# IMMUNOHISTOCHEMISTRY AND BIOMECHANICAL PROPERTIES OF THE NON-PATHOLOGICAL ELBOW JOINT CAPSULE

By

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## ABSTRACT

One of the most important complications in elbow joint (EJ) pathology is post–traumatic contracture of soft tissues surrounding the joint. Insidious stiffness takes over the joint, decreasing the Range of Movement (ROM). Post-traumatic contracture of the EJ, with subsequent stiffening, is very often the result of contracture of the anterior capsule. However, despite the critical role it plays in the pathogenesis of the EJ contracture, very little is known about the structural and biomechanical properties of the EJ capsule.

The type of collagen (notably type I and type III) and its organization in extracellular matrices plays a critical role in determining biomechanical properties (elasticity and structural stiffness) of biological structures. In order to determine whether there were changes in expression of the type of collagen, between normal and contracted post-traumatic EJ capsules (n=3), we performed immunohistochemical analysis. The results indicated that collagen type III was not expressed in capsules derived from cadavers (n=10) with no history of trauma to the EJ (within the limits of detection by fluorescence immunohistochemistry). In contrast, isles of collagen type III fibrils were detected in capsules from patients with contracted post-traumatic EJ (n=3). This observation supports the hypothesis that contracture of the EJ is associated with expression of collagen type III, which is not expressed in normal EJ capsules (in this age group). From these studies, we propose that expression of collagen type III in post-traumatic contracture of the EJ may lead to changes in biomechanical properties of the joint. This in turn may affect the ROM of the contracted EJ.

We initiated studies to determine biomechanical properties of the EJ capsule. These initial studies were performed with non-pathological EJ capsules, in order to establish experimental protocols most appropriate for such studies. Moreover, although the non-pathological EJ capsule plays a critical role in stabilizing the elbow joint (while allowing

physiological motion) there is no data in the literature that documents its biomechanical properties. Thus, our aim was to determine biomechanical properties (Modulus of Elasticity and Structural Stiffness) of normal elbow joint capsules.

The anterior portion of ten non-pathological EJ capsules, were excised from fresh cadavers (aged 75 to 93 years) and cleaned of unrelated soft tissues, such as muscle and fat. The capsules were sectioned into 3 mm wide strips, producing a total of 87 samples. The samples were grouped according to their anatomical locations (radial, mid-capsular and ulnar regions).

Each sample was loaded in tension at 1 mm/sec, to the stage of failure, in a servo-hydraulic materials testing machine (DynaMight, Instron, Canton, MA). The width and thickness were measured using callipers at 25%, 50% and 75% of the initial length. The cross sectional area was approximated as an ellipse and samples were coated with blue chalk, prior to testing, to determine the failure location. Structural stiffness was calculated from the linear region of the load-displacement curve and the intrinsic mechanical property, modulus of elasticity (Young's Modulus), was calculated using the initial cross-sectional area, closest to the point of failure (i.e. at 25%, 50% or 75%). A two-factor repeated measures ANOVA was performed to determine statistical significance for p less than 0.05.

No significant statistical differences were found for Young's Modulus ($p = .1536$) or structural stiffness ($p = .2$) between the three different regions of the capsules. The mean Young's Modulus of the pooled samples was 17.7 MPa and the mean structural stiffness was 5.8 N/mm. Our studies are the first to evaluate Modulus of Elasticity and Structural Stiffness of normal EJ capsules. These results represent reference data for further comparison with biomechanical properties EJ capsules in pathological states, including post-traumatic contracture.

Further studies would be useful to elucidate the correlations between induction of expression of collagen type III and change in biomechanical properties of the EJ capsule. Revelation of an association between these two parameters may lead to novel methods of prevention and/or treatment of decreased ROM of joints due to trauma (either through accidents, vocation or sports) or as a result of diseases associated with inflammation of joints and/or aging (such as rheumatoid arthritis and osteoarthritis).

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## LIST OF ABBREVIATIONS:

| | |
|---|---|
| **ADL** | **Activities of Daily Living** |
| **BSA** | **Bovine Serum Albumen** |
| **CP** | **Coronoid Process** |
| **EJ** | **Elbow Joint** |
| **H&E** | **Hematoxylin-Eosin** |
| **IgG** | **Immunoglobulin G** |
| **IHC** | **Immunohistochemistry** |
| **IVD** | **Intervertebral disc** |
| **Lo** | **Original Length** |
| **NaCl** | **Sodium Chloride** |
| **NGS** | **Normal Goat Serum** |
| **Na/KPO4** | **Sodium Potasium Phosphate** |
| **ORIF** | **Open Reduction and Internal Fixation** |
| **OCT** | **Optimum Cutting Temperature** |
| **PBS** | **Phosphate Buffer Saline** |
| **ROM** | **Range Of Movement** |
| **TPBS** | **Tris Phosphate Buffer Saline** |

**Acknowledgement**

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Respectfully yours,

*Dedicated To My Mother, Ioana*

# SECTION 1: INTRODUCTION

The Elbow Joint (EJ) is one of the most inherently stable articulations of the skeleton and its structure reflects a balance between functional requirements and stability. The forces applied to the elbow are shared between different stabilizing structures such as osseous, articular, ligamentous and capsular components of the joint.

The anterior aspect of the capsule, associated with the Coronoid Process of the Ulna and Brachialis muscle, forms the Anterior Column of the EJ and this column represents one of the most important anatomical restraints that contribute to the joint's stability [1]. When the elbow is fully extended, the Anterior Capsule is responsible for as much as 40 percent of the resistance to the laterally directed (Valgus) stress and one third of the resistance to the medially directed (Varus) stress [2]. During trauma, at least one of the component structures is disrupted and this leads to the risk of developing post-traumatic stiffness of the elbow. Although much information has been acquired about the structure of the EJ, very little is known about its biomechanical properties.

The overall objectives of this study were:

1) To determine if post-traumatic contracture of the elbow joint is associated with expression of collagen type III.
2) To determine biomechanical properties (Modulus of Elasticity and Structural Stiffness) of the normal (non-pathological) elbow joint capsule.
3) To compare these properties between different regions of the normal capsule: lateral (radial), midcapsular and medial (ulnar).

This study was based on the hypothesis that:

1) Changes occur in structural properties (elasticity and stiffness) and subsequently in biomechanical properties of the fibrous membrane of the elbow joint capsule, that are manifested in the contracted post-traumatic capsule of the elbow joint.

2) These changes are determined by changes in molecular composition of the fibrous membranes of the capsules, notably changes in levels of expression and organization of collagen type III.

The rationale in searching for collagen type III was that during the healing process this type was mentioned in the specialty literature as being synthesized and then totally replaced by collagen type I.

However, in order to study these phenomena in capsules derived from post-traumatic elbow joints, it is important to characterize them in comparison with capsules from normal elbow joints. Such analyses have not been performed previously and they will have very important clinical implications.

We also propose that further studies involving comparative analyses of biomechanical properties and molecular composition of capsules of normal elbow and post-traumatic joints may lead to a better understanding of post-traumatic clinical features associated with a decrease in "Range Of Movement" (ROM) in the contracted elbow joint. This, in turn, may lead to novel approaches aimed at prevention and/or treatment this ailment, which is a major problem in patients suffering elbow trauma.

## 1.1 ANATOMY OF THE NORMAL ELBOW JOINT

Articulatio Cubiti, the Latin name for the elbow joint (EJ) is a highly constrained joint. Its stability is maintained by ligaments, osseous and capsular structures [2]. There are three articulations in the EJ: the ulnohumeral joint, the radiohumeral joint and the proximal radioulnar joint. The ulnohumeral joint is composed of a spool shaped pulley called Humeral Trochlea that articulates with the trochlear notch of the ulna [2]. This allows 0 to 150 degrees flexion [22].

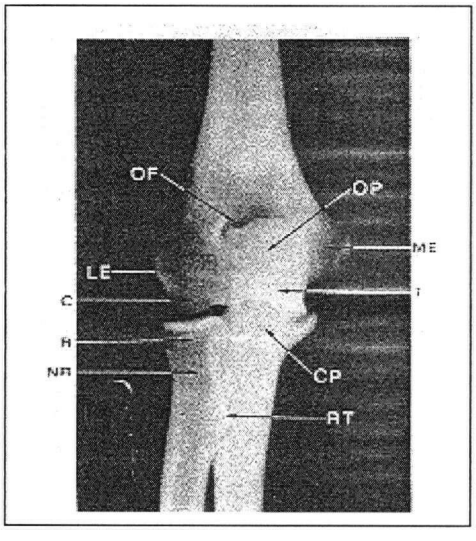



**Figure 1: X-ray of the Human Right Elbow Joint (Antero-Posterior view)** C, capitulum of humerus; CP, Coronoid Process of ulna; LE, Lateral Epicondyle of humerus; ME, Medial Epicondyle of humerus; OF, Olecranon Fossa of humerus; OP, Olecranon Process of ulna; R, head of Radius; RT, Radial Tuberosity; T, Trochea of humerus; NR, Neck of the Radial head [2].

The Coronoid Process (CP; Figure 1) is a triangular shaped anterior projection of the proximal end of the ulna and guards the humeral trochlea [2]. The radiocapitellar joint, (Figure 1) allows 75 degrees pronation and 85 degrees supination [36]. In this joint, the disc shaped proximal end of the radius articulates with the humeral capitulum, which laterally merges into the lateral epicondyle of the humerus [2]. The third joint, the proximal radio-ulnar, lies between the radial notch of the ulna and the radius [2]. The annular ligament of the head of the radius, inserts on the radial notch and circles the radial head. It helps keep the head of the radius in permanent contact with the capitulum.

## 1.2 LIGAMENTS OF THE ELBOW JOINT

The Radial (or Lateral Collateral) and Ulnar (or Medial Collateral) ligaments are located on the lateral and medial sides, respectively. These provide lateral stability to the joint [2]. The relative importance of these ligaments depends on the position of the arm [22; 36]. The Lateral Collateral ligament is fan-shaped and has three components: two of which insert on the annular ligament and one, that is more anterior, is called Lateral Ulnar Collateral ligament. The Lateral Ulnar Collateral ligament is the most important of these. It plays an important role in rotational stability. It originates from the lateral epicondyle and inserts on the tubercule of the supinator crest of the Ulna [2]. Its function is to prevent Varus and Postero-lateral instability of the elbow, which are the clinical results of medially and/or posteriorly directed forces [22; 34; 36]. The Medial Collateral ligament (Figure 2) is attached between the Medial Epicondyle and the medial margin of the trochlear notch.

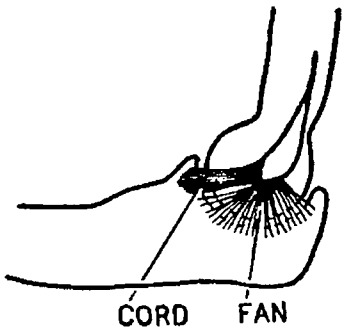



**Figure 2: Medial or Ulnar Collateral Ligament of Elbow Joint**

The anterior fibers form the anterior strong band or cord (Figure 2) that is the most important of the three distinct bands and inserts on the medial aspect of the coronoid process [2]. This band is the primary constraint to the Valgus instability, which is defined as the clinical result of the force acting toward the lateral side of the EJ. A lot of tension develops on the medial collateral ligament [12; 36]. The radial head is of secondary importance [22; 36]. The other

two bands are: the posterior band (which is weaker) and the oblique band, which partially cover the humeral trochlea [2].

## 1.3 NORMAL CAPSULAR STRUCTURE OF THE ELBOW JOINT

The joint capsule is composed of two histologically distinct membranes: the synovial membrane, which lines the synovial cavity, and the outer fibrous capsular membrane. The fibrous layer attaches proximally to the upper margins of the coronoid process and radial fossae on the anterior aspect of the joint but not as far on the olecranon fossa, on the posterior aspect [2; 8]. Distally, the fibrous capsule inserts to the margins of the trochlear notch and Annular ligament (Figure 3) [2].

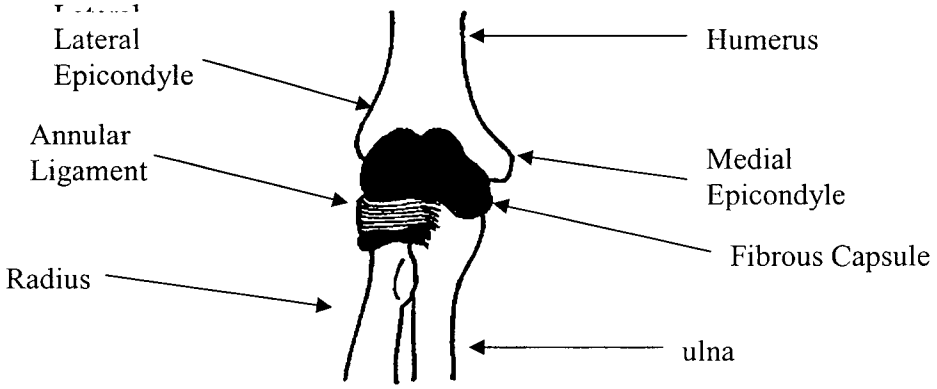



**Figure 3: Synovial Capsule of the Elbow Joint – Antero Posterior view**

The synovial membrane, at the level of the radial head, drops approximately 0.5 cm below the lower free margin of the Annular ligament and surrounds the radial neck in a sac-like manner. This allows the radius to rotate without tearing the synovium [2]. In the fossae, the spaces between the two capsules are occupied by fat-filled synovial pads. Folds of the

synovial membrane project into the joint assisting the fat pads to fill the unoccupied space [2]. In full extension, the capsule of the EJ serves as an important constraint to instability [5; 10].

## 1.4 MUSCLES AND NERVES

The muscles that act on the EJ are grouped by the anatomical side of the joint where they act. On the humeroulnar side, the Brachialis muscle is the main flexor [2]. Three muscles are located on the humeroradial side: the brachioradialis, the biceps and the pronator teres. They act to flex the radius [2]. On the posterior aspect of the EJ, extension is accomplished by the Triceps and the Anconeus muscles [2]. The nerves associated with the anterior side of the elbow joint are the Musculocutaneous, the Median and the Radial nerve. The latter lies directly on the capsule. The Ulnar nerve passes posterior and medial to the joint and is constantly in contact with the Medial Collateral ligament [2].

