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A Biomechanical Model Study of the Rat as a Medial Collateral Ligaments of the Knee

Dr. Fakhir Salman
Medical College, University of Al-Nahrain /Baghdad
Dr. Sadiq Jafer Abbass
Engineering College, University of Al-Nahrain / Baghdad
Noor Ali Sadek,
Engineering College, University of Al-Nahrain / Baghdad
Email: Noor-Ali-sadex@yahoo.com

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ABSTRACT

Ligament primarily stabilizes the diarthrodial joints and function to provide stability and support during the motion of diarthrodial joints. These functions are assisted by the congruent geometry of the articulating joint surfaces and musculotendinous forces. Ligament exhibits viscoelastic, or time-dependent behavior, like many tissues in the body. From the medical point of view an understanding of the biomechanics of ligaments are crucial for the understanding of injury mechanisms and to evaluate existing surgical repair techniques. The mode of failure in ligaments depends strongly on the rate of loading. Thus, ligament viscoelasticity is an important determinant of tissue response to loading, and viscous dissipation by the tissue modulates the potential for injury. Many mathematical models have been developed to describe the complexity of these behaviors that could include the microphysical interactions of various constituents but none of them seems to represents the overall properties of these structures. Models can be an important tool in understanding tissue structure-function relationships and elucidating the effects of injury, healing, and treatment. The main objective of this work is to study from the biomechanical point of view, the behaviour of an example of the medial collateral ligament in response to stress and strain effects to evaluate the biological behaviour of the ligament. The strain effect as example of the modified superposition method and analyze the results and the model that can express the medial collateral ligament behaviour.

Keywords: Ligament, viscoelastic behaviour, biomechanical model, modified superposition method.

دراسة ميكانيكية حيوية في الجرذ كنموذج للروابط الأنسجة الجانبية للركبة

الخلاصة

تعد الروابط من الأنسجة الحيوية التي تعمل على تثبيت عظام المفاصل الزلالية , و وظيفتها الأساسية تثبيت ودعم هذه المفاصل اثناء الحركة . هنالك بعض العوامل التي تدعم الرابط لأداء وظائفه منها التناسق في شكل العظام في منطقة التقائها عند المفصل و قوة العضلات المتصلة بالمفصل. و للروابط سلوك لزوجة مرنة (viscoelastic behaviour) يعتمد على الزمن

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كمعظم الانسجة الحية. من وجهة النظر الطبية فانه من الضروري فهـم الميكانيكيـة الحيويـة للروابط لفهم ميكانيكية حدوث الجروح وايجاد التقنيات الجراحية البديلة. ان الفشل الذي يحدث في الرابط يعتمد بصورة كبيرة على معدل تحميل القوة. لهذا فان خاصية اللزوجة المرنـة تعطـي نظرة مهمة عن استجابة النسيج لتحميل القوة وكيفية تجنب الجروح. ولهذه الأهمية تـم تطـوير بعض النماذج الرياضية لوصف و فهم عمل الروابط من الناحية الوظيفية و التركيبية. يهدف هذا العمل دراسة الناحية الميكانيكية الإحيائية كمثال للرابط الموجود على الناحية الداخلية لمفصـل الركبة واستجابته لتأثير الإجهاد و المطاوعة, ثم تطبيق النموذج الرياضي لوصـف و تحليـل النتائج.

INTRODUCTION

The word "ligament" is derived from the Latin word "ligare" which means to bind. Ligaments attach one articulating bone to another across a joint. The major functions of ligaments are as follows: To attach articulating bones to one another, to guide joint movement and possibly to act as a strain sensor for the joint. Ligaments are well suited to the physiological functions they perform and consisting of a ground substance matrix reinforced by collagen and elastin [1].

The ground substance matrix is composed of proteoglycans, glycolipids, and fibroblasts and holds large amounts of water. Ligaments are relatively hypo-cellular with interconnected, elongated fibroblastic cells in their midsubstance and more rounded cells found near their insertions to bone. The primary function of the cells is to maintain the collagen scaffold. This is consistent with other musculoskeletal soft tissues that have mechanical function as their main purpose [2].

Collagen is the main protein present in ligaments and the primary component resisting tensile stress in ligaments. It is found chiefly in fibrillar form, oriented between insertions such that it will resist tensile forces.

Ligaments with larger bimodal collagen distribution tend to be stronger and able to sustain higher stresses [1]. Those with smaller unimodal diameters are more suited to resisting lower stresses. The hierarchical structure of the collagen in the ligament midsubstance includes fibers, fibrils, subfibrils, microfibrils, and tropocollagen (figure (1)).

