CARPAL TUNNEL SYNDROME
THE ROLE OF THE SUBSYNOVIAL CONNECTIVE TISSUE

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Carpal Tunnel Syndrome: The Role of the Subsynovial Connective Tissue

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To my parents

CONTENTS

CHAPTER 1

General introduction

General introduction

1.1 Subsynovial Connective Tissue in the Carpal Tunnel

The carpal tunnel is a narrow (1-1.5 cm diameter) space at the base of the palm, bounded dorsally, medially and laterally by the carpal bones and palmarly by the flexor retinaculum (Figure 1).

The contents of the carpal tunnel include the flexor digitorum profundus (FDP) tendons, the flexor digitorum superficialis (FDS) tendons, the flexor pollicis longus (FPL) tendon, the subsynovial connective tissue (SSCT) associated with these tendons, the radial bursa and the ulnar bursa and the median nerve.

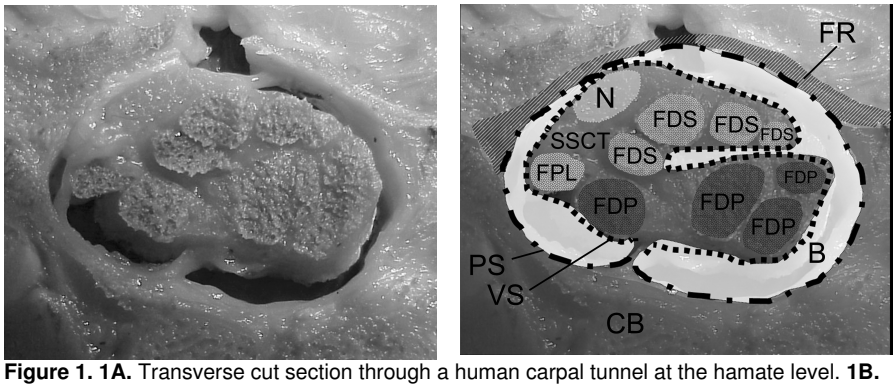

Figure 1. 1A. Transverse cut section through a human carpal tunnel at the hamate level. **1B.** Scematic overview of figure 1A showing the structures within the carpal tunnel. The flexor retinaculum (FR) and carpal bones (CB) surrounding the carpal tunnel and within the carpal tunnel are the median nerve (N), the flexor pollicis longus (FPL) tendon, the flexor digitorum profundus (FDP) and superficialis (FDS) tendons, the bursa (B), the SSCT, the parietal (PS) and the visceral synovial (VS) layer.

The tenosynovium in the carpal tunnel is part of the visceral synovial layer of the wrist bursa. It is connected to the flexor tendons and the median nerve[1] by the subsynovial connective tissue (SSCT). The SSCT loosely connects to the finger flexor tendons and the visceral synovial membrane, which in turn encloses the tendons within the ulnar tenosynovial bursa.

Guimberteau[2] reported that the SSCT consists of multiple layers of collagenous fibers in which the blood and lymphatic vessels are richly represented, and that the flexor tendon, SSCT, and visceral synovium form a sliding unit that moves against the parietal synovium, which is fixed to the flexor retinaculum and carpal bones.

1.2 Tendon Gliding

Gelberman et al.[3] have identified two main classes of tendon, intrasynovial and extrasynovial, that differ significantly in their response to injury or loading.

All tendons are exposed to tensile loading, but intrasynovial tendons, i.e. those located within the synovial fluid environment, are also exposed to significant

compression and shearing forces, such as the flexor tendons in the fingers[4-6].The closed synovial fluid environment of intrasynovial tendons is enveloped by a layer of parietal synovium, which is attached to soft tissues such as pulleys, capsule, or the bone, and a visceral synovial layer which covers the tendon surface. These two layers are continuous at the proximal cul-de-sac, the vincula origins, and the tendon insertions[5, 7].

The nutrition of the intrasynovial tendon is based on both the vascular perfusion[8] and synovial fluid diffusion[9, 10], with synovial fluid playing the dominant role[11, 12].