## 1.5 HISTOLOGY AND MOLECULAR COMPOSITION OF THE FIBROUS MEMBRANE OF THE ELBOW JOINT CAPSULE

Morphologically the normal capsule of the EJ in humans is a complex structure that consists of collagen fibrils embedded in a matrix of proteoglycans associated with a relative paucity of cells [3]. Fibroblasts are the predominant type of cells and they are arranged in parallel rows between fibers of collagen that are synthesized by the fibroblasts.

## 1.6 COLLAGENS

Collagen is the major protein of ligaments and tendons. Type I, II, III, V and XI make up the group of fibrillar collagens. By forming highly organized fibers, these collagens provide the

structural support for the body in skeleton, skin, blood vessels, nerves, intestines and the fibrous capsules of the organs [25; 26].

### 1.6.1 CHARACTERISTICS OF HUMAN FIBRIL-FORMING COLLAGENS

In humans, the fibril-forming collagens are type I, type II, type III, type V and type XI [23; 25]. Collagen type I predominates in bones, tendons, joint capsules and the annulus fibrosus of the intervertebral disc (IVD) [23; 25]. Collagen type II predominates in articular cartilages and nucleus pulposus of the IVD [23; 25]. Collagen type III is found in embryonic tissues and in adult tissues that need to present adaptability to volume such as artery, skin and soft organs where they form reticular fibers [25; 26]. Collagen type III is also predominant in the initial stages of the healing and formation of scar-tissue [23; 25; 39]. Collagen type III is not expressed in bone, tendons, capsules and cornea [1; 3]. Type V and type XI collagens usually combine with type I and II to form heterotopic fibrils, but in a very small percent [23].

In adults, the major constituent of the EJ fibrous membrane is collagen type I (86% of the membrane's dry weight). Collagen type I contains high concentrations of the amino acids glycine (33%), proline (15%) and hydroxiproline [3; 25]. Hydroxiproline is a derivative of proline and it is almost unique to collagens [3]. Hydroxilysine, a derivative of lysine, is also present in collagen type I [3]. Thus, the primary structure of the collagen chains consists of these amino acids [3; 25; 44]. The secondary structure relates to the arrangement of each chain in a left-handed configuration and the tertiary structure is formed when the 3 chains are combined in a right handed triple helix giving to collagen molecule a rod-like shape. This constitutes a stable low energy biological unit [25; 44].

Every third amino acid is represented by glycine, which contributes to the stability of the molecule by forming hydrogen bonds among the three chains of the superhelix [25; 44]. The adjacent molecules are arranged so that oppositely charged aminoacids are aligned. This results in a very strong bond and a lot of energy is required to separate the molecules [3]. The collagen molecules then combine to form units called microfibrils in a quaternary structure. Five molecules of collagen combine to form microfibril, subfibrils and fibrils [1; 25; 44].

Collagen type III is a homotrimer of three alpha 1 chains as opposed to collagen type I, which is a heterotrimer of two alpha 1 chains and one alpha 2 chain. Both types have approximate chain lengths of 1000 amino acids but collagen type III shows several unique features which include high levels of 4-hydroxyproline, more than 333 residues and half-cystines which participate in the intramolecular disulfide cross-links [3].

In addition to collagen, elastin fibers are also found in fibrous membranes of capsules, tendons and ligaments, but only in a small quantities [3]. The protein elastin is a naturally occurring rubber-like elastomer found in extracellular matrices in mammalians. In elastin, one third of the amino acid residues are represented by glycine, one ninth are proline. In contrast to collagen, it contains very little hydroxiproline, does not contain any hydroxilysine and contains many molecules of non-polar amino acids like alanine, valine, leucine and isoleucine [1; 3]. Elastin fibers are branched together to form a network inside the tissue and after stretching they return to their initial length [1; 3].

### 1.6.2 COLLAGEN AND AGE

In newborns, collagen type III is produced and the fibers formed from this type of collagen are supple and elastic. In adult age, collagen type III only represents a very small percentage

of total collagen. Only during pathological post-traumatic processes, healing of collagenous structures implies producing more collagen type III [23; 25]. With each passing decade, collagen-producing cells (fibroblasts) make less collagen type III and progressively convert to synthesizing collagen type I [25], Figure 4. Collagen type I consists of sulfur groups that have a tendency to cross-link and form bridges between collagen filaments, causing the fibers they comprise to be relatively tough and non-elastic.

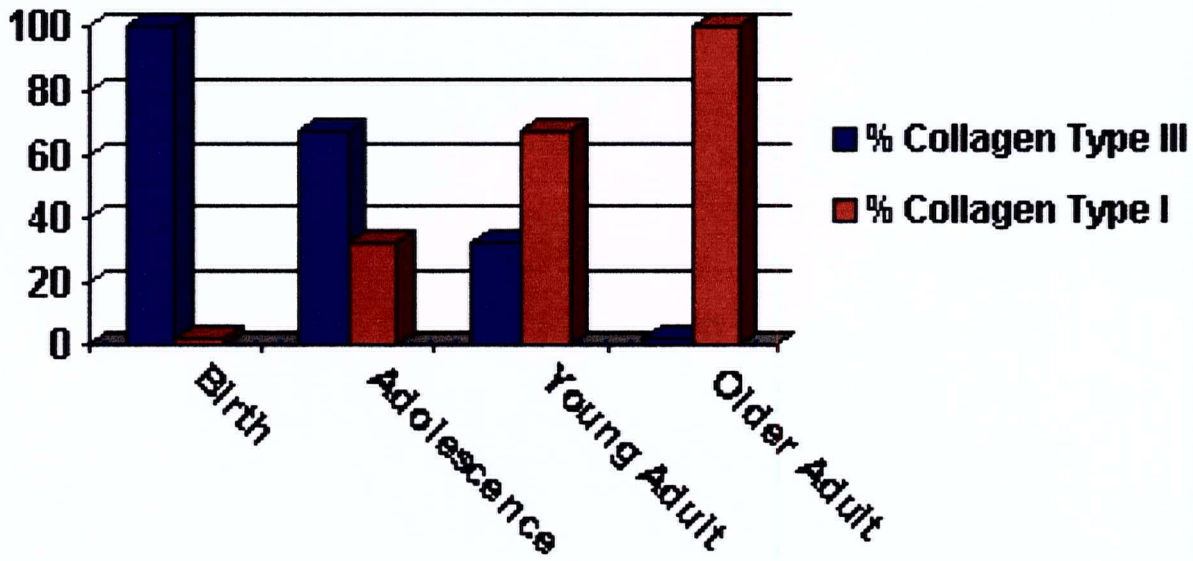



**Figure 4: Age-specific Changes in Expression of Collagen Types I and III**

## 1.7 BIOMECHANICAL PROPERTIES OF THE NORMAL ELBOW JOINT CAPSULE

One of the most inherently stable joints, the elbow joint has two degrees of freedom: the first, represented by flexion and extension motions, is allowed by the articulation between humerus and ulna, while the second is allowed by articulation of the capitulum and the head of the radius and is called pronation and supination [2; 4; 36; 40]. Due to evolution and the use of the upper limb for purposes other than locomotion, some of the muscles around the elbow joint have migrated to the front of the joint. As a result, all the flexor muscles are

the main flexor of the forearm. The biceps muscle is a flexor of the elbow only during supination; it does not flex when pronated, and it is only a partial flexor when the forearm is semipronated. Flexion of the biceps can supinate the forearm [1; 2; 4; 23]. The brachioradialis muscle and the pronator teres muscle are the weakest flexors of the elbow, even if the brachioradialis has a more advantageous origin and insertion, being situated on top of the pronator teres [1; 2]. Extension is mainly a passive action, which is helped by gravity [2]. The use of the triceps muscle is to prevent flexion at the level of the elbow, or to regulate flexion as when pushing [1; 2].

The Collateral ligaments are considered the most important stabilizers and prevent medial and lateral movements [33; 36; 40]. The Medial Collateral ligament guards against Valgus instability and the Lateral Collateral ligament guards against Varus instability as well as Posterolateral rotatory instability [33; 36; 40]. These ligaments are very strong and the average failure load is around 260 Newtons [45]. Functional studies done on cadavers show gross instability of the EJ if one or both of the ligaments are sectioned. In 1980, Schwab *et al.* conducted studies with cadavers by sectioning the EJ capsule and they demonstrated that it forms a major constraint to instability in full extension. More detailed measurements estimate that the EJ capsule contributes 40% to Valgus stability and 30% to Varus stability when the EJ is fully extended.

As a complex, which needs to share the forces applied to it, the stabilizing factors of the EJ are divided into four columns: Lateral Column - radius, capitulum, Lateral Colateral ligament; Anterior Column - coronoid process, Brachialis muscle, anterior capsule; Medial Column - medial epycondile, Medial Collateral ligament, coronoid process; and the Posterior Column - Olecranon process, Triceps muscle, posterior capsule. [12; 42; 47] Injuries to the

capsular, osseous and articular components, by overload, affect the stability and the potential for recurrent and chronic instability become greater as more components are injured [4; 40; 47].

Biomechanical properties of the capsule depend on the material properties and the architecture of collagen fibers, elastin fibers, and proteoglycans [1]. Under normal physiological conditions, maintenance of the tissue is regulated and controlled through a balance between synthesis and degradation [23; 25]. The collagen fibers contribute to transmit the force generated by the muscles, lend to the tensile strength of the bone, resist extension by the surface layers of the articular cartilage and contribute to limit the range of motion (ROM) of the joints [23; 25]. Arrangement and alignment of collagen fibers reflect the applied mechanical stress on the tissue [23].

These collagen structures form either tendons, capsules or ligaments [1; 44]. Because collagen is the strongest of the fibrous proteins and because of the parallel arrangement of the fibers with the direction of the force, tendons or ligaments possess one of the highest tensile strength of the materials present in the body [23; 44].

From a mechanical point of view, a traumatic event represents excessive tensile loading on the collagenous tissue and could result in its partial or complete rupture [1; 23].

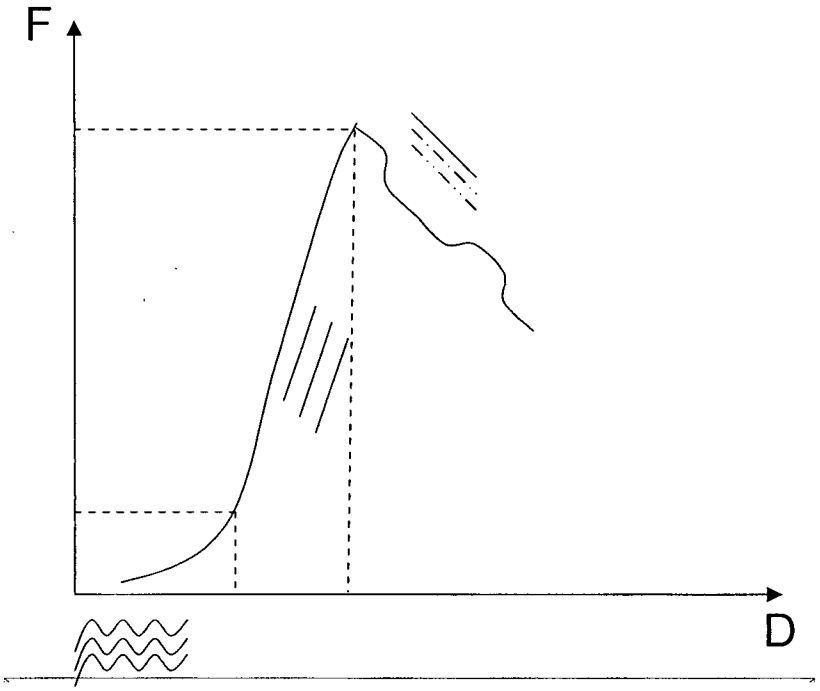



**Figure 5: Stress - Strain Curve for a Tendon**

When collagen is subjected to increasing tension, a stress-strain-curve similar to that seen in Figure 5 is obtained. The stress-strain curves begin with a toe region, where the collagen structure stretches easily, at relatively low levels of force. This is because of the orientation of the fibers in the loading direction, and also because the fibers are straightening. This region is a short interval and is believed to be the result of a change in the wavy pattern of the relaxed collagen fibers as loading progresses. As loading continues, stiffness of the tissue increases and progressively greater force is required to produce equivalent amounts of elongation. The elongation is often expressed as strain [1; 44]. The next region of the curve is a linear one. The slope of which is represented by the Modulus of Elasticity (Young's Modulus) of the capsule. It is followed by maximum stress, which represents the ultimate tensile strength, and then, by an abrupt downward slope called failure region.

Viscoelasticity property of collagenous tissues display time and history behavior of force application. This becomes important as the loading of the capsule, tendon or ligament is cyclic during activities of daily living (ADL). The viscoelastic property is thought to be a result of the matrix of mucopolysaccharides (ground substance) and water content. Because the capsules contain more collagen fibers and less ground substance than other soft tissues, they show higher plastic deformation, and thus they are less elastic than other soft tissues [1]. The rate of loading is also an important factor. By increasing the elongation speed, the capsule will become stiffer [1]. Other factors which can influence tensile properties of a collagenous structure (tendons, capsules and ligaments) are anatomic location, the amount of activity, and age.

Different anatomic locations have different biomechanical and biological environments (for example, biochemical analysis has shown that capsule from the flexors side of a joint at the same location, have a much higher collagen concentration than the capsule from the extensors side). Exercise has been found to have a positive effect on structural and mechanical properties of capsules, as well as on collagen synthesis - thickening of the collagen fibrils will resist bigger tensile forces due to more intrafibrillar covalent crosslinks [1 23; 47]. It has been found that thickness of capsules increases with age and there are significant differences between age groups [46].

Deformational properties of an object are to be defined as intrinsic material properties and are represented by the Modulus of Elasticity (Young's Modulus) and Structural Stiffness. These are discussed below.

## 1.8 ELASTICITY AND STRUCTURAL STIFFNESS

*a)* ***Elasticity*** - Two important mechanical parameters that need to be studied to get information about intrinsic properties of a tissue are Stress and Strain. Stress is defined as the amount of force applied to a specific area and it can be compressive or tensile in nature. It is calculated by dividing the applied force by the cross sectional area [1; 44]. Strain is defined as the ratio of the change in length (dL) created by a force and the initial length (Lo). It is dL divided by Lo [1; 44].