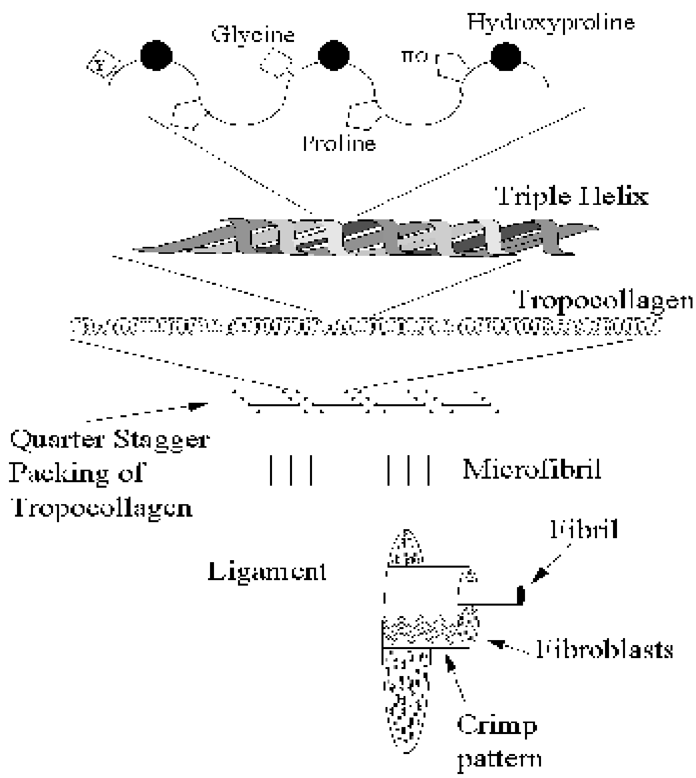

Figure (1) Schematic illustration depicting the hierarchical structure of collagen ligament midsubstance [6]

Fibroblasts tend to align in rows between fibre bundles and are elongated along the long axis in the direction of normal tensile stress as in figure (2) [3].

Figure (2) Photograph illustrating crimped pattern of collagen in ligament [1].

Proteins are also present in ligament and tendon which distributed in the matrix and blood vessels forming part of an important matrix-cell feedback mechanism [4].

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 [2]. 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.

Elastin is an elastic substance that is found in very small amounts in most skeletal ligaments (approximately l.5%) in fibular form. In elastic ligaments (e.g., ligamentum flavum), however, elastin fibers are about twice as common as collagen fibers. The role of elastin is probably related to recovering ligament length after stress is removed. Elastin "protects" collagen, at least at low strains [5].

CONSTITUTIVE EQUATIONS

The elastic modulus was computed from the stress- strain curve, which represent the slope of the linear part of the curve.

$$E = \frac{\Delta s}{\Delta e} \qquad (1)$$

Where, E is the elastic modulus. σ is the measured stress and ε is the measured strain, both can be calculated by,

$$s = \frac{F}{A} \qquad (2)$$

Where, F is the measured force and A is the calculated area.

$$e = \frac{\Delta l}{l_o} \qquad (3)$$

Where, Δl is the measured change in length and l_o is the original length [1].

The first constitutive model considered was nonlinear (modified) superposition, 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:

$$s\,(e,t) = \int_0^t E(t-t\,,e(t))\frac{de(t)}{dt}dt \qquad (4)$$

The form of the relaxation function will be chosen as a nonseparable strain-dependent power law:

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

The function A (e) represents the initial modulus (E_0), which can be obtained from the stress–strain curve or isochronal curve describing the nonlinear elastic behavior.

The function B (e) describes the strain-dependent rate of stress relaxation and can take the form B (e) =g (e) n_0, where n_0 is some initial relaxation rate and g(e) accounts for strain-dependent nonlinearity in relaxation rate. Substituting a Heaviside function, as described above, into equation (4) results in:

$$s\ (e,t) = E_0 e t^{g(e)n_0} = s_0 t^{g(e)n_0} \quad (6)$$

Where, E_0 and s_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.

A polynomial describing the rate function was obtained by fitting rate versus strain data (figure (3). [7]

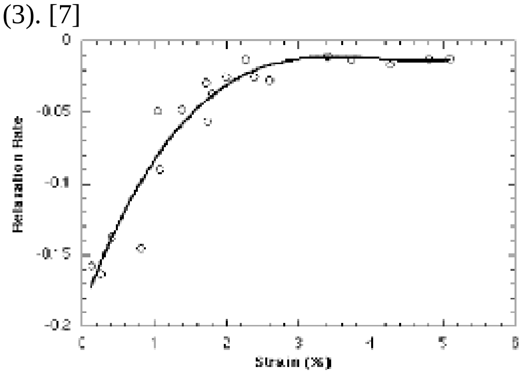

Figure (3) Fitting of a polynomial function (curve) to experimental stress relaxation rate of Provenzano et al. (2001) (points) for multiple rat ligaments tested at multiple strain levels. Rate was defined as n in a t^n time dependence. [7]

The second constitutive model considered was Schapery's model, which has been more frequently used to describe polymer behavior than biological phenomena.