The extrasynovial tendons primarily experience tensile loads, and are surrounded by a loosely arranged matrix of collagen with abundant vasculature (paratenon) that supplies the tendon nutrition[3, 7, 13, 14]. The sliding mechanism of the extrasynovial tendon relies on its paratenon[15], which functions as an elastic sleeve, permitting free movement of the tendon against the surrounding tissue[3, 16]. Guimberteau et al. have shown that the paratenon also plays an important role in maintaining the tendon physiologically[2]. The gliding resistance of extrasynovial tendons is much greater than intrasynovial tendons[4].

A tendon can also be intrasynovial in one location and extrasynovial in another. For example, the flexor tendons of the fingers are intrasynovial in flexor tendon zone 2, but extrasynovial more proximally in flexor tendon zone 3 (Figure 2).

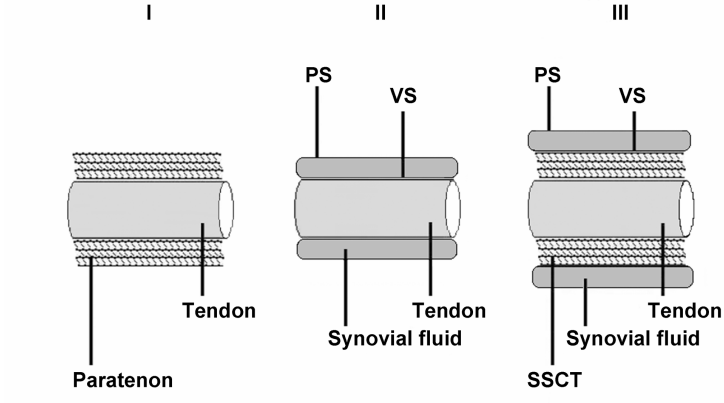

Figure 2. (I) Extrasynovial tendon with the paratenon. (II) Intrasynovial tendon with parietal synovium (PS) and visceral synovium (VS) sheets. (III) Flexor tendon in the carpal tunnel with SSCT and synovial fluid in the bursa.

While the anatomical structure of the carpal tunnel, flexor tendon zone 4, has been well studied[17, 18], the flexor tenosynovial organization in this area has not been examined in detail. The structure of the flexor tenosynovial organization in this area is neither typically intrasynovial nor typically extrasynovial. The difference is the subsynovial connective tissue (SSCT), which lies between the flexor tendons, median nerve[1] and the visceral synovial layer of the ulnar bursa. The surface of the

tenosynovium in the carpal tunnel is part of the visceral synovial layer of the ulnar wrist bursa. The gliding mechanism of the flexor tendons in the carpal tunnel appears instead to be a hybrid of two mechanisms, one paratenon (the SSCT) and the other synovial.
Guimberteau has hypothesized that, during tendon motion, the SSCT serves as a sliding unit to reduce the friction and protect the blood supply to the tendon and synovium[2].

1.3 Carpal Tunnel Syndrome

Carpal tunnel syndrome (CTS), a compression neuropathy of the median nerve, is the most common and best known of the compression neuropathies of the upper extremity, affecting as much as 5% of the adult population in some studies[19-22].
CTS causes nocturnal pain, paraesthesiae and numbness involving the median nerve innervated fingers, and patients are often awakened by these symptoms[19, 23]. In more advanced cases patients notice clumsiness or weakness on prolonged grip which is improved with rest. Nerve conduction studies (NCS) are helpful in diagnosing and assessing the severity of the carpal tunnel syndrome[24], but the need for confirmatory NCS to support the diagnosis has been challenged. CTS is an increasing recognized cause of disability[25, 26].

Analgesics, splinting, activity modification, and steroid injection would be the first-line treatment for patients with mild to moderate CTS. Carpal tunnel release can be subsequently offered to those who do not respond to splinting and steroid injection.