Since collagen fibers are arranged in an orderly manner and they are oriented in the direction of loading of tendons, capsules and ligaments, they are anisotropic materials. Their intrinsic material properties depend upon the direction of loading [1; 44]. Modulus of Elasticity has been determined by several investigators (for example, Fung 1972, Vidiik1968) and it is based on a linear relationship between stress and strain (Modulus of Elasticity = Stress/strain) and it is expressed in MegaPascals or GigaPascals [1].

*b)* ***Structural Stiffness*** - Tensile testing of tendons, capsule and ligaments involves measuring the force and the elongation produced. This represents the second important intrinsic material property called Structural Stiffness. When testing a ligament, relatively low stiffness was observed in the toe region of the Load/Displacement graph. This is attributed to the wavy pattern (crimping) of collagen fibers within the tissue and to the non-uniform recruitment of the fibers [1; 44].

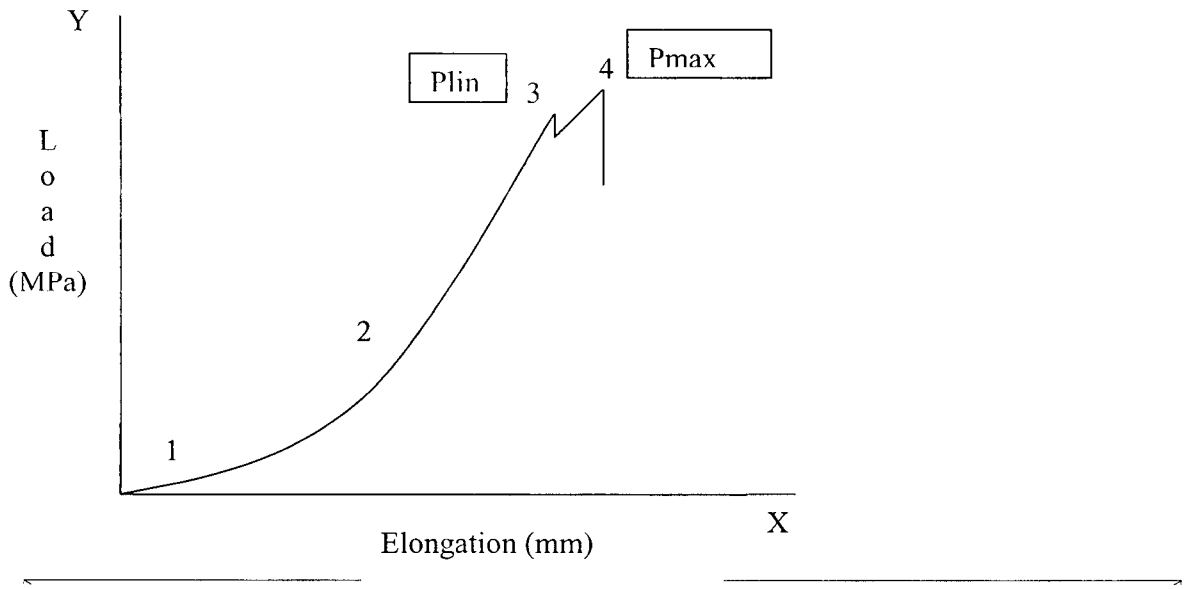



**Figure 6: Load-elongation Curve for Rabbit Tendon** The numbers indicate the four characteristic regions of the curve. (1), the primary or toe region, in which the tissue elongated with a small increase in load as the wavy collagen fibers straighten out. (2), secondary or linear region, in which the fibers straighten out and the stiffness of the specimen increased rapidly. Deformation of the tissue began and had a more or less linear relationship with load. (3), end of secondary region. The load value at this point is designated as $P_{Lin.}$ Progressive failure of the collagen fibers took place after $P_{Lin}$ was reached, and small force reductions (dips) occurred in the curve. (4), maximum load ($P_{max}$) reflecting the ultimate tensile strength of the tissue. Complete failure occurred rapidly and the specimen lost its ability to support loads [44].

Because of variable degrees of crimping and different orientation, each fibril unfolds and begins to resist stretching at a different stage of elongation of the ligament [1; 44]. As

elongation increases, more fibers become unfolded and oriented in the direction of loading. Thus, gradual increase in the sample stiffness is produced, as shown in Figure 6 [1; 44]. Stiffness is calculated by dividing the force by the elongation. Because of the inherent complexity of biological material, various technical difficulties must be taken into consideration when determining intrinsic material properties. These are outlined below.

## 1.9 STUDYING BIOMECHANICAL PROPERTIES OF ELBOW JOINT

Technical difficulties during tensile testing should take in consideration the following: the environment (temperature and hydration); gripping the tissue to avoid slippage during testing; initial length; specimen geometry; and cross sectional area [1]. Hydration and temperature can affect the measurements during the tensile test. Collagenous tissues swell in water or in physiologic saline, which will decrease the stiffness or will stiffen the tissue and increase the failure stress if the tissue dehydrates. Increased temperature between 39° C and 45° C can create irreversible structural damage. Gripping has to be strong enough in order not to give false elongation by slippage of the sample or even to suggest failure at low forces. Care must be taken when gripping the end of the tissue that is sampled so that it is not damaged [1].

Initial length affects the strain measurements and also the measurement of stiffness and calculation of elasticity. Therefore, to help standardize the test, the gauge length is defined either by observation of the specimen *in situ* or by application of a small initial force (a preload) [1]. The effect of geometry has to be eliminated, and this is done by constructing very precise specimens for testing by precise measurements of the sample. Cross-sectional area needs to be measured very accurately because tensile stress is calculated by dividing the force by the cross sectional area. Measurements of cross-sectional area is difficult to achieve

because the sample is small and the shape is not uniform. Thus, sometimes the area is approximated by assuming cross-sectional shape such as rectangle or ellipse [1].

## 1.10 PATHOPHYSIOLOGY OF A POST-TRAUMATIC ELBOW JOINT

Depending on the force of the impact acting on the elbow, the Lateral Column and the Medial Column, which share the loading forces at the EJ level, are both affected [4]. Simple or complex fractures of the radial head associated with ligamentous apparatus destruction and/or posterior dislocation lead to loss of motion. According to the Mason's modified classification of the proximal extremity of the radius in type I fracture, there is minimal displacement of the bone fragments [12]. In the type II fractures, the displacement of the bone fragments is bigger than 2 mm, the motion of the joint can be mechanically impaired and they require surgical repair [12]. In type III fractures, the comminution is very significant. The comminuted fragments are very difficult to repair and may need excision if movement of the joint is to be obtained following healing [12]. All of these fractures are associated with collateral ligament injuries, posterior dislocation and/or with coronoid process fracture [4; 12].

Treatment for type I fractures involves the use of casts or braces. For type II and type III fractures, surgical intervention is certain and consists of Open Reduction and Internal Fixation (ORIF) and repair of the ligaments and other associated injuries [4; 12; 38]. Excision of the radial head in the type III fractures is not accepted anymore because of loss of permanent radiocapitellar contact inside of the Lateral Column and it should be repaired [4; 12; 38]. If neglected or unrecognized, complications such as Postero-lateral Chronic instability, Proximal migration of the radius, Valgus deformation may follow and they are very difficult to be treated [4; 38]. Therefore, surgical repair with restitution of normal

anatomy of the region must be achieved [4; 12]. After conservative or surgical treatment, the EJ is immobilized by a cast for 4 to 6 weeks [4]. By casting the EJ at 90 degrees, with the hand in the neutral position, the best alignment of the bone fragments with concomitant continuous relaxation of the muscles is achieved, allowing fractured bones and other collagenous soft tissues to repair and heal [4; 25; 39].

## 1.11 THESIS PROBLEM

One of the most important complications in EJ pathology is post–traumatic contracture of soft tissues surrounding the joint [4]. Insidious stiffness takes over the joint, decreasing the ROM. Even with early physiotherapy, contracture of the EJ becomes so stable that within 3 to 6 months from the initial trauma the patient has to be surgically intervened in order to achieve a functional arc of ROM [41; 43]. Epidemiology of the stiff EJ puts trauma as the first cause at 44% overall [22]. Though individual functions differ, it is well recognized that most activities of daily living (ADL) require an arc of motion of approximately 100 degrees, with a regular ROM between 30 and 130 degrees [37]. Any loss of this arc of movement may limit one's function of the upper limb. Trauma, either occurring through accident or provoked (with sport activities, for example), could inflict various degrees of lesions and depending on the level of damage, a large number of complications can occur. When a functional arc of ROM cannot be achieved following a traumatic event, surgical intervention is required [4; 17].

If the two goals of treatment, anatomical repair and healing of the fracture are achieved and confirmed by X-ray examination, the only logical explanation for stiffening of the capsule after trauma is that changes in the composition of the capsule and its biomechanical properties are taking place.

Therefore, the purpose of this study was to initiate experiments that would verify that changes in expression of collagen and in biomechanical properties of the EJ capsule are associated with contracture of the elbow. It has previously been demonstrated that during the initial stages of healing, the rupture sites are bridged by newly synthesized collagen type III, which partially replace type I collagen [23; 25].

The major intrinsic material properties of capsules that could contribute to contracture of the EJ, and result in decreased range of movement, are elasticity and structural stiffness. Measurement of these two properties in biological samples is challenging because of their inherent complexity. (The parameters that have to be addressed when studying biomechanical properties of the EJ capsule are described above.) Studies of biomechanical properties of the EJ capsule (normal or pathological) have not been documented previously. Therefore, it became critical to design and build equipment and to establish protocols and methodologies that are suitable for this purpose. This was accomplished in the studies described here, in which we determined biomechanical properties of the non-pathological EJ capsule. In addition, we applied our system to determine if there are any inherent variations in biomechanical properties in different regions of the normal capsule: lateral (radial), midcapsular, and medial (ulnar).

# SECTION 2: MATERIALS AND METHODS

## 2.1 SELECTION OF CADAVERS

The cadavers were donated through the Body Donation Program to the Department of Anatomy, Faculty of Medicine, University of British Columbia. Ten cadavers were used for these studies. They did not have a history of fractures, trauma or clinical signs of limitations of flexion or extension movements in the elbow joint (determined by questioning the family physician). They did not show any signs of limitations in range of motion (ROM), prior to death, suggesting that the elbow capsule was not associated with any apparent pathology. The ages of the donors fell in the range of 75 to 93 years (Table 1).

**Table 1: Profile of Cadavers Selected for the Study – Normal EJ Capsules**

| Cadaver Number | Initials | Age at Death | Month & Year of Surgical Removal | Number of Sections |
|---|---|---|---|---|
| 1 | M.M. | 84 | September 2001 | 9 |
| 2 | T.J.G. | 93 | October 2001 | 6 |
| 3 | H.L. | 79 | January 2002 | 6 |
| 4 | A.S. | 78 | January 2002 | 7 |
| 5 | G.C. | 83 | June 2002 | 9 |
| 6 | W.E. | 85 | July 2002 | 6 |
| 7 | R.E.H. | 84 | September 2002 | 8 |
| 8 | A.W.R. | 76 | October 2002 | 5 |
| 9 | G.J.M. | 77 | November 2002 | 10 |
| 10 | S.Y. | 75 | November 2002 | 9 |

**Table 2: Profile of Patients with Post-Traumatic Contracted EJ Capsule**

| Patient Number | Initials | Age of Patient | Gender | Month & Year of Surgery |
|---|---|---|---|---|
| 1 | P.D. | 30 | M | June 2000 |
| 2 | S.T. | 40 | F | March 2001 |
| 3 | U.A. | 44 | M | March 2001 |

## 2.2 SURGICAL REMOVAL ELBOW JOINT CAPSULES

For harvesting the capsules, the cadavers were placed in dorsal decubit. A bag was placed underneath the right shoulder and another one was placed under the right elbow. A lateral approach described by Kocher in 1911 was used, alternatively, one of the Extensile approaches to the anterior or anterolateral aspects of the elbow was used [41; 42]. This approaches the joint between the brachioradialis laterally and biceps and brachiallis medially. The dissection was carried toward the capsule by retracting the neurovascular package and the medial muscles. The anterior capsule was retrieved by reaching medially.

When sampling the capsules, we followed the orientation rule, whereby the capsule was removed from the lateral (radial) aspect to the medial (ulnar) aspect. Ten right anterior capsules were used for histology, immunohistochemistry and biomechanical analyses.

The orientation rule and the surgical removal approach for normal EJ capsules have been suggested and agreed upon by the surgeon who provided the pathologic samples of the contracted post traumatic EJ capsule (Table 2).

## 2.3 SAMPLE PREPARATION

The dissected capsules were cleaned of unrelated soft tissue, such as muscles, fascia and fat. They were divided into 3 fragments: 2 small ones, each measuring approximately 1 square cm, and a larger one measuring approximately 30X50 mm. The larger fragment was frozen in saline, at -20° C and was later used for biomechanical analyses. The pathologic samples of EJ capsules have been treated exactly the same method as the non pathologic ones. Unfortunately, because of surgical concerns, they were not large enough to provide also the 3rd large fragment, which would have allowed mechanical testing. The first two small fragments suffered immunohistochemistry tests and H-E staining. One of the small

fragments was fixed in 3.7% paraformaldehyde in PBS and it was used for immunohistochemistry. The other one was also fixed in 3.7% paraformaldehyde in PBS for histological characterization following staining with Hematoxylin-Eosin.

## 2.4 HISTOLOGICAL CHARACTERIZATION OF SPECIMENS

For histological assessment, the capsular samples were fixed in 3.7% paraformaldehyde for 12 hours, at room temperature, dehydrated by emersion in high percentage ethanol (95%-100%) for 24 hours, further dehydrated with xylene and then embedded in paraffin wax. 5 μm sections were obtained using a microtome and they were processed for standard histology using staining with H-E. The morphology of the specimens was determined by light microscopy.

