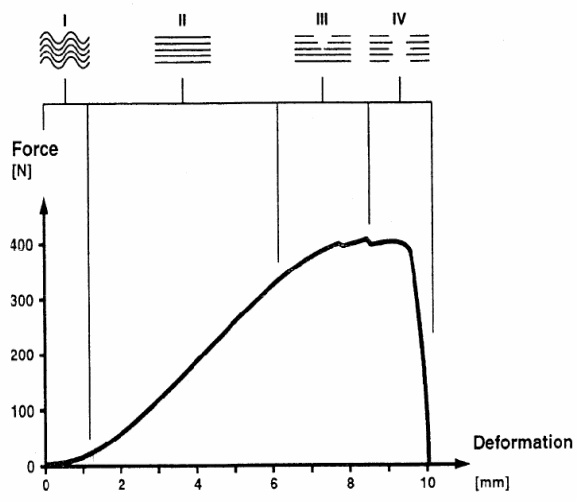

Figure (2) A typical force-deformation curve. I =toe region; II = linear region; III = region of micro- failure; IV = failure region [Benno M. Nigg and Walter Herzog, 1999]

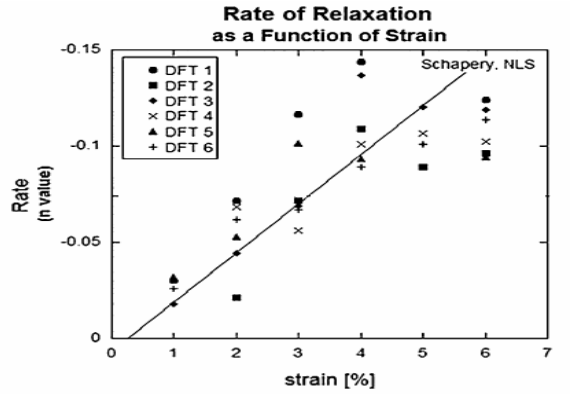

Figure (3) The Rate of Relaxation (defined as n term of power law equation, $E = At^n$) as a Function of Strain for Digital Flexor Tendon [Sarah E. Duenwald, 2010].

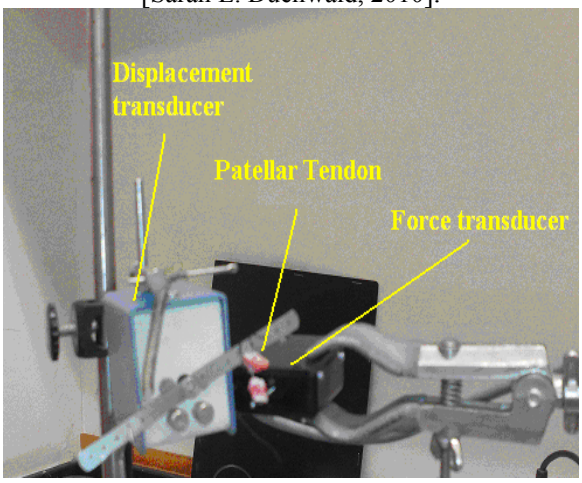

Figure (4) Connection of the patellar tendon with the force and displacement transducer.

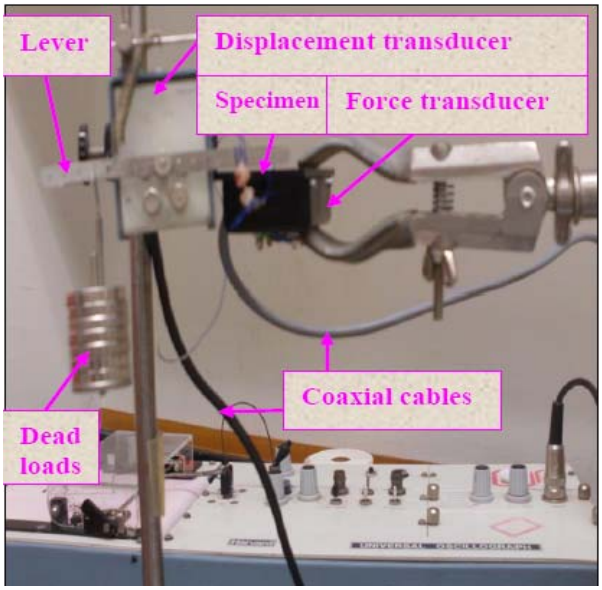

Figure (5) Dead Loads Technique.