The general form of Schapery's model describing nonlinear stress relaxation is as follows:

$$s(e,t) = h_e(e)E_e e + h_1(e)\int_0^t \Delta E(r(t) - r'(t))\frac{dh_2(e)e}{dt}dt. \quad (7)$$

With the reduced time, ρ, defined as

$$r = \int_0^t \frac{dt'}{a_e}[e(t')] \quad (8)$$

And reduced time variable of integration r',

$$r' = \int_0^t \frac{dt'}{a_e}[e(t')]. \qquad (9)$$

In equation (7), E_e is the equilibrium, or final, value of the elastic modulus, and ΔE is the transient modulus. The terms h_e, h_1, h_2, and a_e are strain-dependent material properties; (a_e is a function of strain and time and may also be temperature dependent) that have thermodynamic significance. In the constant strain application of Schapery's theory the form of the transient modulus will be modeled as a power law:

$$\Delta E(r) = Cr^n \qquad (10)$$

Where, C and n are assumed to be material constants at any strain level, for a constant temperature.

When equation (10) is substituted into equation (7) the stress is:

$$s(e,t) = h_e(e)E_e e + h_1(e)C\int_0^t (r - r')^n \frac{dh_2(e)e}{dt}dt \qquad (11)$$

Substituting a Heaviside function into equation (11) for a particular strain, e_0 and setting $h_1 = a_e = 1$;

$$s\ (e_0, t) = h_e E_e e_0 + h_2 C e_0 t^n \qquad (12)$$

Noting that E_e and e are known from experimental data, and that C and n are constants determined by curve fitting. [8]

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*", medial collateral ligament 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.

Medial collateral ligament 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 ligament itself. Intact bones remained attached to the distal and proximal ends of the specimens as shown in the figure (4). Specimens were kept hydrated in a physiological normal saline solution. Twenty medial collateral ligaments were used in the experimental work.

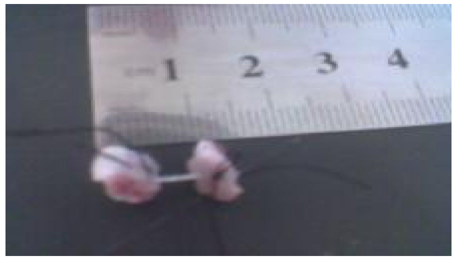

Figure (4) Medial collateral ligament with the remaining bony ends

After the preparation of the specimens, all of the instruments were connected together as in figure (5). In which, the ligament from the bone side was fixed with the force transducer and the other end of the ligament from the bone side was fixed with the displacement transducer and both transducer were connected to the polygraph.

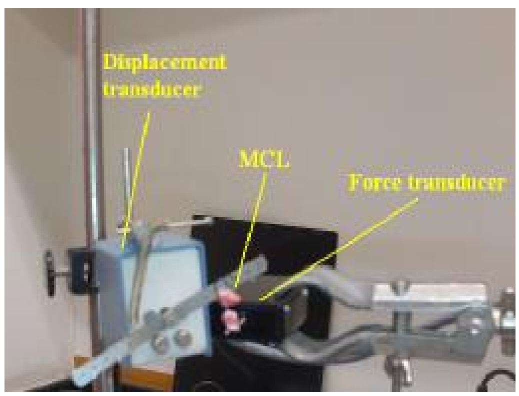

Figure (5) Connection of the medial collateral ligament (MCL) with the force and displacement transducers

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 figure (6).

The second technique, in which, a continuous loads or weights were used as shown in figure (7).

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.