The underlying disease mechanism for CTS is increased carpal tunnel pressure[27-33]. The carpal tunnel pressure can be increased, as a result of either a reduction in the size of the space in the carpal tunnel or an increase in the volume of its contents. The latter is thought to be a main factor as the most common pathological finding in CTS is non-inflammatory fibrosis and thickening of the synovium[34-40]. Also, any condition that increases the volume of the contents of the carpal tunnel tends to compress the median nerve. Benign tumors, such as lipomata, hemangiomata, and ganglia may encroach upon the carpal tunnel[34]. Although there are many conditions, e.g. diabetes mellitus, rheumatoid arthritis, thyroid dysfunction, pregnancy and use of oral contraception, that are associated with carpal tunnel syndrome most of the patients are idiopathic CTS patients.

The effect of synovial bulk seems clearly to be a factor in CTS, as it is so commonly observed during surgery, but there is a dynamic component to the pathofysiology aswell. Many studies noted that the resting carpal tunnel pressure is higher than normal in patients with acute CTS[41] or chronic CTS[27, 42],and that with activity the pressure in CTS patients rises much higher[28]. Most investigators believe that the repetitive of forceful hand use causes a variety of the CTS.

However, the relationship between the repetitive movement and the tenosynovial thickening and its relationship to the development of carpal tunnel syndrome remain poorly understood.

Similar fibrotic changes in the tendon gliding environment are also noted in such conditions as de Quervain's syndrome[43-45],ulnar tunnel syndrome[46], trigger finger[47], lateral epicondylitis[48, 49]and tibialis posterior dysfunction[50]disorders of the long head of the biceps[51, 52].

How this fibrosis might affect tendon function, if at all, is unknown. In the carpal tunnel, such changes may affect nerve function, as the median nerve is often found to be thetered to the thickened SSCT in patients operated on for carpal tunnel syndrome[53-58].

We believe that the relative motion between the flexor tendon and the visceral synovium is an important index of the behavior of the subsynovial connective tissue (SSCT). If the stiffness of the SSCT is increased in CTS, it is likely that the gliding characteristics will be affected.

1.4 Hypothesis:

It is generally accepted that repetitive, forceful hand or wrist motion, often associated with awkward wrist posture, is a risk factor for carpal tunnel syndrome[59-63] but how these mechanical factors relate to the pathological changes of non-inflammatory synovial thickening seen typically in cases of carpal tunnel syndrome is unknown.

Both clinical and animal studies have shown convincingly that CTS is a compression neuropathy of the median nerve, but have not identified how the compression is generated or maintained. Animal models of CTS have focused on nerve pathology rather than changes in the surrounding tissues[60, 64-69]. This thesis investigates the etiology of CTS by identifying the functional structure and mechanical properties of the subsynovial connective tissue (SSCT) within the carpal tunnel, which, we believe, may play a very important role in the etiology of CTS.

We believe that forceful and repetitive motion leads to damage of the SSCT due to shear strain that goes beyond the physiological limit of elastic deformity of this tissue. This damage stimulates a healing process. The resulting tissue, however, is more fibrous and less elastic than the original tissue, so that the threshold for shear is decreased, and a vicious cycle ensues.

These pathological changes cause SSCT thickening, which leads to increased carpal tunnel pressure, both by a direct bulk effect and by impeding the normal fluids through the synovium. This latter effect, we believe, explains the edema typically seen in histologic samples of CTS synovium[37, 40, 70-72], as well as the elevation of the baseline carpal tunnel pressure and the delay in recovery of carpal tunnel pressure after exercise seen in CTS patients[28]. At the same time, increased carpal tunnel pressure increases the load at the gliding interface of the tendon and synovium. Thus, the frictional force between the visceral and parietal synovium could increase, which would further aggravate the vicious cycle. The increased carpal tunnel pressure will affect the median nerve circulation and thus initiating the symptoms of carpal tunnel syndrome. Such changes may also affect nerve function, as the median nerve is often found to be tethered to the thickened SSCT in patients operated on for carpal tunnel syndrome[53-58].

If this hypothesis regarding the etiology of CTS is correct, it may lead to new therapies and prevention strategies, based on evaluation of CTS synovial compliance and job modification to avoid activities likely to cause to the synovial system.