## 2.5 IMMUNOHISTOCHEMICAL ANALYSES

For immunolocalization of collagen type III, fragments measuring 1 square cm that were derived from the dissected EJ capsule were fixed in freshly prepared 3.7% para-formaldehyde in PBS (1.5M NaCl, 0.2M Na/KPO4 and 1.5M KCl, pH 7.3). The specimens were mounted on a stub using OCT compound (Tissue-Tek), and snap frozen in liquid nitrogen. 15 μm frozen sections were obtained using a BI HI Cryostat (Bright Industries Comp). The sections were transferred onto slides that had previously been cleaned with chromic acid (for one hour), rinsed thoroughly and coated with polylysine. 5 sections were obtained for each specimen: two were probed for the expression of collagen type III, the third served as a control for the primary antibody, the fourth served as a control for the secondary antibody and the fifth one was used to assess autofluorescence.

For IHC, the slides were coated with 5% goat serum in TPBS/BSA for 20 minutes. The specimens were then incubated with 50 µl of mouse monoclonal anti-collagen type III antibodies (clone FH-7A; ascites fluid; SIGMA-ALDRICH; Ontario; Canada) at a concentration of 1 µg/ml in 1% NGS/TPBS/BSA. The sets of slides that served as controls for the specificity of the primary antibodies were incubated with 50 µl of normal mouse IgG at a concentration of 1 µg/ml in 1% NGS/TPBS/BSA. The sets of slides that were used to evaluate autofluorescence, were incubated with 1% NGS/TPBS/BSA only. The slides were left overnight at 4° C. They were then washed three times with TPBS/BSA (10 minutes per wash).

The slides (except those that were used to evaluate autofluorescence, and those that were used to determine the specificity of the secondary antibodies, which were incubated with 1% NGS/TPBS/BSA) were then probed with 50 µl of secondary antibodies at 10 µg/ml. These were goat anti-mouse antibodies tagged with fluorochrome Alexa 488 (Molecular Probe; Eugene; Oregon). The slides were kept in a humidified chamber at 37° C for 1 hour, and then they were washed three times with TPBS/BSA (10 minutes per wash). The slides were mounted with Vectashield for fluorography, examined using a Zeiss Axioplot microscope at 10X magnification and photographed using a high-speed film, Ektachrom 1600.

## 2.6 BIOMECHANICAL PROPERTIES OF THE NORMAL EJ CAPSULES

In order to compare the material properties of the two separate sample sets, the following mechanical analyses were carried out:

*Testing the sets for Modulus of Elasticity and Structural Stiffness*

Ten right non-pathological EJ capsules were surgically removed from 10 different fresh cadavers as described above. The interval between death and surgical removal was less than

72 hours but more than 12 hours. Since strain is defined as the ratio of change in length and the original length (dL/Lo), the original length was a very important factor. Therefore, slippage of the capsule from the holding device had to be addressed. A special holding device was designed and built (Figure 7).

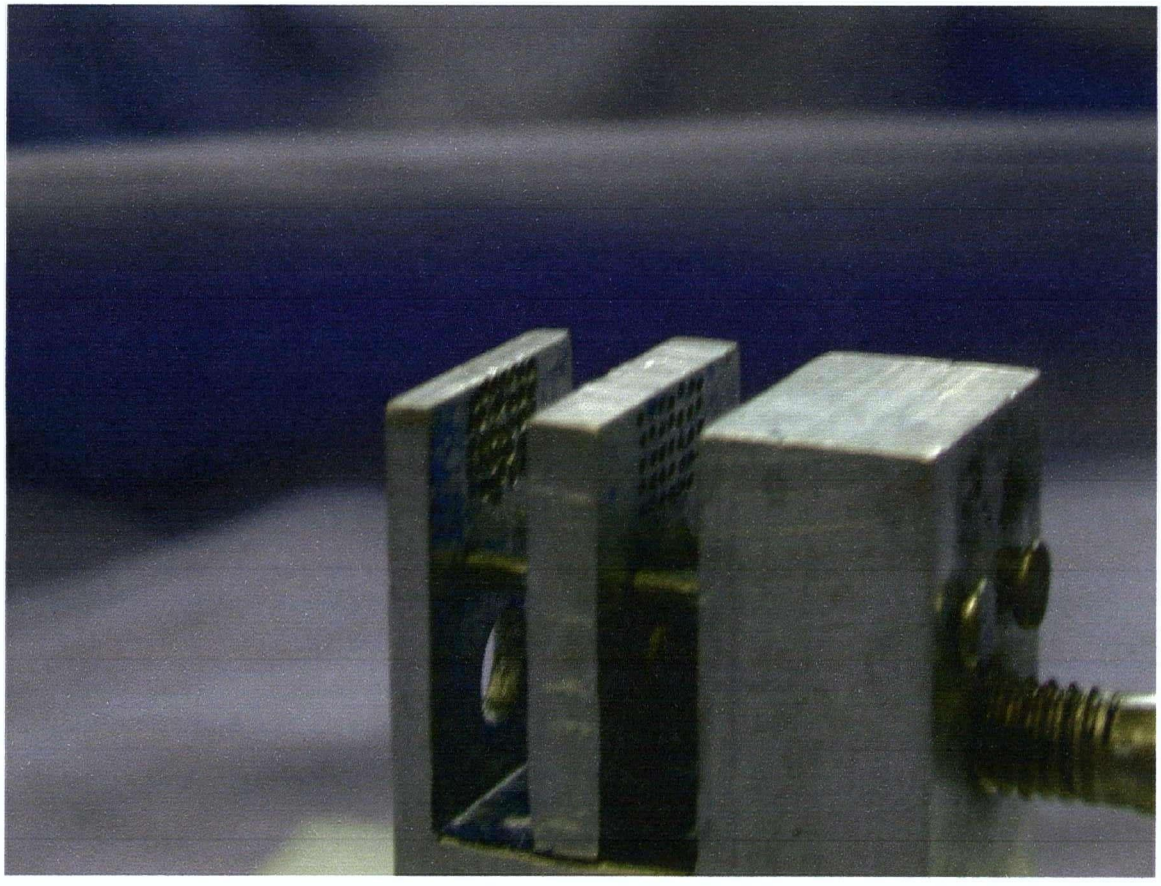


**Figure 7: Specimen Holding Device Designed to Avoid Slippage**

The clamp has a rectangular shape measuring 70 by 50 by 20 mm. One end was attached by a 6 mm diameter screw to the mobile arm of the Instron and at the other end, a canal 15 mm wide and 20 mm deep, was cut to form a thin arm of the clamp which became the built-in blade. The other blade, the mobile one, was cut separately from the same metal and adjusted to the size of the canal to match the size of the fixed one.

The mobility of the second blade was assured by a 3 mm diameter screw which held the blade at one end. The screw was 50 mm long and could be screwed in and out of a predrilled

and threaded hole into the thick arm of the clamp. Two guide pins, 1 mm in diameter were implanted through each corner of the free end of fixed blade, mobile blade and thick arm of the clamp, in order to prevent rotation of the mobile blade during ascending / descending movements.

The sample was held between the 2 blades by applying pressure with the mobile blade on the fixed blade. Each of these blades had multiple 0.3 mm diameter holes drilled into them in order to hold one end of the sample. The second clamp, identical to the one described above, was mounted on the adjustable bench of the Instron.

Alignment of the clamps was very important . Thus, a level was used until perfect alignment in both planes, anteroposterior and lateromedial was obtained. This assured that pulling was unidirectional and no bending moments on the capsule were present. Further control of slippage was done by visual means using blue chalk to color the tissue sample, which is white, at the beginning of each test. Using this device, slippage was not experienced with any of the 87 sample slices that were tested.

To eliminate variable geometry of each specimen (slice), a special cutter with 6 microtome blades mounted together was designed and built (Figure 8).

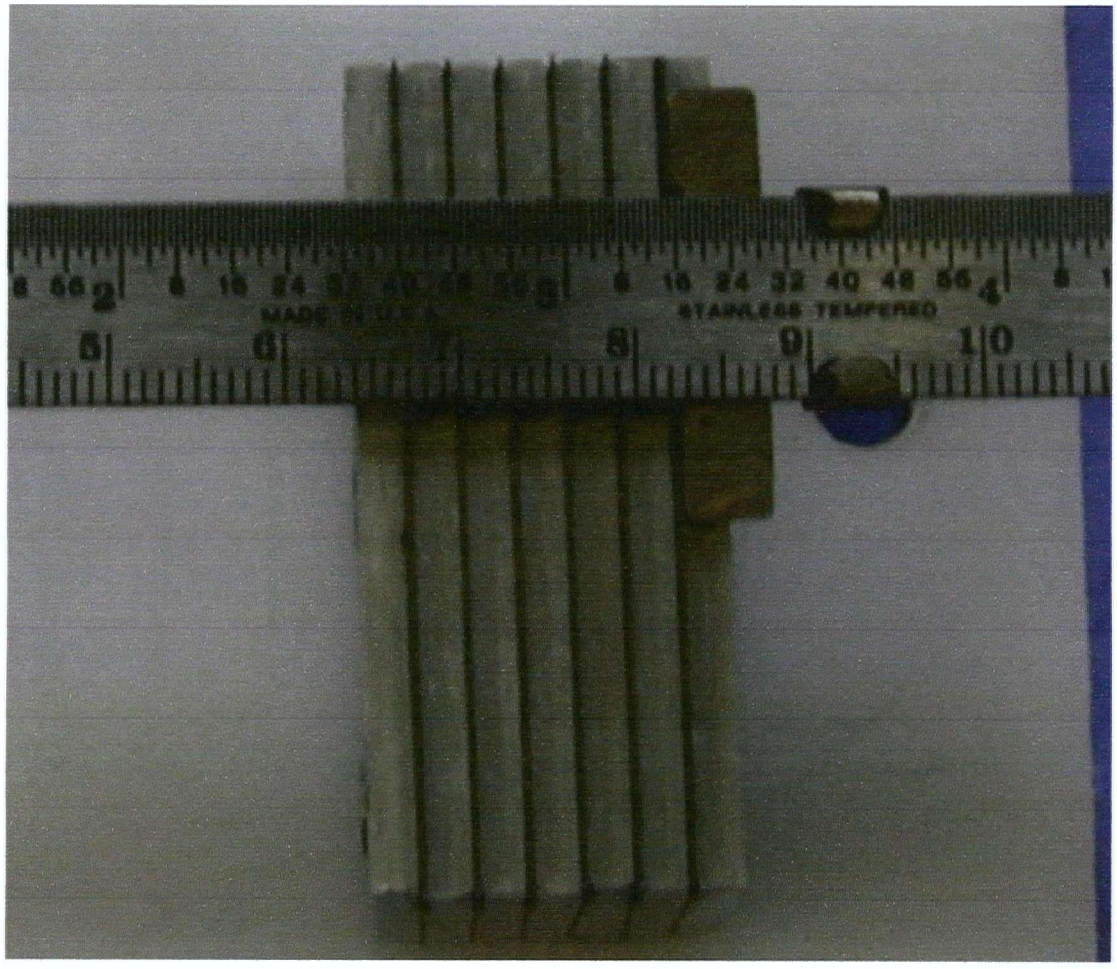


**Figure 8: Specimen Slicing Device: the ruler measures precisely the interval between the blades**

The distance between the blades was 3 mm and multiple of 6 slices were obtained and each of them numbered from 1- 11 (the number of cut slices varied in each) starting at the lateral side (radial) and sectioning towards the medial (ulnar) side.

Cleaning the capsule became a very important issue with regard to measurement of thickness. Therefore muscle rests, fat, fat pads or fascia were carefully removed from the outer and intra-articular surfaces of the capsule. Any residual muscle, fat pads and other debris were

removed. Before starting the test, the initial length (Lo) and thickness were measured using calipers, the width was rechecked and the data were recorded on separate test file sheets.

The tests were performed at room temperature and a good level of hydration was maintained even during testing by spraying normal saline solution from a syringe [1; 24]. The tests were performed by a computer assisted machine, Dynamight made by Instron, which was equipped with a standard 1 KiloNewton capacity Load cell (Figure 9).

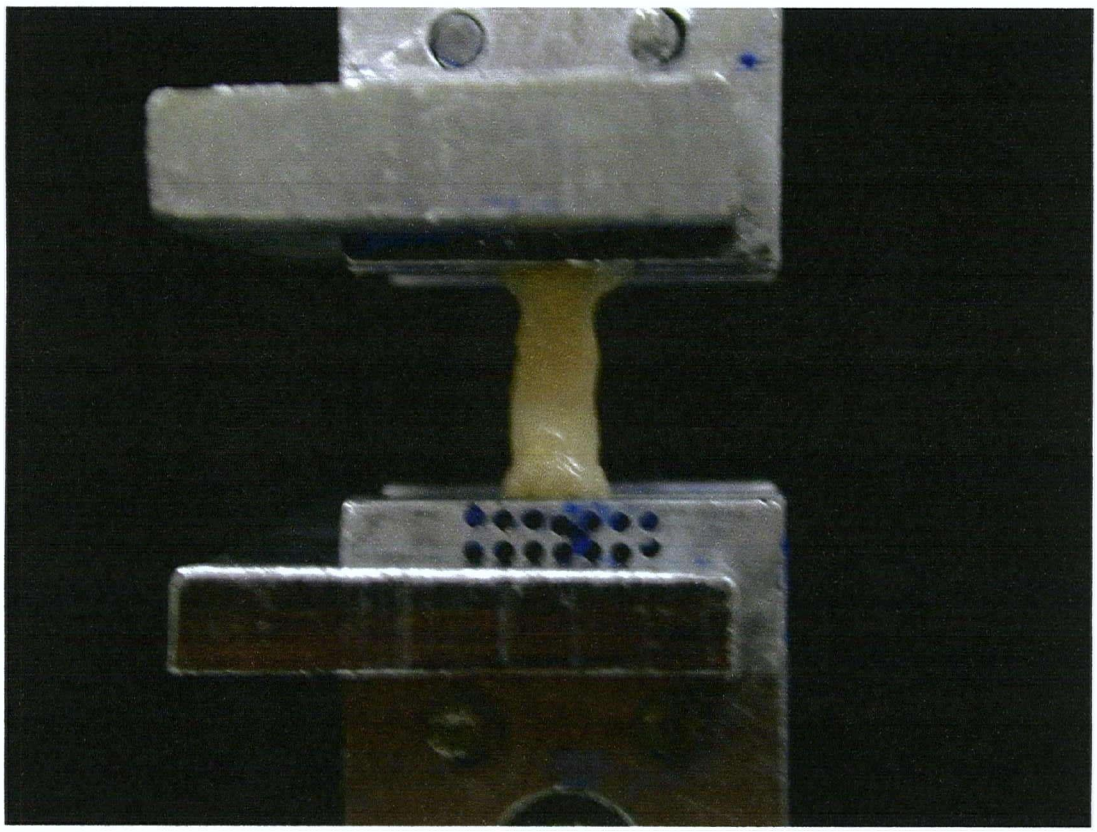


**Figure 9: Illustration of Tensile Testing of a Specimen using Dynamight.**

The specimen was loaded into the upper clamp by trapping at least 5mm of the total length of the specimen between the fixed and the mobile blades. A good strong grip of the specimen

was required during tensile test. Then, the mobile arm of the Dynamight was lowered until at least 5 mm length of the specimen was trapped between the blades by tightening the mobile blade (Figure 9).