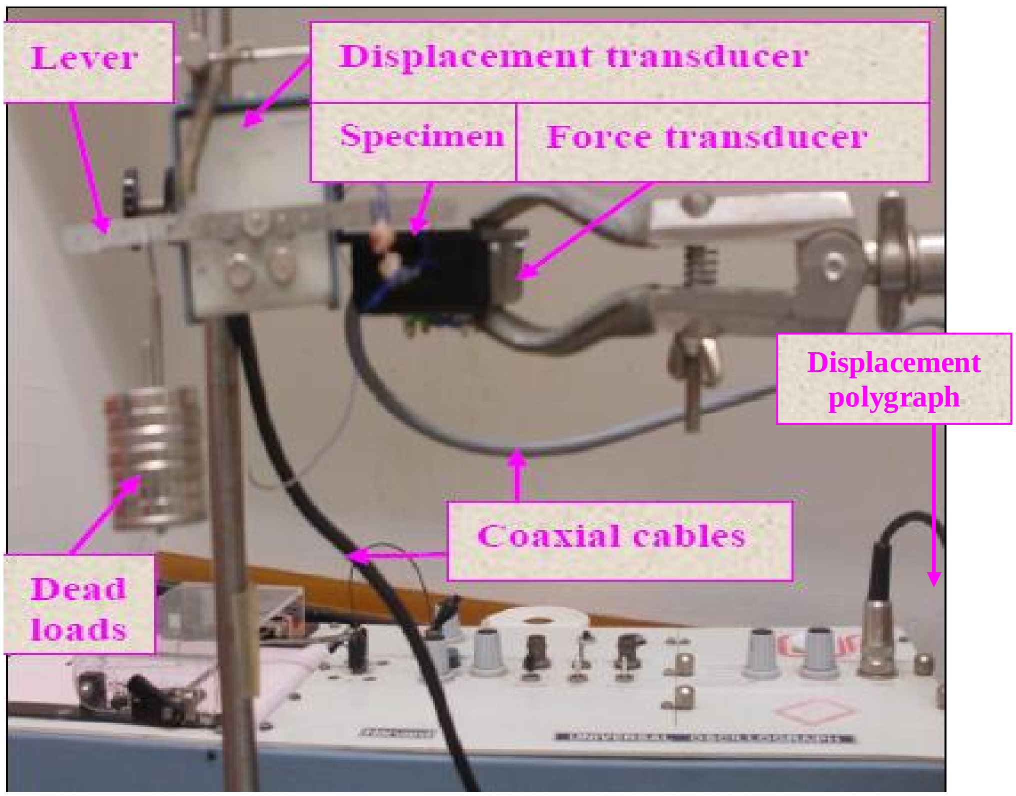

Figure (6) Dead Loads Technique.