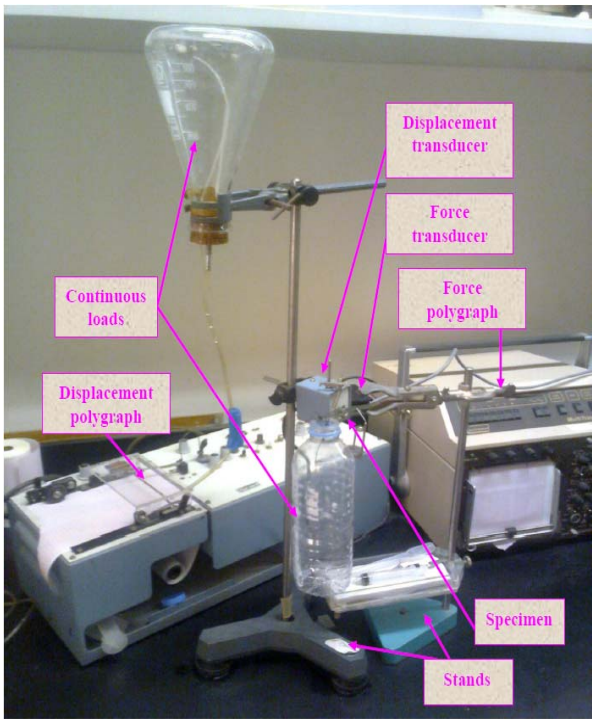

Figure (6) Continuous Loads Technique.

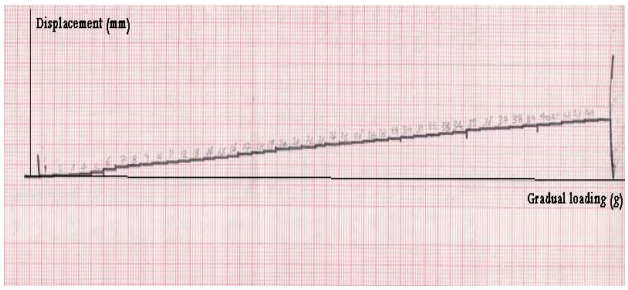

Figure (7) Raw data shows the relationships between the steps of gradual add on increases of displacement with the gradual loading by weights of the rat patellar tendon each 5mm=1mm displacement.

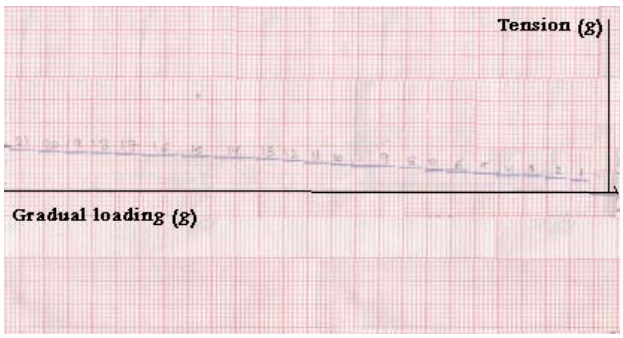

Figure (8) Raw data shows the relationships between the steps of gradual add on increases of tension with the gradual loading by weights of the rat patellar tendon[each 4mm=10gm of tension]

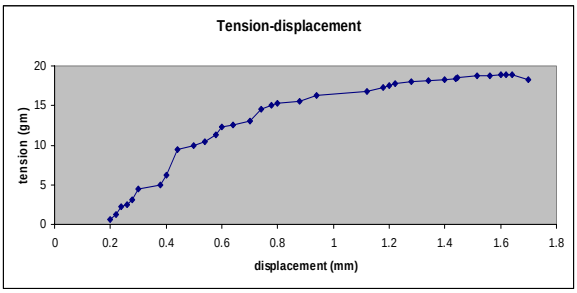

Figure (9) The gradual add on increases of the tension and displacement with gradual loading for one rat patellar tendon.