The test was done using a computer program called Waveform, which holds for 0.5 sec in the beginning of the test and then starts displacing continuously for the next 10 mm. The load limits were set for +/- 50 Newtons and the position limits were set for +/- 23 mm. Some slices failed at different length close to the limits and a total of 5 slices failed within 10 mm data recording interval. The data obtained from these slices were eliminated from the data set. The data and the graphs obtained through the tests were automatically recorded and displayed. Test data were selected to plot Load/ Displacement curves, with displacement on the X axis (expressed in millimeters) and load on the Y axis (expressed in Newtons).

Stiffness was calculated by determining the slope of the linear region and the load-displacement plot was obtained by using the formula dY/dX (dY represents the change in the Force vector and dX represents change in displacement). Each specimen was considered as an ellipse, therefore the area was calculated by using the formula A=3.14(a x b), in which a and b are the small and the large diameters, respectively.

Stress is defined as force divided by the initial cross sectional area. Strain was calculated by dividing the change in distance of the region of interest by their initial length of the specimen (Lo). Modulus of Elasticity was calculated dividing stress by the strain and it was expressed in MegaPascals. Since we analyzed more than two groups, ANOVA model was used with repeated measurements.

# SECTION 3: RESULTS, DATA ANALYSIS AND STATISTICS

## 3.1 HISTOLOGY

In order to confirm the morphology of the anterior fibrous capsules, we performed histological analysis. The specimens consisted mainly of bundles of collagen (fibers stained blue with H-E, data not shown). This morphology is typical of normal capsules. There were no overt signs of injury or inflammation. The analysis also confirmed that the specimens were free of muscles, fat and other debris.

## 3.2 IMMUNOHISTOCHEMICAL (IHC) ANALYSIS

In order to determine whether there were changes in expression of the type of collagen, between normal and contracted post-traumatic EJ capsules, we performed immunohistochemical analysis. As shown in Figure 10A, collagen type III was not observed in capsules derived from cadavers with no history of trauma to the elbow joint. In contrast, isles of collagen type III fibrils (stained green, Figure 11A), surrounded by type I collagen fibers (not stained with antibodies to collagen type III), were detected in capsules from patients with contracted post-traumatic elbow joint. This observation supports the hypothesis that contracture of the elbow joint is associated with expression of collagen type III, which is not expressed (within the limits of detection of the IHC methodology) in normal EJ capsules.

**Figure 10: Immunohistochemistry of a Normal Elbow Joint Capsule**

In panel A, the specimen was probed with anti-collagen type III antibodies; panels B, C, and D, were controls for the specificity of technique and the procedure was performed with NMIgG replaceing the primary antibody (B), primary antibody with buffer (C) and in the absence of both primary and secondary antibodies (D). The background fluorescence demonstrates autofluorescence that is typical of collagen (presumably type I).

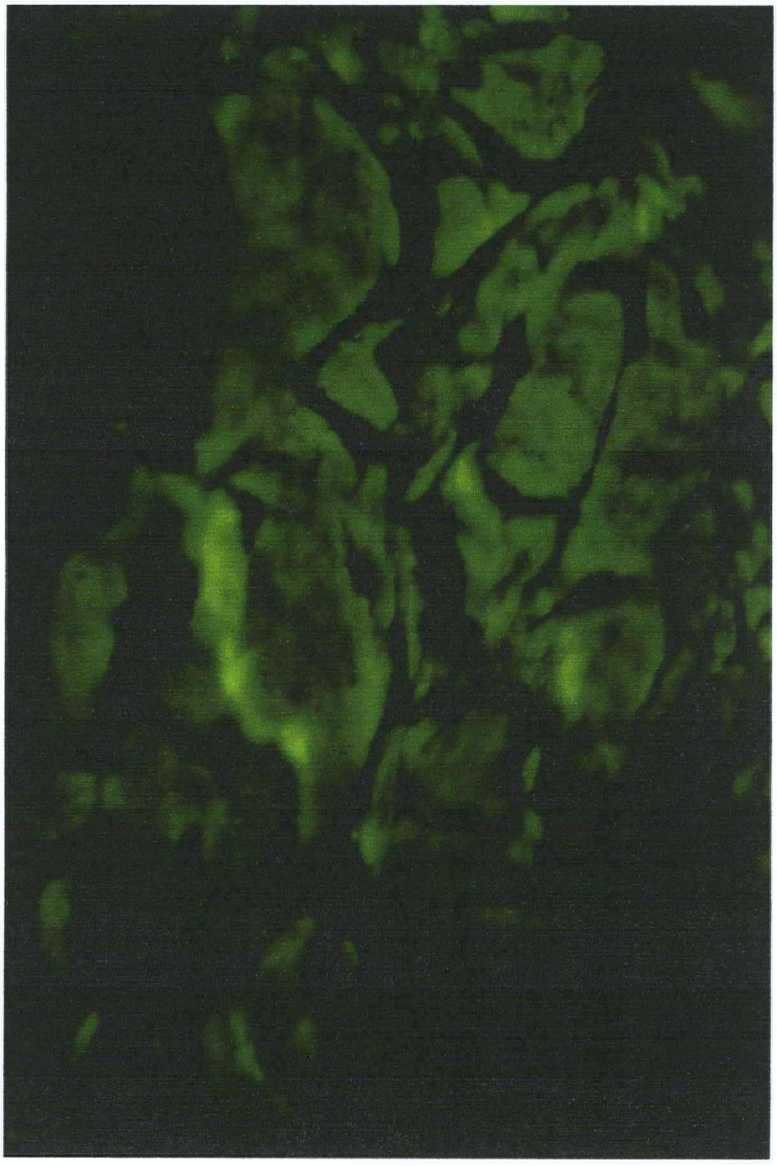


**Figure 10 A**

**Magnification 230 X**

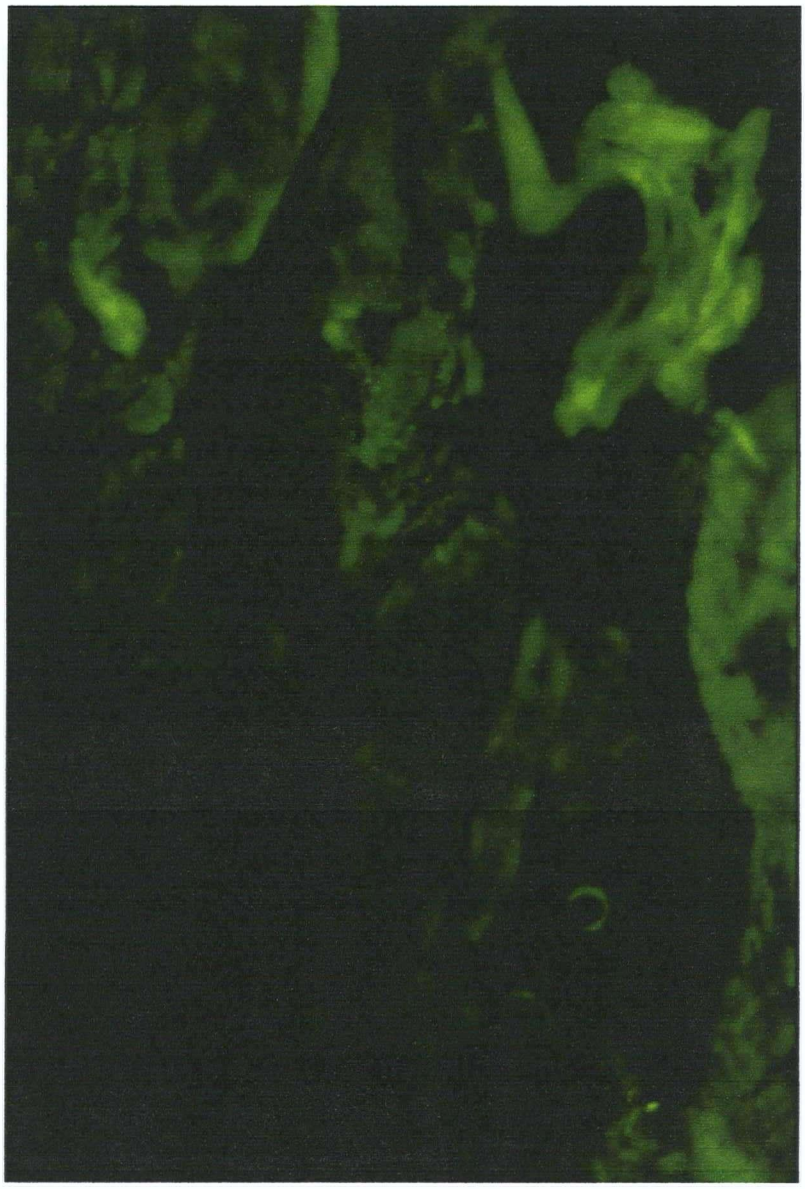


**Figure 10B – Normal Mouse Ig G**

**Magnification 230 X**

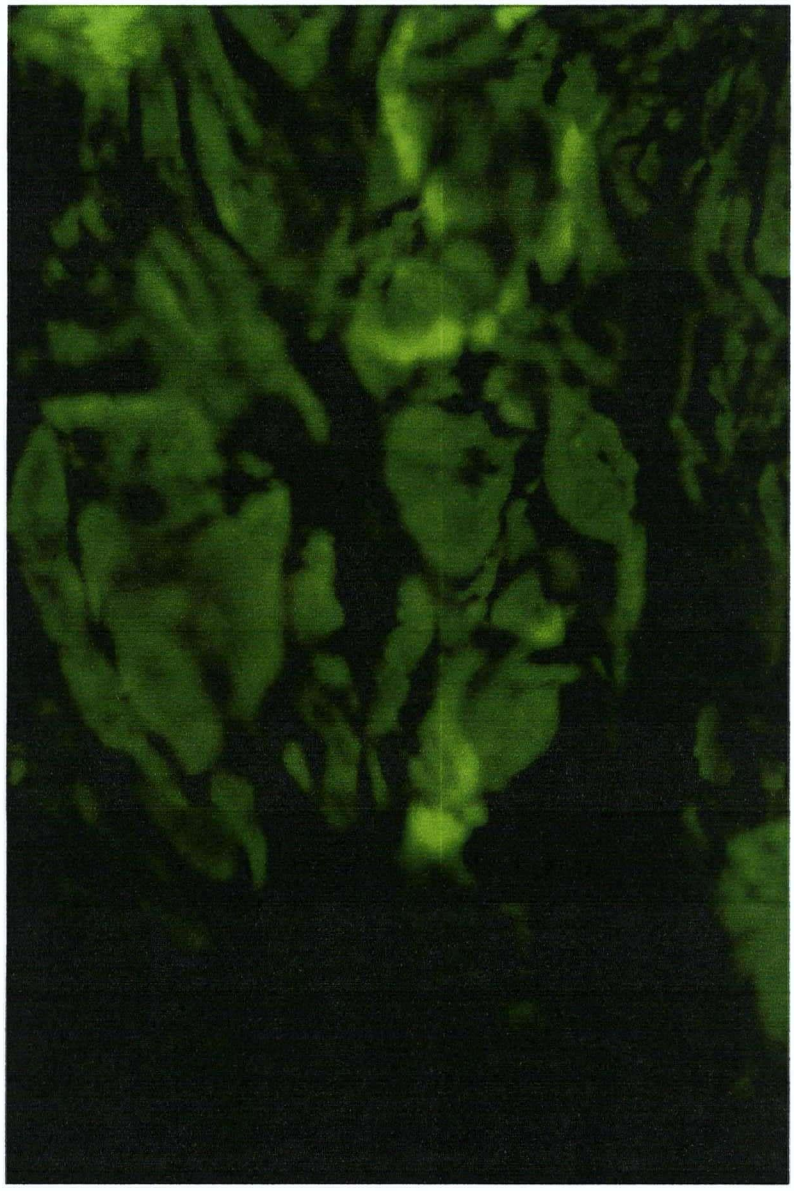


**Figure 10 C – primary Antibody replaced with buffer**

**Magnification 230 X**

**Figure 10D – Absence of primary and secondary Antibodies**

**Magnification 230 X**

**Figure 11: Immunohistotochemistry of a Contracted Post-Traumatic Elbow Joint Capsule**

Panel (A) shows positive staining when the contracted post-traumatic elbow joint capsule was probed with antibodies to collagen type III (isles of fluorescent green material). Panels B, C, and D, were controls for the specificity of technique (as described above).