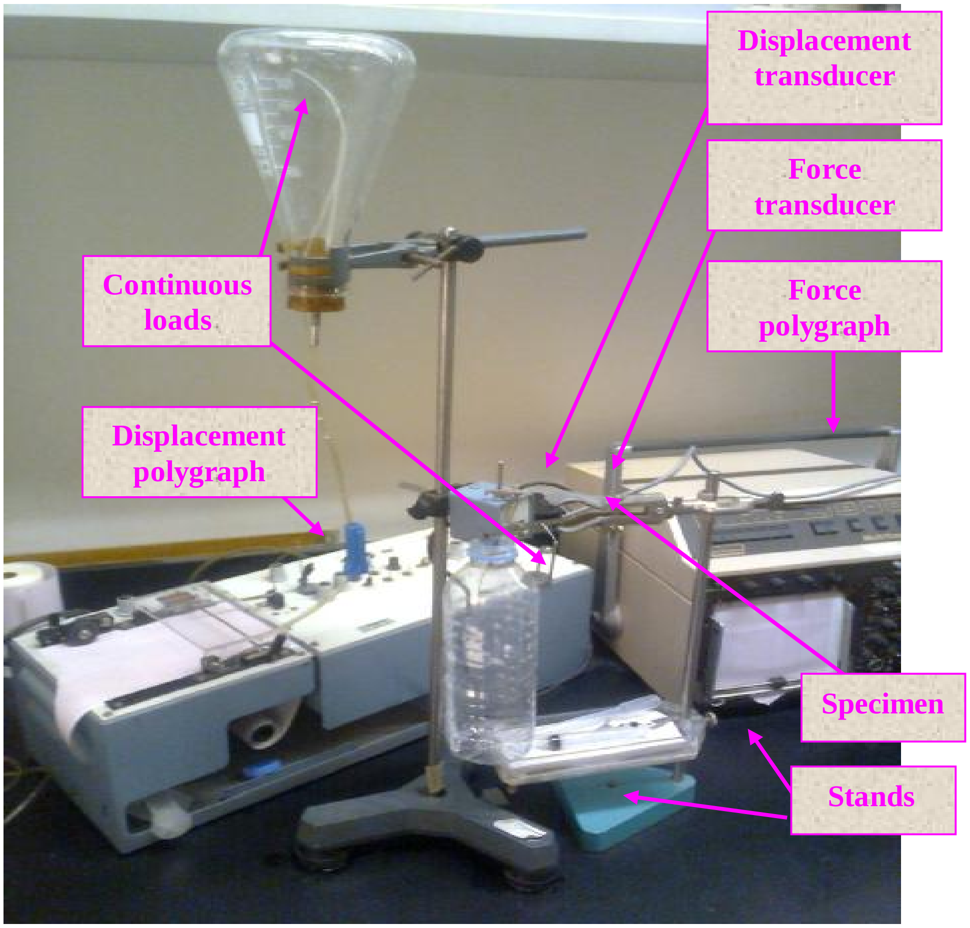

Figure (7) Continuous Loads Technique

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 average and mathematical expressions in Microsoft Office Excel program.

First technique figure (6) was used to show the force- displacement curve of the rat medial collateral ligament

Figure (8) shows the relationships between the steps of gradual add on increases of displacement with the gradual loading of the MCL by weights. And figure (9) shows the relationships between the steps of gradual add on increases of tension with the gradual loading of the MCL by weights.

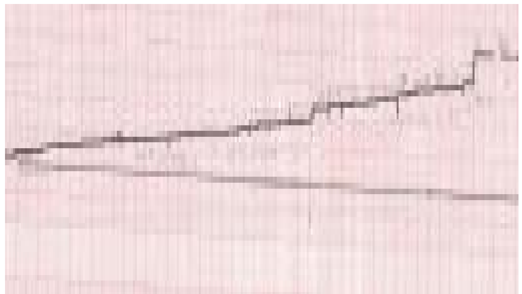

Figure (8) Steps of gradual add on increases of displacement with the gradual loading by weights of the MCL each 5mm=1mm of displacement

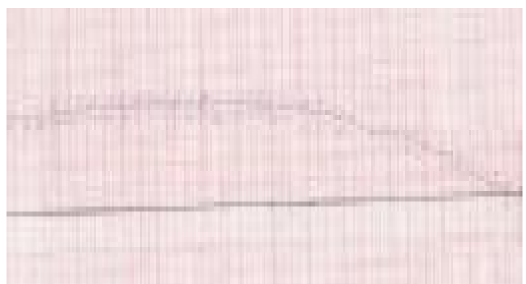

Figure (9) Steps of gradual add on increases of tension with the gradual loading by weights of the MCL each 4mm=10gm of tension.

Figure (10) shows the gradual add on increases of the tension and displacement with gradual loading for the rat medial collateral ligament and figure (11) shows the average of all of the gradual increases of the displacements with the gradual loading for the experiments of the medial collateral ligaments using dead loads technique.

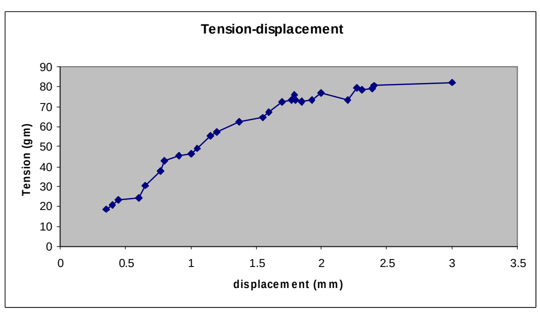

Figure (10) The gradual add on increases of the force and displacementwith gradual loading for the rat MCL.

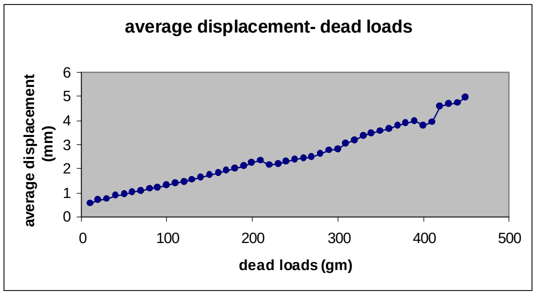

Figure (11) The average of all of the gradual increases of the displacements with the gradual loading for the experiments of the rat MCLs.

Also, figure (12) shows the average of all of the gradual increases of the tensions with the gradual loading for the experiments of the rat MCL, and figure (13) shows the average of all of the gradual increases of the tensions and displacements at gradual loading in all the experiments of the rat MCLs.

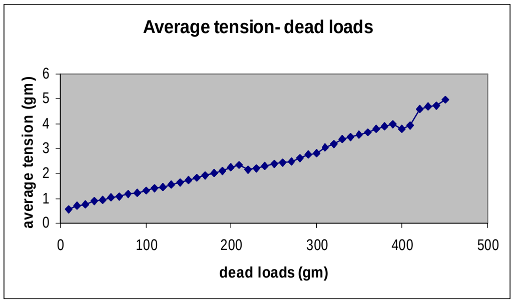

Figure (12) The average of all of the gradual increases of the tensions with the gradual loading for the experimentsof the rat MCLs.