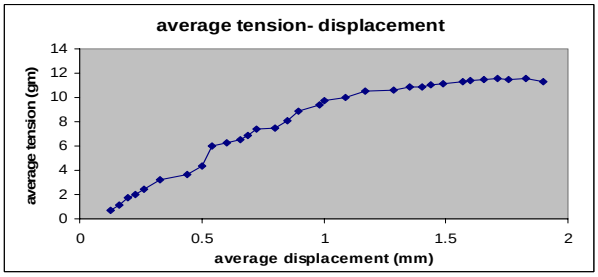

Figure (10) The average of all of the gradual increases of the tensions and displacements at gradual loading in all the experiments of the rat patellar tendons.

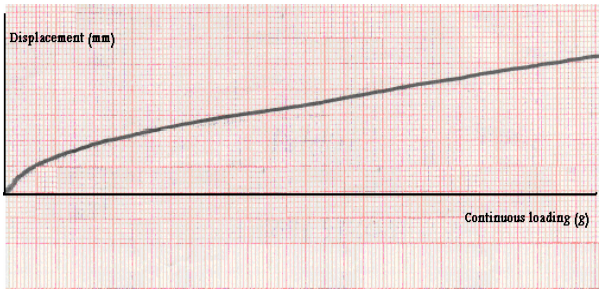

Figure (11) Raw data shows the relationships between the continuous add on increases of displacement with the continuous loading by weights of the rat patellar tendon [each 5mm on the curve =1mm of displacement].

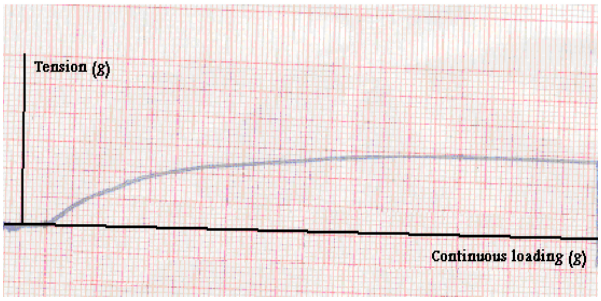

Figure (12) Raw data shows the relationships between the steps of continuous add on increases of tension with the continuous loading by weights for the rat patellar tendon [each 4mm on the curve = 10gm of tension].

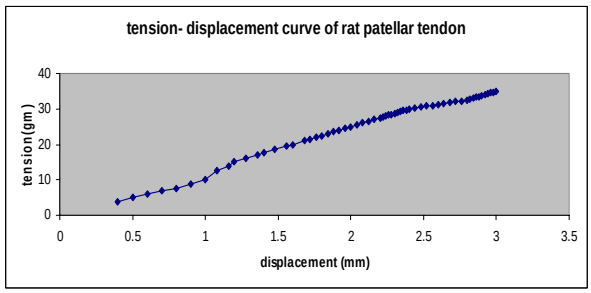

Figure (13) The continuous add on increases of the tension and displacement with continuous loading for one rat patellar
tendon.

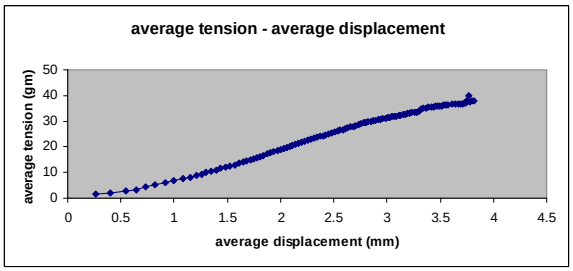

Figure (14) The average of all of the continuous increases of the tensions and displacements at continuous loading in all the experiments of the rat patellar
tendons.

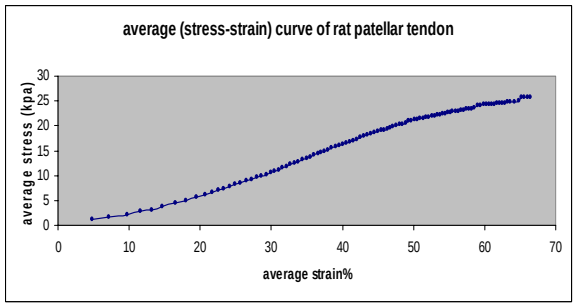

Figure (15) Average stress-strain curve for rat patellar tendons under continuous loading.