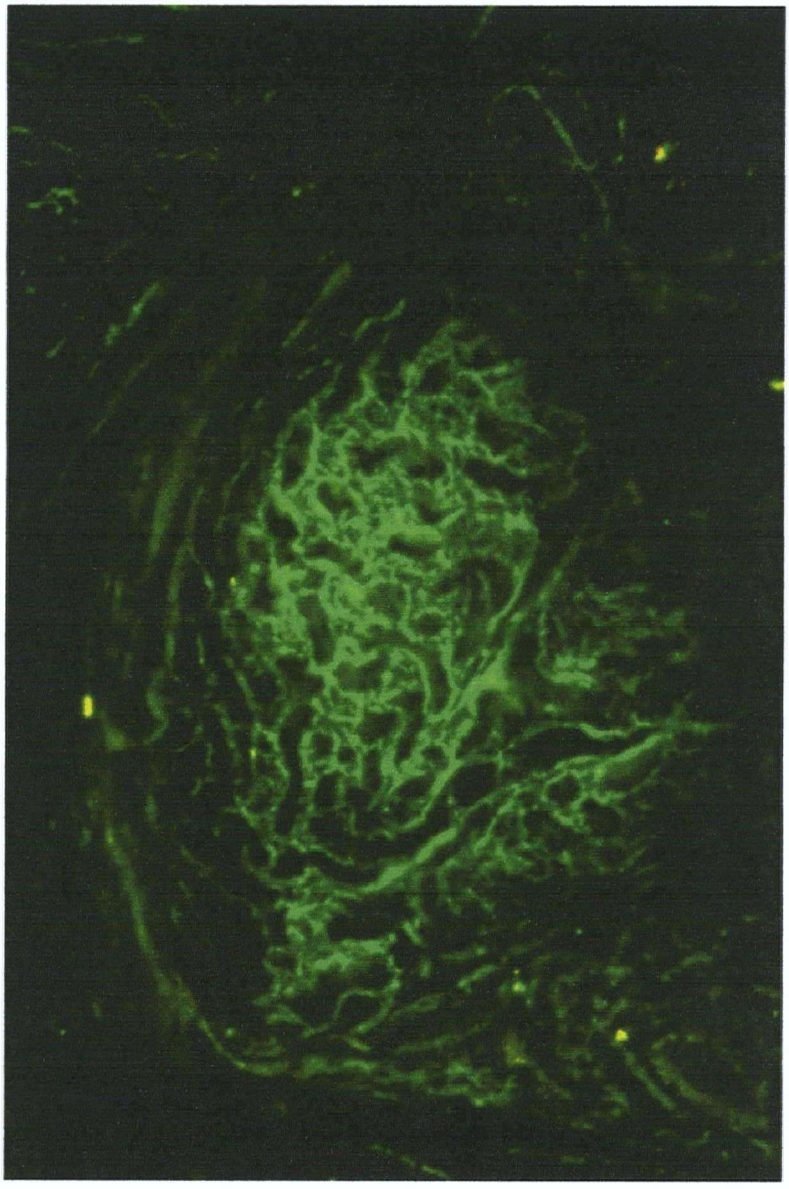


**Figure 11 A Magnification 230 X**

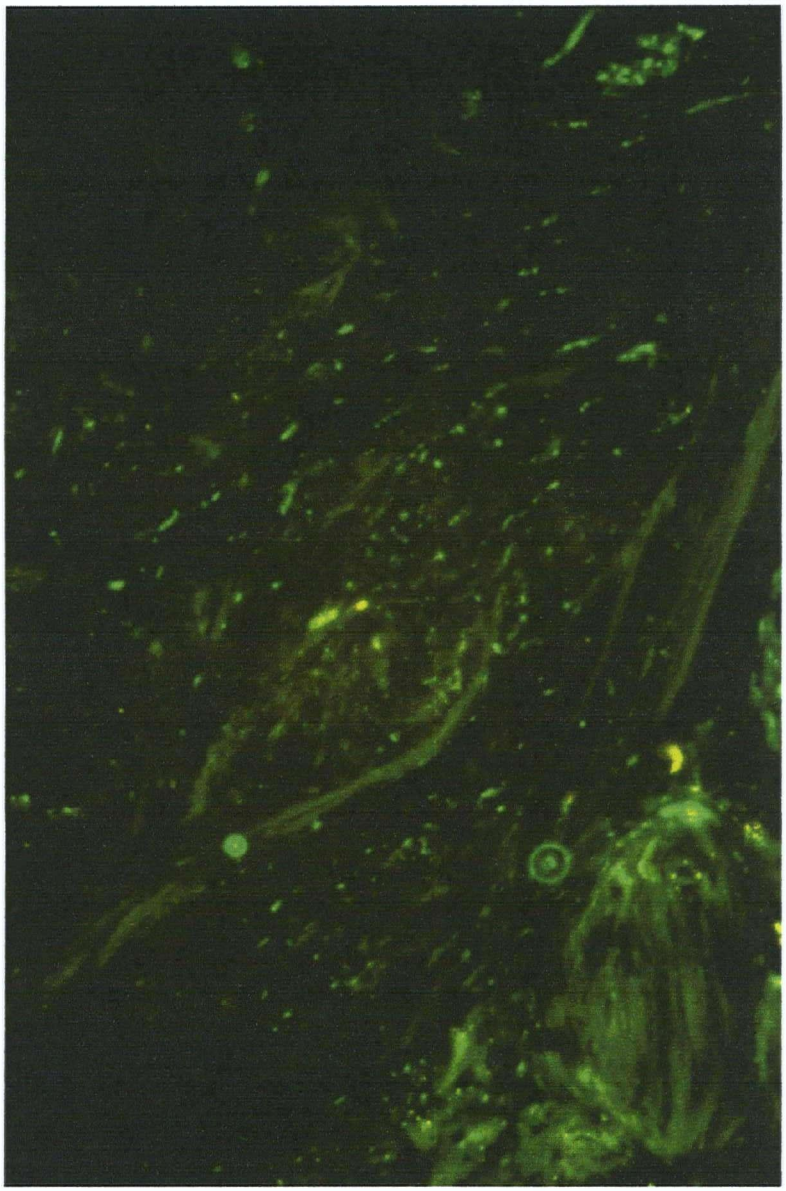


**Figure 11B**

**Magnification 230 X**

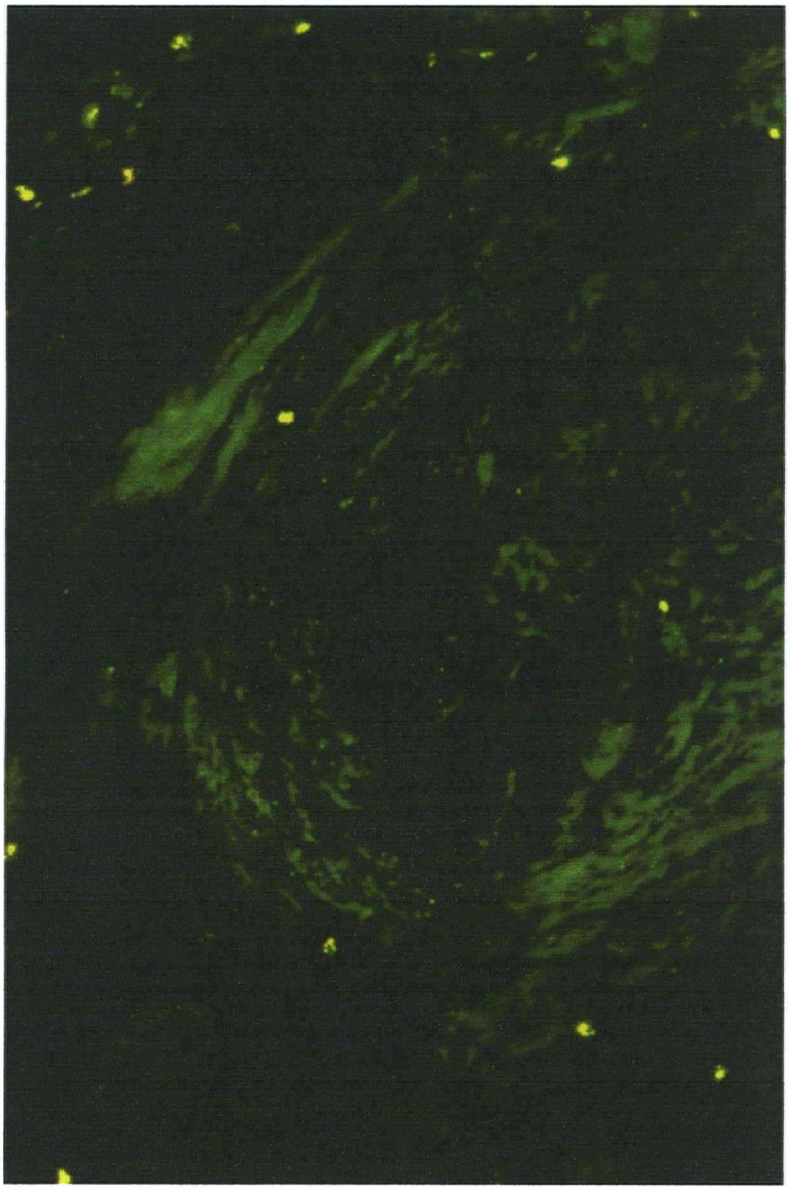


**Figure 11C**

**Magnification 230 X**

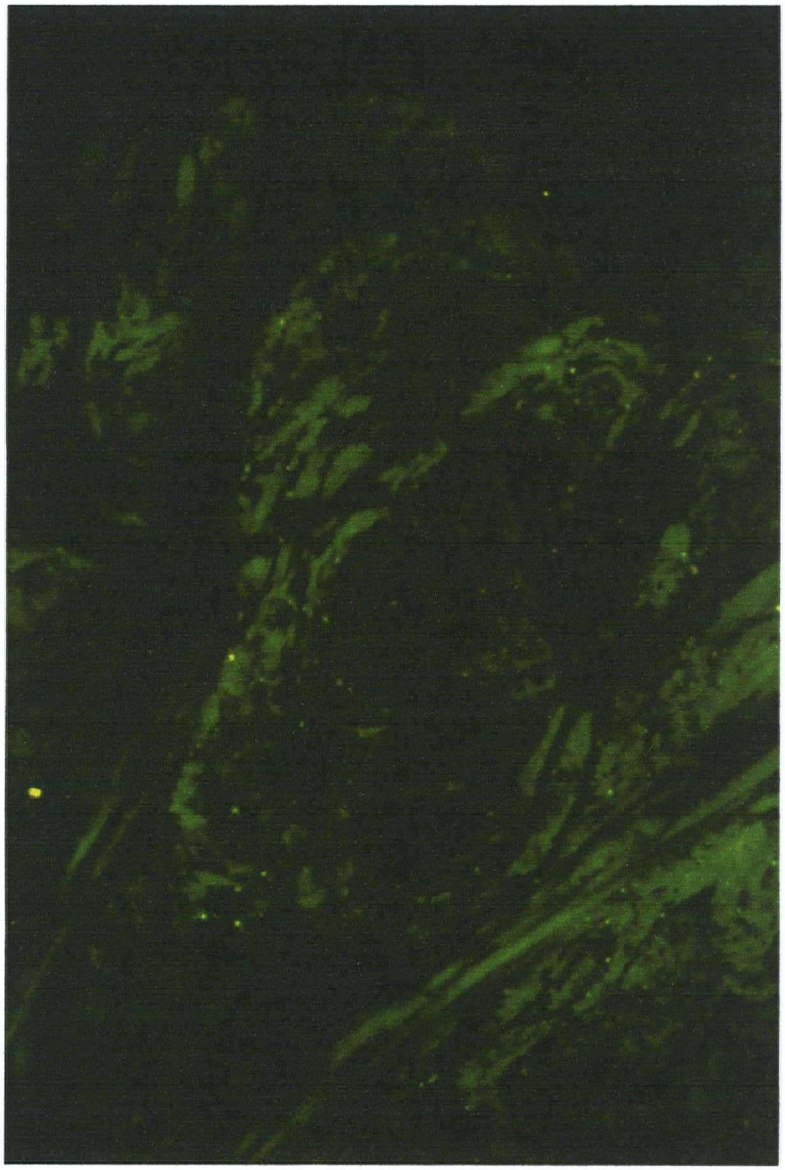


**Figure 11D**

**Magnification 230 X**

## 3.3 BIOMECHANICAL PROPERTIES OF THE NON-PATHOLOGICAL ELBOW JOINT CAPSULES

As stated previously, one of the most important complications in EJ pathology is the post-traumatic contracture of the soft tissues surrounding the joint [4]. In addition, post-traumatic contracture of the elbow joint with subsequent stiffening is very often the result of contracture of the anterior capsule and it is managed by a number of surgical options. However, despite the critical role it plays in the pathogenesis of the elbow joint contracture, very little is known about the capsule and its biomechanical properties. Therefore, the objective of this study was to establish experimental procedures that will facilitate determination of the biomechanical properties of the non-pathological and pathological elbow joint capsules. We initiated the study by analyzing biomechanical properties of normal elbow joint capsules. The study was also aimed to compare these properties in different regions of the normal capsule: lateral (radial), midcapsular and medial (ulnar). The Tables below list and summarize data of modulus of elasticity and structural stiffness obtained from 10 normal elbow joint capsules.