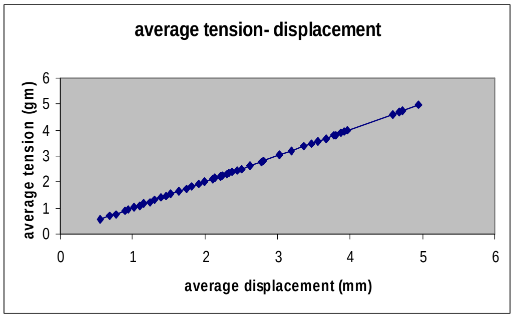

Figure (13) The average of all of the gradual increases of the tensions and displacements at gradual loading in all the experiments of the rat MCLs.

After that the continuous loads technique in figure (7) was used to show the effect of continuous loading on the force- displacement curves.

Figure (14) shows the relationships between the continuous add on increases of displacement with the continuous loading by weights of the rat MCL and figure (15) shows the relationships between the steps of continuous add on increases of tension with the continuous loading by weights for the rat MCL.

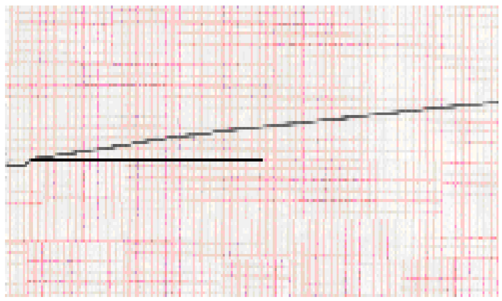

Figure (14) The relationships between the continuous add on increases of displacement with the continuous loading by weights of the rat MCL each 5mm = 1mm of displacement

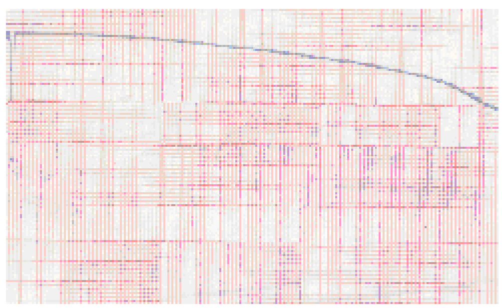

Figure (15) The relationships between the steps of continuous add on increases of tension with the continuous loading by weights for the rat MCL each 4mm= 10gm of the tension

Figure (16) shows the continuous add on increases of the tension and displacement with continuous loading for the rat MCL.

Also, figure (17) shows the average of all of the continuous increases of the displacements with the continuous loading for the experiments of the rat MCLs using continuous loads technique.

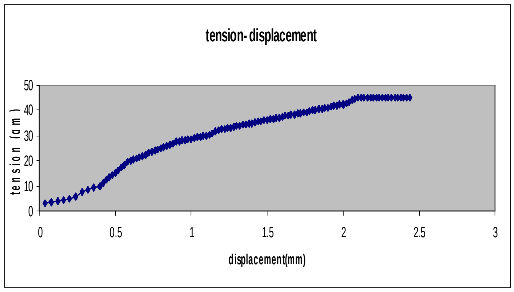

Figure (16) The continuous add on increases of the tension and displacement with continuous loading for the rat MCL.

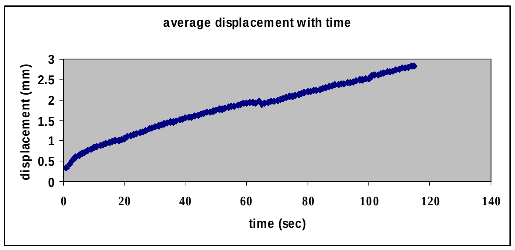

Figure (17) The average of all of the continuous increases of the displacements with the continuous loading for the experiments of the rat MCLs.

Also, figure (18) shows the average of all of the continuous increases of the tensions with the continuous loading for the experiments of the rat MCLs, and figure (19) shows the average of all of the continuous increases of the tensions and displacements at continuous loading in all the experiments of the rat MCLs.