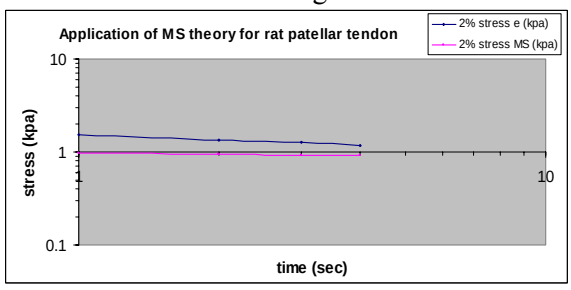

Figure (16) Application of the modified superposition theory to the rat patellar tendon at strain level of 2%. The blue line represented the experimental results and the pink line represented the theoretical results.

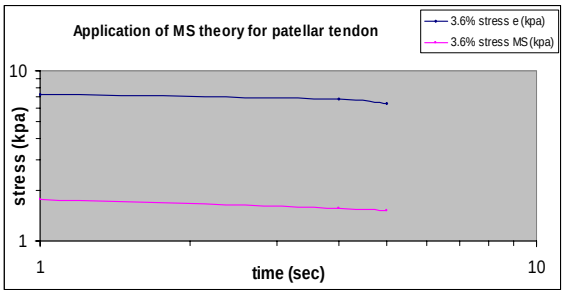

Figure (17) Application of the modified superposition theory to the rat patellar tendon at strain level of 3.6%. The blue line represented the experimental results and the pink line represented the theoretical results.

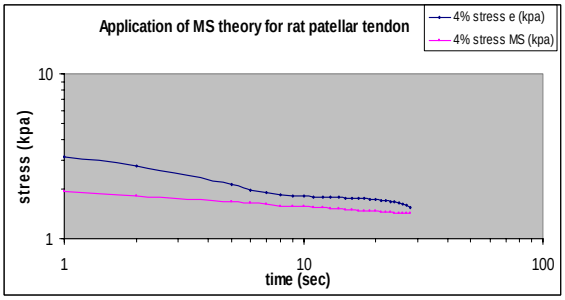

Figure (18) Application of the modified superposition theory to the rat patellar tendon at strain level of 4%. The blue line represented the experimental results and the pink line represented the theoretical results.

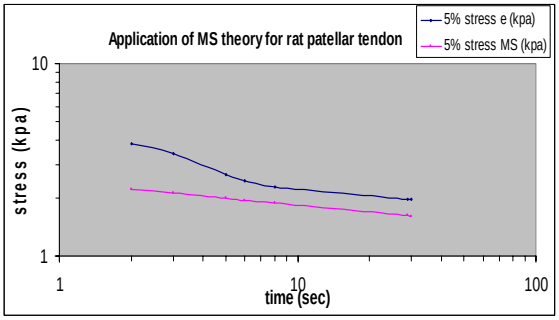

Figure (19) *Application* of the modified superposition theory to the rat patellar tendon at strain level of 5%. The blue line represented the experimental results and the pink line represented the theoretical results.

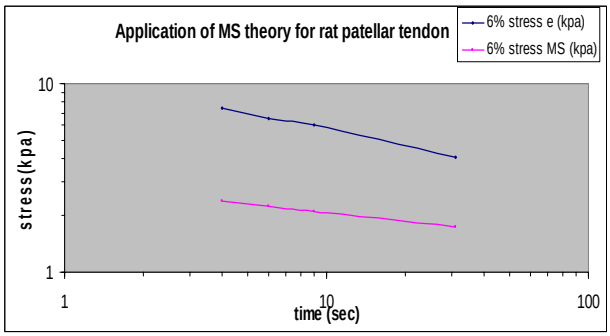

Figure (20) Application of the modified superposition theory to the rat patellar tendon at strain level of 6%. The blue line represented the experimental results and the pink line represented the theoretical results.

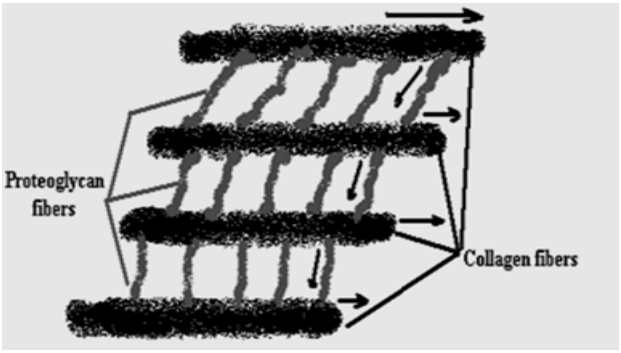

Figure (21) Illustration of collagen interaction with Proteoglycan.