1.5 Aims and Purpose

Based on the hypothesis mentioned above, the following aims with corresponding studies have been designed.

1.5.1 SSCT Morphology and Histology

Dissectioning of cadaver wrists will give us more information of the SSCT in relation to the carpal tunnel. The methods of histology, immunohistochemical staining, and scanning electron microscopy will be used to assess the SSCT morphology in 1) CTS patients undergoing carpal tunnel release surgery, 2) normal subjects from human cadaver, 3) subjects with an antemortem diagnosis of CTS in human cadavers, 4) candidate animal models.

- To characterize the histology and immunohistochemistry of the subsynovial connective tissue in normal human cadaver wrists and in patients with carpal tunnel syndrome (*Chapter 2.1*).
- To assess information about the morphology of the functional structure of the SSCT in the carpal tunnel in normal human cadaver wrists, human cadaver wrist with an antemortem diagnosis of CTS and in patients with carpal tunnel syndrome (*Chapter 2.2*).

1.5.2 Motion Characteristics of the SSCT

- To identify the displacement of the FDS tendon, visceral synovium, and parietal synovium in the normal human cadaver wrist and in carpal tunnel syndrome patients. This is a critical step, in which we will validate our hypothesis by showing that the displacement of the tendon and synovium varies in CTS patients as our hypothesis predicts (*Chapter 3.1 and 3.2*).
- To use the ultrasound to study flexor tendon and SSCT motion (*Chapter 3.3*).

1.5.3 Gliding Characteristics of the SSCT

- To measure the gliding resistance of the flexor tendons and SSCT in the carpal tunnel (*Chapter 4.1*).

1.5.4 Search for an Animal Model

- To identify an animal with anatomical and histological comparable carpal tunnel and carpal tunnel contents for an animal model (*Chapter 5.1*).
- To create in an animal model the pathologic conditions seen in the SSCT in CTS patients (*Chapter 5.2*).

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CHAPTER 2

Morphology and Histology

2.1

A Histological and Immunohistochemical Study of the Subsynovial Connective Tissue in Idiopathic Carpal Tunnel Syndrome.

Ettema AM, Amadio PC, Zhao C, Wold LE, An KN.
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Abstract

Background: The most common histological finding in carpal tunnel syndrome is noninflammatory synovial fibrosis. The accumulated effect of minor injuries is believed to be an important etiologic factor in some cases of carpal tunnel syndrome. We sought evidence of such injuries in the synovial tissue of patients with carpal tunnel syndrome and in cadaver controls.
Methods: We compared synovial specimens from thirty patients who had idiopathic carpal tunnel syndrome with specimens from a control group of ten fresh-frozen cadavers of individuals who had not had an antemortem diagnosis of carpal tunnel syndrome and who met the same exclusion criteria. Analysis included histological and immunohistochemical examination for the distribution of collagen types I, II, III, and VI and transforming growth factor- (TGF-) RI, RII, and RIII.
Results: Histological examination showed a marked increase in fibroblast density, collagen fiber size, and vascular proliferation in the specimens from the patients compared with the control specimens ($p < 0.001$). Collagen types I and II were not found in the synovium of either the patients or the controls, but collagen type VI was a major component of both. Collagen type-III fibers were more abundant in the patients than in the controls ($p < 0.001$). Expression of TGF- RI was found in the endothelial cells and fibroblasts in the patient and control specimens, with a marked increase in expression in the fibroblasts of the patients compared with that in the control tissue ($p < 0.001$).
Conclusions: These findings are similar to those after injury to skin, tendon, and ligament and suggest that patients with idiopathic carpal tunnel syndrome may have sustained an injury to the subsynovial connective tissue.
Clinical Relevance: The changes demonstrated in the subsynovial connective tissue not only could lead to an increase in the volume of the contents in the carpal tunnel but also may alter its material properties, such as compliance and permeability to fluid flows, and vascularity. These changes may, in turn, predispose the subsynovial connective tissue to additional injury and contribute to the elevation in carpal tunnel pressure seen in patients with carpal tunnel syndrome.