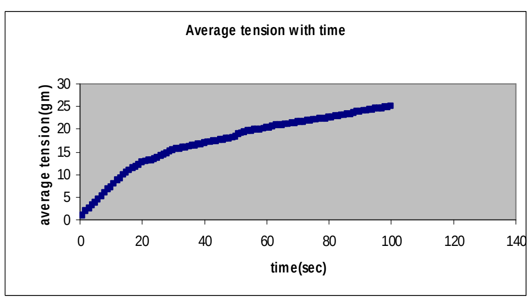

Figure (18) The average of all of the continuous increases of the tensions with the continuous loading for the experiments of the rat MCLs

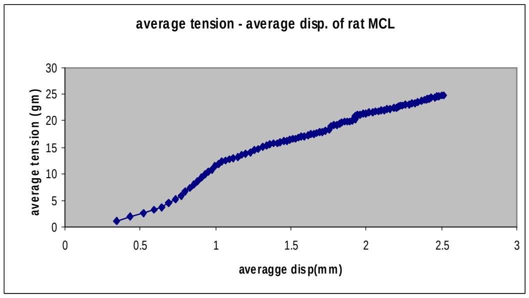

Figure (19) The average of all of the continuous increases of thetensions and displacements at continuous loading in all the experiments of the rat MCLs.

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.

For rat medial collateral ligament with cross sectional area of 1.372 mm^2, original length of 5 mm and rat weight of 215.45 gm the stress- strain curve shown in the figure (20).

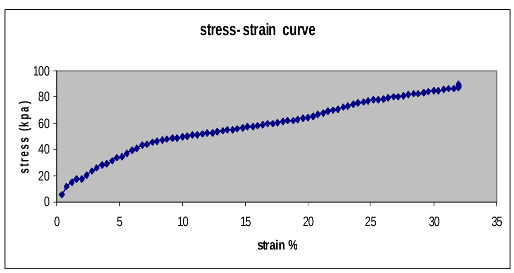

Figure (20) Stress- strain curve for rat MCL under continuous loading.

The average for all of the stress curves under continuous loading with respect to time for the rat medial collateral ligaments shown in figure (21).

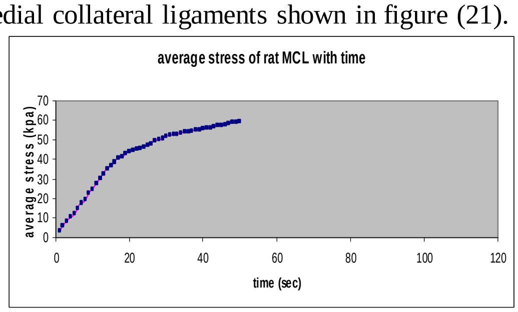

Figure (21) Average stress curve with time respect for rat MCLs under continuous loading.

Also, the average of all of the strain curves with respect to time in continuous loading of the medial collateral ligaments shown in figure (22)

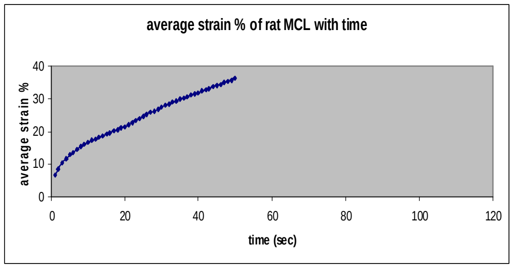

Figure (22) Average strain curve with time respect for rat MCLs under continuous loading .

As well as; an average of all of the stress- strain curves for the rat medial collateral ligaments in continuous loading shown in the figure (23).

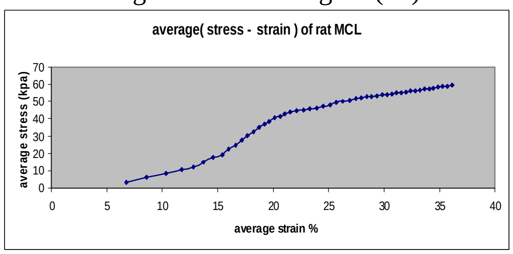

Figure (23) Average stress-strain curve for rat MCLs under continuous loading.

The third part of the results was the application of the modified superposition method. One of the major reasons that the modified superposition theory would be preferred among the other theories discussed, was that the variables of the theory could be clearly established from the experimental results without complications as found in the Schapery's single integral theory which contains a strain dependent variables with values different for each strain level that made the application of the Schapery's theory to be less common in the description of the medial collateral ligament.

The modified superposition theory 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.

The modified superposition theory was applied to the rat medial collateral ligament, and the equation (6) was used. The elastic modulus was determined from the slope of the linear region of the stress- strain curve in figure (23) and shown to be equal to 267.5153 Kpa also From the figure (2) the relaxation rate was shown to be equal to -0.03, -0.02, -0.015, -0.013 and -0.012 for the corresponding strain levels of 2%, 2.5%, 3%, 3.5% and 4% respectively. For each strain level the theoretical results of the modified superposition theory and the experimental results were shown in the figures from (24) to (28) 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.