**Table 3A-J: List and Summary of Modulus of Elasticity and Structural Stiffness of Elbow Joint Capsules (10 Cadavers)**

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 1 | 1 | 3.109125 | 16.836916 |
| 1 | 2 | 3.595104 | 19.921915 |
| 1 | 3 | 2.02237 | 13.954783 |
| 1 | 4 | 0.396622 | 8.0465798 |
| 1 | 5 | 1.150389 | 4.7383319 |
| 1 | 6 | 15.90078 | 72.186008 |
| 1 | 7 | 4.301137 | 9.3928387 |
| 1 | 8 | 2.117118 | 11.237358 |
| 1 | 9 | 3.463601 | 12.381261 |
| | Mean | 4.00625 | 18.743999 |
| | StDev | 4.630404 | 20.551403 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 2 | 1 | 0.972106 | 2.84873 |
| 2 | 2 | 1.156537 | 7.43071 |
| 2 | 3 | 3.655358 | 7.160226 |
| 2 | 4 | 4.127428 | 17.99509 |
| 2 | 5 | 4.154413 | 9.454774 |
| 2 | 6 | 7.610205 | 14.89329 |
| | Mean | 3.612675 | 9.963805 |
| | StDev | 2.430874 | 5.549081 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 3 | 1 | 5.95238 | 10.105074 |
| 3 | 2 | 2.59691 | 5.6577691 |
| 3 | 3 | 2.276092 | 7.8023352 |
| 3 | 4 | 4.550508 | 14.261874 |
| 3 | 5 | 2.498137 | 6.3614528 |
| 3 | 6 | 3.290839 | 28.911186 |
| | Mean | 3.527478 | 12.183282 |
| | StDev | 1.447356 | 8.7650553 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 4 | 1 | 5.887904 | 19.30971 |
| 4 | 2 | 4.522105 | 7.389077 |
| 4 | 3 | 11.97459 | 24.39443 |
| 4 | 4 | 5.815994 | 24.90825 |
| 4 | 5 | 6.754398 | 19.54538 |
| 4 | 6 | 0.683202 | 2.97868 |
| 4 | 7 | 0.531598 | 1.985431 |
| | Mean | 5.167113 | 14.35871 |
| | StDev | 3.911761 | 9.954908 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 5 | 3 | 10.11437 | 18.397158 |
| 5 | 4 | 7.738831 | 22.377652 |
| 5 | 5 | 21.56488 | 16.779435 |
| 5 | 6 | 13.32474 | 38.778487 |
| 5 | 7 | 19.20453 | 25.228225 |
| 5 | 8 | 25.17726 | 48.08502 |
| 5 | 9 | 12.06467 | 32.55115 |
| 5 | 10 | 18.50823 | 54.38172 |
| 5 | 11 | 14.15752 | 28.916577 |
| | Mean | 15.76167 | 31.721714 |
| | StDev | 5.702687 | 13.085683 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 6 | 1 | 5.583998 | 14.69352 |
| 6 | 4 | 4.465499 | 19.98592 |
| 6 | 5 | 9.431759 | 67.38321 |
| 6 | 6 | 21.97476 | 59.45566 |
| 6 | 7 | 3.861235 | 7.210539 |
| 6 | 8 | 9.466766 | 45.20048 |
| | Mean | 9.13067 | 35.65489 |
| | StDev | 6.742164 | 25.13268 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 7 | 1 | 4.337479 | 20.835453 |
| 7 | 2 | 3.134441 | 19.455611 |
| 7 | 3 | 3.792294 | 17.060695 |
| 7 | 4 | 2.666779 | 11.884072 |
| 7 | 5 | 2.772574 | 8.1389597 |
| 7 | 6 | 0.191311 | 0.7632332 |
| 7 | 7 | 0.406881 | 1.161703 |
| 7 | 8 | 0.449024 | 1.1434291 |
| | Mean | 2.218848 | 10.055394 |
| | StDev | 1.640397 | 8.4981502 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 8 | 1 | 0.350247 | 0.654057 |
| 8 | 6 | 2.200822 | 7.783817 |
| 8 | 7 | 1.766918 | 7.873983 |
| 8 | 8 | 1.528162 | 8.496292 |
| 8 | 9 | 2.428081 | 9.274585 |
| | Mean | 1.461537 | 6.202037 |
| | StDev | 0.791456 | 3.712193 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 9 | 1 | 1.6936 | 7.5799271 |
| 9 | 2 | 2.814149 | 14.549501 |
| 9 | 3 | 2.426791 | 7.621719 |
| 9 | 4 | 3.207728 | 14.824158 |
| 9 | 5 | 3.851971 | 15.04041 |
| 9 | 6 | 1.945961 | 10.060858 |
| 9 | 7 | 4.255322 | 15.892932 |
| 9 | 8 | 7.041761 | 24.995698 |
| 9 | 9 | 8.394685 | 32.777896 |
| 9 | 10 | 1.718017 | 4.5369271 |
| | Mean | 3.40466 | 13.82065 |
| | StDev | 1.713453 | 5.6532106 |

| Specimen | Section | Stiffness | Modulus |
|---|---|---|---|
| 10 | 1 | 7.523026 | 26.50083 |
| 10 | 2 | 5.291472 | 16.09469 |
| 10 | 3 | 11.94379 | 31.42843 |
| 10 | 4 | 19.37826 | 46.1831 |
| 10 | 5 | 4.297248 | 11.44025 |
| 10 | 6 | 3.56207 | 10.58253 |
| 10 | 7 | 2.671979 | 8.865998 |
| 10 | 8 | 3.467528 | 9.231351 |
| 10 | 9 | 1.400125 | 3.38712 |
| | Mean | 7.266921 | 20.0409 |
| | StDev | 5.739827 | 13.49894 |

**Table 4: Mean Value of Modulus of Elasticity and Structural Stiffness of Elbow Joint Capsules (10 Cadavers, 87 Sections)**

| | | Stiffness | Modulus |
|---|---|---|---|
| Overall | Mean | 5.794887 | 17.70179 |
| | STDev | 5.70555 | 15.27065 |

The results show that the overall mean modulus of elasticity of the normal elbow joint capsule was 17.7018 MegaPascals (0.017 GPa)( Table 4). This indicates that the elbow joint capsule is less rigid than tendons and ligaments (Table 5).

**Table 5: Biomechanical Properties of Collagenous Biological Materials (Including data for normal elbow joint capsules obtained in this study)**

| TISSUE | MODULUS OF ELASTICITY (MPa) |
|---|---|
| Achilles Tendon (Rabbit) | 300-2000 [49] |
| Anterior Cruciate Ligament (Rabbit) | 70-350 [48] |
| Normal Elbow Joint Capsule (Human) | 18 MPa |
| Normal Hip Joint Capsule Ligaments (Human) | 76.1-285.8 (Anterior-Posterior) [24] |
| Normal Anterior And Posterior Cervical Longitudinal Ligaments (Human) | 56 [52] |

The Modulus of Elasticity of the EJ capsule is also much lower than that of typical materials used in engineering (Table 6)

Typical Properties of Selected Materials Used in Engineering
(U.S. Customary Units)

| Material | Specific Weight,* lb/in.³ | Ultimate Strength: Tension, ksi | Ultimate Strength: Compression,† ksi | Ultimate Strength: Shear, ksi | Yield Strength:‡ Tension, ksi | Yield Strength:‡ Shear, ksi | Modulus of Elasticity, $10^6$ psi | Modulus of Rigidity, $10^6$ psi | Coefficient of Thermal Expansion, $10^{-6}$/°F | Ductility, Percent Elongation in 2 in. |
|---|---|---|---|---|---|---|---|---|---|---|
| MAGNESIUM ALLOYS | | | | | | | | | | |
| Alloy AZ80 (Forging) | 0.065 | 50 | | 23 | 36 | | 6.5 | 2.4 | 14 | 6 |
| Alloy AZ31 (Extrusion) | 0.064 | 37 | | 19 | 29 | | 6.5 | 2.4 | 14 | 12 |
| TITANIUM | | | | | | | | | | |
| Alloy (6% Al, 4% V) | 0.161 | 130 | | | 120 | | 16.5 | | 5.3 | 10 |
| MONEL ALLOY 400(Ni-Cu) | | | | | | | | | | |
| Cold-worked | 0.319 | 98 | | | 85 | 50 | 26 | | 7.7 | 22 |
| Annealed | 0.319 | 80 | | | 32 | 18 | 26 | | 7.7 | 46 |
| CUPRONICKEL (90% Cu, 10% Ni) | | | | | | | | | | |
| Annealed | 0.323 | 53 | | | 16 | | 20 | 7.5 | 9.5 | 35 |
| Cold-worked | 0.323 | 85 | | | 79 | | 20 | 7.5 | 9.5 | 3 |
| TIMBER, air dry: | | | | | | | | | | |
| Douglas-fir | 0.017 | 15 | 7.2 | 1.1 | | | 1.9 | .1 | Varies | |
| Spruce, Sitka | 0.015 | 8.6 | 5.6 | 1.1 | | | 1.5 | .07 | 1.7 to 2.5 | |
| Shortleaf pine | 0.018 | | 7.3 | 1.4 | | | 1.7 | | | |
| Western white pine | 0.014 | | 5.0 | 1.0 | | | 1.5 | | | |
| Ponderosa pine | 0.015 | 8.4 | 5.3 | 1.1 | | | 1.3 | | | |
| White oak | 0.025 | | 7.4 | 2.0 | | | 1.8 | | | |
| Red oak | 0.024 | | 6.8 | 1.8 | | | 1.8 | | | |
| Western hemlock | 0.016 | 13 | 7.2 | 1.3 | | | 1.6 | | | |
| Shagbark hickory | 0.026 | | 9.2 | 2.4 | | | 2.2 | | | |
| Redwood | 0.015 | 9.4 | 6.1 | 0.9 | | | 1.3 | | | |
| CONCRETE | | | | | | | | | | |
| Medium strength | 0.084 | | 4.0 | | | | 3.6 | | 5.5 | |
| High strength | 0.084 | | 6.0 | | | | 4.5 | | 5.5 | |
| PLASTICS | | | | | | | | | | |
| Nylon, type 6/6, (molding compound) | 0.0412 | 11 | 14 | | 6.5 | | 0.4 | | 80 | 50 |
| Polycarbonate | 0.0433 | 9.5 | 12.5 | | 9 | | 0.35 | | 68 | 110 |
| Polyester, PBT (thermoplastic) | 0.0484 | 8 | 11 | | 8 | | 0.35 | | 75 | 150 |
| Polyester elastomer | 0.0433 | 6.5 | | 5.5 | | | 0.03 | | | 500 |
| Polystyrene | 0.0374 | 8 | 13 | | 8 | | 0.45 | | 70 | 2 |
| Vinyl, rigid PVC | 0.0520 | 6 | 10 | | 6.5 | | 0.45 | | 75 | 40 |
| Rubber | 0.033 | 2 | | | | | | | 90 | 600 |
| Granite (Avg. values) | 0.100 | 3 | 35 | 5 | | | 10 | 4 | 4 | |
| Marble (Avg. values) | 0.100 | 2 | 18 | 4 | | | 8 | 3 | 6 | |
| Sandstone (Avg. values) | 0.083 | 1 | 12 | 2 | | | 6 | 2 | 5 | |
| Glass, 98% silica | 0.079 | | 7 | | | | 9.6 | 4.1 | 44 | |

**Table 6: Typical Mechanical Properties of Selected Materials used in Engineering**

**(Derived from reference 50)**

As shown in Figure 12, in the measurement of elasticity, no statistically significance difference was observed in Young's Modulus ($p = .15$) between the three different regions of the capsule (radial, midcapsular and ulnar). Neither was there any statistically significant difference in structural stiffness ($p = .2$) between these regions, Figure 13.

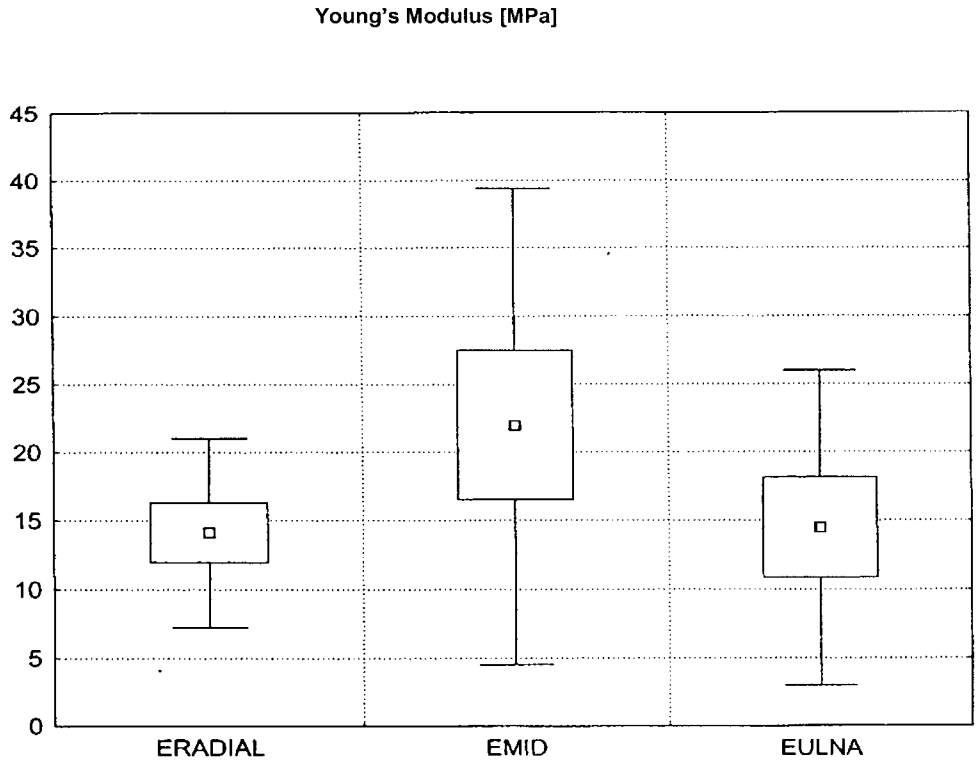



**Figure 12: Modulus of Elasticity of the Radial (ERADIAL), Mid-capsular (EMID), and Ulnar (EULNAR) regions of Normal Elbow Joint Capsules**

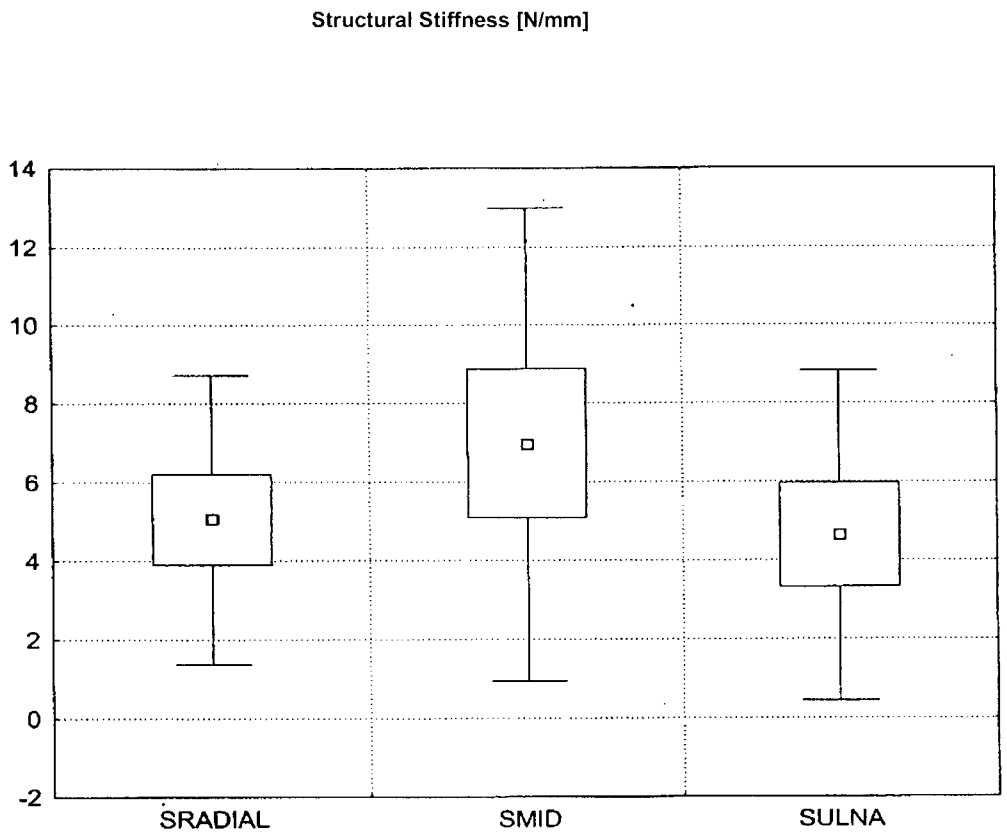



**Figure 13: Structural Stiffness of the Radial (SRADIAL), Mid-Capsular (SMID), and Ulnar (SULNAR) Regions of Normal Elbow Joint Capsules**

When sections of the normal capsules were subjected to mechanical testing, stress-strain curves were obtained, an example of one is presented in Figure 14. Similarly, a load-displacement curve is presented in Figure 15.