Introduction

Idiopathic carpal tunnel syndrome is the most common entrapment neuropathy. The underlying disease mechanism is increased carpal tunnel pressure[1-5], as a result of either a reduction in the size of the space in the carpal tunnel or an increase in the volume of its contents. The most common histological finding in carpal tunnel syndrome is noninflammatory synovial fibrosis [6-10]. The etiology of this finding and its relationship to the development of carpal tunnel syndrome remain poorly understood.

There are two bursae in the carpal tunnel. The radial bursa envelopes the flexor pollicis longus, and the ulnar bursa surrounds the other flexor tendons. The median nerve is extrabursal. Palmarly, the ulnar bursa is fixed to the undersurface of the flexor retinaculum. Dorsally, the bursa is separated from the flexor tendons by the

subsynovial connective tissue, a meshwork of areolar connective tissue and its associated vasculature, which makes up the vast majority of the tenosynovial mass (Figs. 1-A and 1-B). Guimberteau reported that the subsynovial connective tissue consists of multiple layers of collagenous fibers in which the blood and lymphatic vessels are richly represented[11] and that, during tendon motion, this tissue serves as a sliding unit to reduce friction and protect the vascular supply to the tendons and synovium. As a potential explanation for the characteristic histopathology of carpal tunnel syndrome, we propose that the fibrosis and thickening of the subsynovial connective tissue seen typically in patients with carpal tunnel syndrome are the result of some sort of injury.

In this study, we sought evidence of increased fibroblast density, abnormal blood vessels, the presence of collagen type III, and expression of transforming growth factor- (TGF-), all typical findings in healing soft-tissue wounds[12-15].

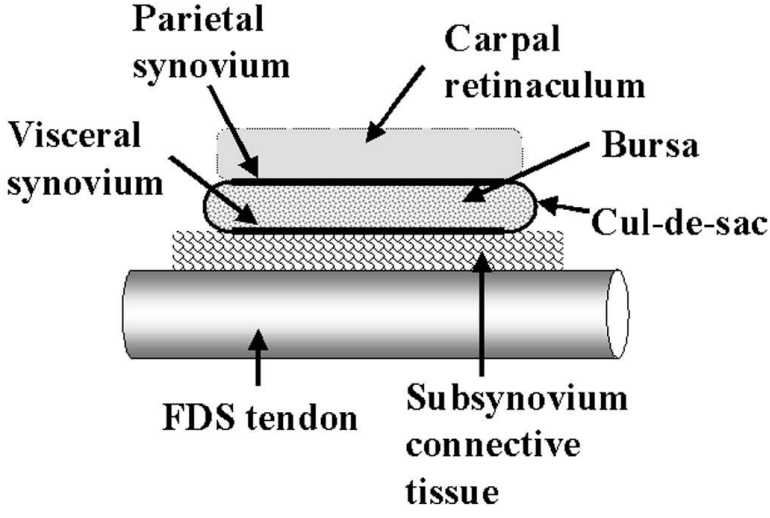

Fig. 1-A
Figs. 1-A and 1-B The structure of the sliding unit in the carpal tunnel region. **Fig. 1-A** Schematic diagram. FDS = flexor digitorum superficialis.

Our specific hypothesis was that patients with carpal tunnel syndrome, but not unaffected individuals, would show evidence of injury in the subsynovial connective tissue in the form of increased fibroblast density, abnormal blood vessels, the presence of collagen type III, and expression of TGF- .