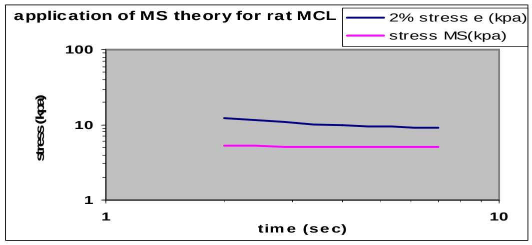

Figure (24) Application of the modified superposition theory to the rat medial collateral ligament at strain level of 2%. The blue line represented the experimental results and the pink line represented the theoretical results.

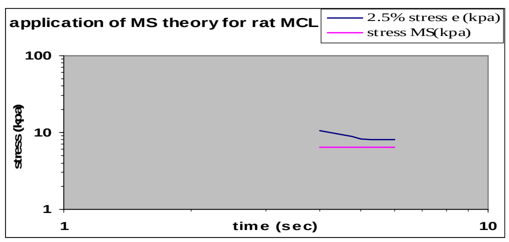

Figure (25) Application of the modified superposition theory to the rat medial collateral ligament at strain level of 2.5%. The blue line represented the experimental results and the pink line represented the theoretical results.

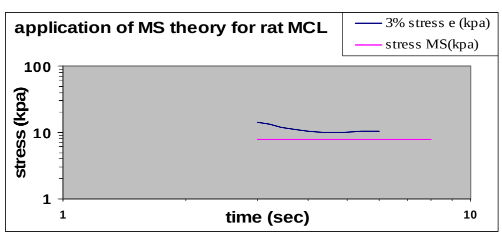

Figure (26) Application of the modified superposition theory to the rat medial collateral ligament at strain level of 3%. The blue line represented the experimental results and the pink line represented the theoretical results.

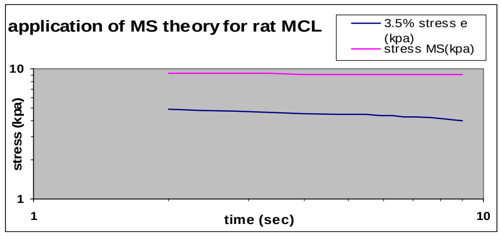

Figure (27) Application of the modified superposition theory to the rat medial collateral ligament at strain level of 3.5%. The blue line represented the experimental results and the pink line represented the theoretical results.

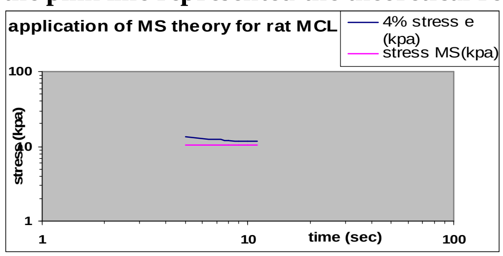

Figure (28) Application of the modified superposition theory to the rat medial collateral ligament at strain level of 4%. The blue line represented the experimental results and the pink line represented the theoretical results.

DISCUSSION

The ligament is formed of two main components named by collagen fibers and Elastin fibers. The collagen fibers represent the tensile component while the Elastin fibers represent the elastic component.

In this study when the modified superposition theory of equation (6) applied to the experimental results, two paths of the behaviour of the fibers are shown in figures (24), (25), (26), (27) and (28); the first path that include a non- parallel lines of the experimental results with the theoretical results [i.e. application of the modified superposition theory]. The second path includes parallel lines between the experimental and the theoretical results. This indicates that the second path has behaviour similar to the polymers [5]. But, in the first path the behaviour was not similar to polymers, this may attributed to two explanations:

- In biological tissues the combination of more than one component will give a behaviour that represents both materials. Since we have collagen component and Elastin component so we assume that the collagen component will perform its changes before the second component [i.e. the Elastin component]. This can be speculated by our results [figure (19)] which shows that there is elongation with little tension in the first portion of this curve which called "toe" region. With increasing of the elongation a much tension are produced in the ligament which is shown in the second portion of the same curve that is called the "linear" region.
- The other explanation that describes the behavioural changes in the ligament stated that the behavioural changes are related to the geometrical architectures of the ligament

[9]. In which the “toe” part of the curve is explained by the initially stretching of the crimp. After that the behaviour will appear more linear due to the removing or stretching out of the crimp appearance.

Finally, a conclusion could be made that the experimental results are typically succeeded to fit the applied theory [modified superposition theory] and show the overall behaviour of the ligament.

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