**Figure 14: A Representative Stress-Strain Curve (Cadaver Number 1)**

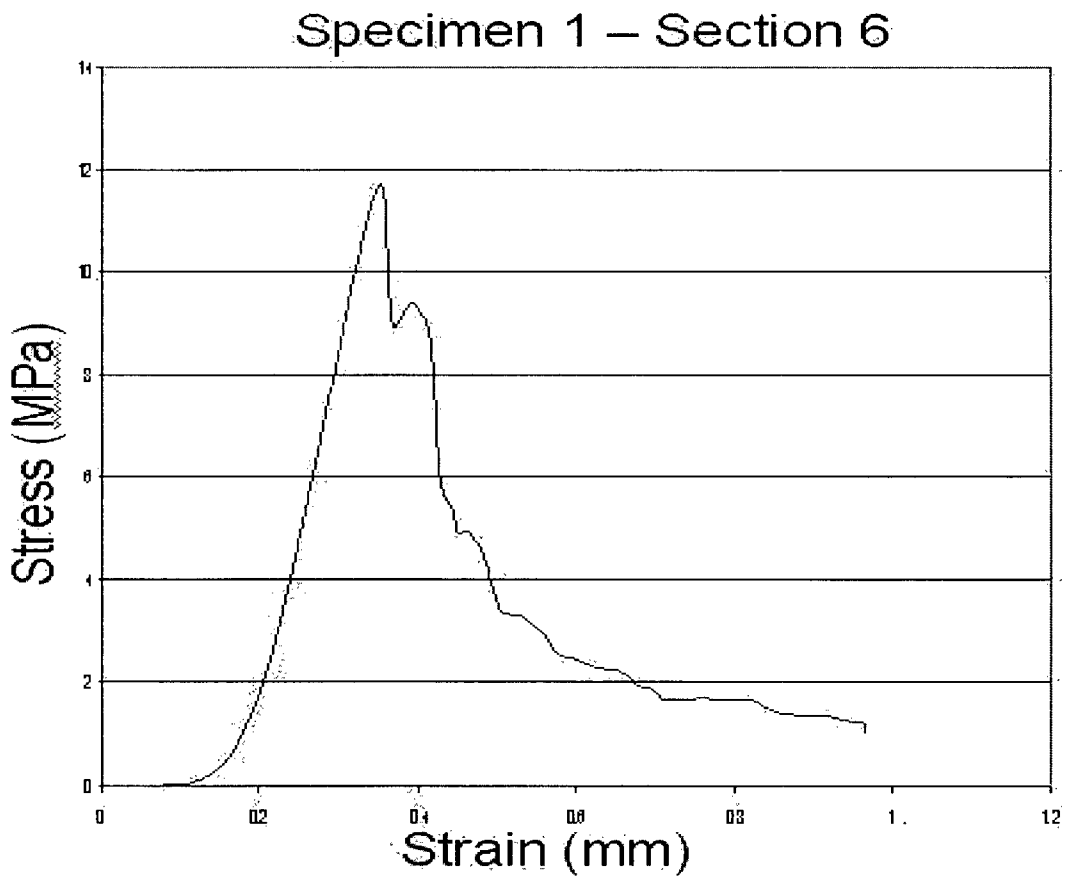
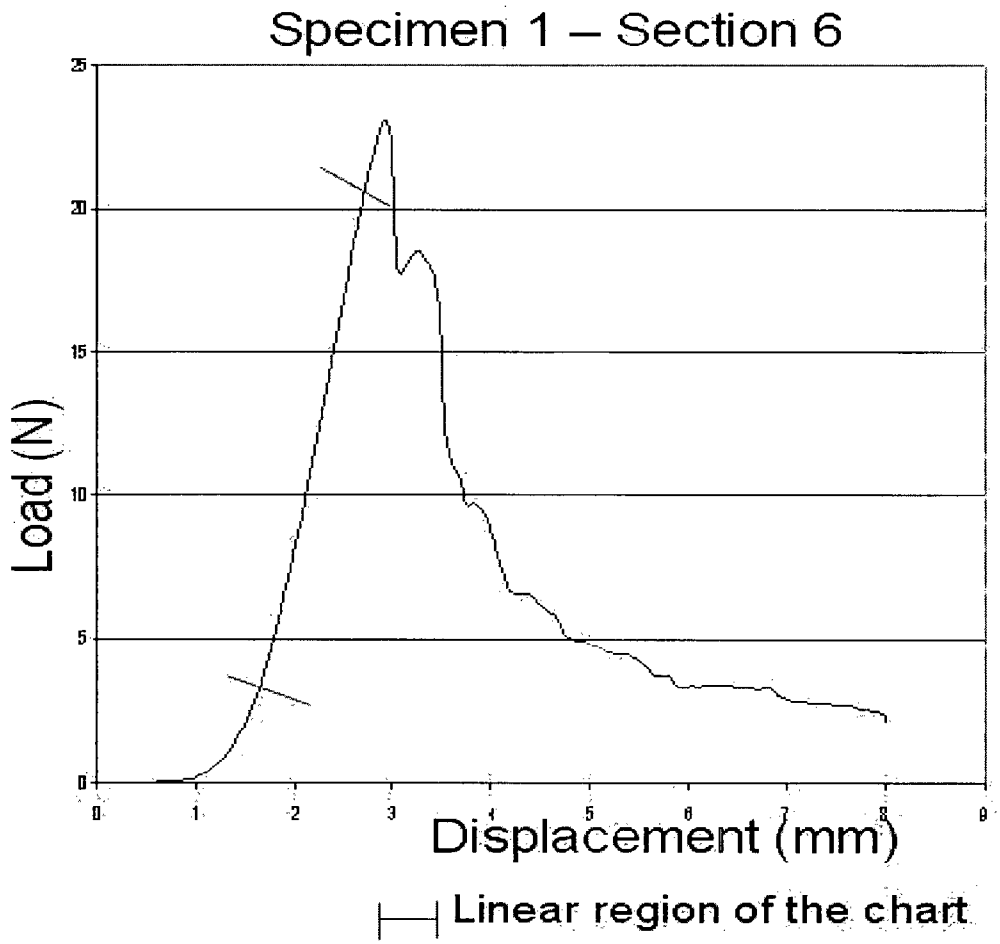


**Figure 15: A Representative Load-Displacement Curve (Cadaver Number 1)**



## SECTION 4: DISCUSSION AND FURTHER STUDIES

One of the most important complications in the EJ pathology is post–traumatic contracture of the soft tissues surrounding the joint [4]. Insidious stiffness takes over the joint, decreasing the ROM. Even with early physiotherapy, contracture of the EJ becomes so stable that within 3 to 6 months from the initial trauma the patient has to be surgically intervened on in order to achieve a functional arc of ROM [41; 43]. If the two goals of treatment, anatomical repair and healing of the fracture are achieved and confirmed by X-ray examination, the only logical explanation for stiffening of the capsule after trauma is that changes in the composition of the capsule and its mechanical properties are taking place. It has been demonstrated that during the initial stages of healing, the rupture sites are bridged by newly synthesized collagen type III which partially replace collagen type I [23; 25].

In this study, we provided evidence that collagen type III is not expressed in the normal atraumatic capsule of the elbow joint (in the age group 75-93). It is likely that the fibrous membrane of the capsule in the normal elbow joint in this age group predominately contains collagen type I. This would be in accordance to previous studies (Rauterberg et al, 1993, for example), that have shown that in certain human tissues, such as tendons and capsules, collagen type I (Hanson and Bentley, 1983) is predominant. We have also shown that collagen type III, which is not usually found in tendons and capsules, including capsules of the elbow joint (as shown in these studies) [3; 23; 25; 26], is expressed during the healing process of the contracted post-traumatic capsule of the elbow joint. This suggests that contracture of the EJ capsule is associated with expression of collagen type III, which may contribute to changes in the biomechanical properties associated with decrease in the range of motion, such as that which is seen in post-traumatic contracture of the elbow joint.

In order to compare and contrast biomechanical properties of the EJ capsule between post-traumatic and atraumatic, it is important to establish methodologies and protocols using capsules derived from normal elbow.

Our study was done on capsules excised from old age cadavers ( aged 75 to 93 years old).

We could not make any age related comparison because of the Acceptance Criteria for the Body Donation Programm, in effect at that time in the Department of Anatomy at UBC. We did not pursue any other comparison, for example male/female considering it less clinically relevant.

In addition to the establishment of protocols, the studies would reveal biomechanical properties (such as elasticity and structural stiffness) of the normal elbow joint. These analyses have not been conducted previously. In addition, our aim was to determine if there were any differences in the biomechanical properties between different regions of the capsule: lateral (radial), midcapsular, and medial (ulnar).

Our results indicate that within the age group 73 to 95, the mean Young's Modulus was 17.7 MPa, and the structural stiffness was 5.8 MPa. We also found that there wasn't any statistically significant difference in elasticity or stiffness between the three regions of the capsule. That implies that none of the 3 mentioned regions of the EJ capsule which where studied, will react differently when same load will be applied upon. The stability of the joint will be equally supported and the plastic deformation will be the same in each region.

No special attention should be offered to any of these regions during surgical repair of the anterior capsule of the EJ.

This is in contrast to the hip capsule ligaments [ 24 ], in which there is significant variation in biomechanical properties from the anterior side (76.1) to the posterior side (285.8). Therefore, repair of the hip capsule requires more reinforcement in the posterior region because this region is subjected to more stress.

Our observation that such regional variation in biomechanical properties does not exist in the EJ capsule suggests that surgical repair does not need differential reinforcement.

Another joint capsule which we can compare and discuss our findings would be the shoulder joint. Shoulder joint Capsules have been stretched to failure and they showed resistance to a bigger mechanical stress than EJ capsules, as well as an inverse relationship to the age patient, becoming weaker with increasing age. [ 53 ]

Also it has been demonstrated that the anteroinferior part of the shoulder capsule will rupture first and this confirms the clinically related feature of the humerus head of being dislocated anteriorly. [ 53 ]

However, our study tested strictly only EJ capsule and not the complex bone-capsule-bone as in the Hip and Shoulder tests. This way the intrinsic material properties are defined better and they could be related to each individual region: radial, midcapsular and ulnar.

Our study was not intended to find the Ultimate Tensile Strength of the EJ capsule.

The immunohistochemistry testing of this study eliminates the presence of Collagen type III in normal non-pathologic EJ capsule and clearly evidentiates its presence in pathologic posttraumatic contracted EJ capsules. This is in disagreement with the histological findings of the Shoulder study which shows Collagens type I, III and V present in atraumatic capsules As well a certain degree of fibrosis associated with chronic inflammation were detected and this is self-explanatory for the presence of type III and V of Collagens. [ 53 ]

In considering analysis of the biomechanical properties of the normal EJ capsule, it is critical to take into consideration the age group that is studied. Particularly because it is known that

collagen type III, which is more supple and elastic than collagen type I, is expressed in newborns, and it is gradually replaced by collagen type I during the process of aging (Figure 4). Therefore, analysis of changes in biomechanical properties of the EJ capsule during the process of healing which follow trauma, may provide further support for the association between these properties and changes in the expression of collagen from type III to type I, during aging.

In order to fully understand the pathophysiologic process that leads to the development of the contracted post-traumatic capsule of the elbow joint, we suggest that other tests should be performed. These include quantification of collagen type I and type III during the process of healing and subsequent instalment of progressive and permanent EJ contracture and correlation of this with clinical findings, such as the arc of ROM and stiffness of the elbow joint.

These studies will be particularly important because of our working active population, which will lead to increase in prevalence of the stiffness of the joints, which is likely to have a major impact in changing the quality of life.

However, in mathematical modeling of the entire elbow joint, it will be important to also consider age-specific biomechanical properties of ligaments, tendons and muscles.

Better understanding of the pathological processes that lead to development of the contracted post-traumatic capsule of the elbow joint, may also lead to novel approaches to prevent or treat this affliction. These may include, for example, selectively blocking the synthesis and/or extracellular processing of collagen type III or blocking migration of fibroblasts (cells that synthesis collagen type III) into the capsule.

# SECTION 5: CONCLUSIONS

Quantification of biomechanical properties of the anterior EJ capsule is essential for understanding its physiological function. A tensile force-displacement (P-Δ) response of the anterior capsule of elbow joints was observed that is typical for collagenous capsules. The response has a concave toe region that has previously bean associated with unfolding of collagen fibers and their reorientation in the direction of the force applied, followed by a relatively linear region which is maintained until the material reaches its maximum loading capacity.

Our studies also suggest that trauma to the anterior Elbow Joint capsule alters the architecture of the tissue by inducing the expression of collagen type III. Because collagen type I is a major component of the capsule, we can infer that expression of collagen type III in the injured tissue alters the biomechanical properties, including elasticity and structural stiffness. Further studies, using the protocols that we have standardized in this study, are required to confirm that biomechanical properties of the capsules from patients with conjuncture of the EJ have different Modulus of Elasticity and Structural Stiffness in comparison to those from cadavers (of the same age group) without a history of trauma to the EJ. In addition, quantitative measurements of the level of expression of collagen type III in patients with trauma and correlation of this with the arc of ROM, will further reveal the significance of changes in collagen expression and stiffness of the elbow joint.

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