Materials and Methods

We retrospectively reviewed the charts of patients who had had synovial biopsy specimens sent to our Department of Laboratory Medicine and Pathology during

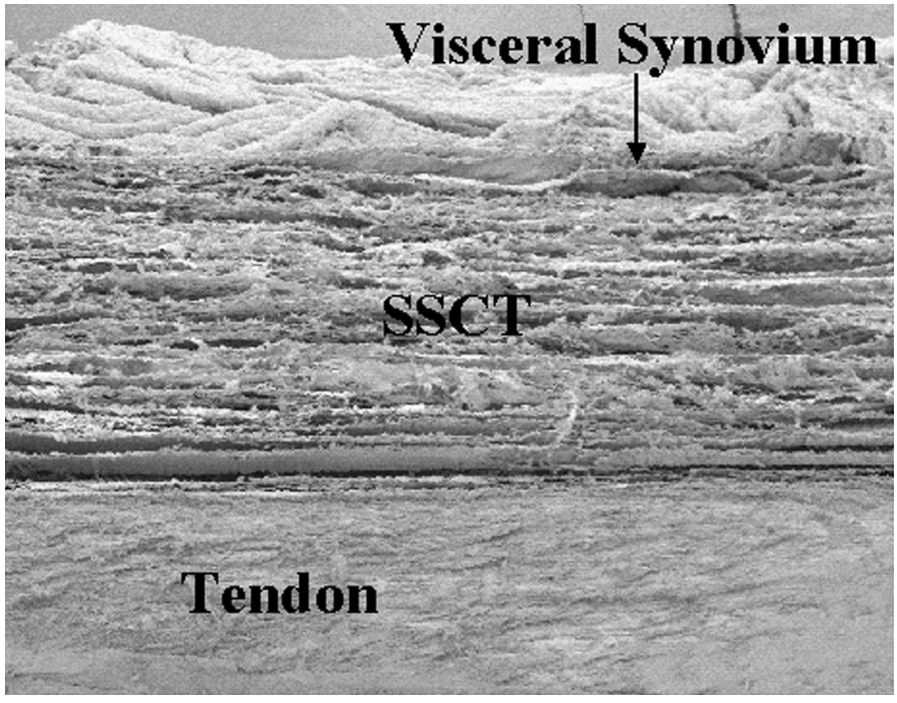

Fig. 1-B
Scanning electron micrograph of normal subsynovial connective tissue (SSCT) (original magnification, ×40).

carpal tunnel release between January 1999 and December 2001. The study was approved by our institutional review board. The medical history of each patient was abstracted to identify patients with a clinical diagnosis of idiopathic carpal tunnel syndrome. Patients with a history of diabetes, glucose intolerance, thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendinitis, gout, hemodialysis, a body mass index of >30, sarcoidosis, amyloidosis, peripheral nerve disease, or traumatic injuries to the ipsilateral arm were excluded, leaving thirty consecutive patients with presumed idiopathic carpal tunnel syndrome. A total of fifty-nine charts were reviewed. Of the fifty-nine patients, twenty-one were excluded because they did not have idiopathic carpal tunnel syndrome, and eight others were excluded because they were younger (mean age, 35.3 years; range, twelve to thirty-seven years) than the control population, leaving a total of thirty patients with idiopathic carpal tunnel syndrome.

There were twelve women and eighteen men with a mean age of 65.3 years (range, 38.9 to 93.4 years). Five patients had involvement of the left hand; nine, involvement of the right hand; and sixteen, bilateral involvement. The tissue was obtained from thirteen left hands and seventeen right hands. The tissue was obtained from the dominant hand of fifteen patients and from one patient whose hand

dominance had not been recorded. The tissue was the result of a limited synovectomy in two patients (one endoscopic procedure and one open release, resulting in 3 and 4 cm^3 of tissue, respectively) and of a synovial biopsy in the rest (resulting in 0.5 to 1.0 cm^3 of tissue). The limited synovectomies were done because, in the opinion of the surgeon, synovial bulk was impinging on the median nerve, even after release. The biopsies were performed as part of the routine practice of one surgeon and to rule out other pathological conditions, such as amyloidosis (tissue from ten patients was stained with Congo red, all with negative findings), in the case of other surgeons.

Demographic data, including age, gender, hand dominance, occupation, side of involvement, and side on which the tissue was obtained, were recorded for the thirty study patients. The results of the Tinel, Phalen, and carpal tunnel compression tests, measures of sensibility and strength, and any notation of thenar atrophy were also sought from the medical records of each patient. The duration of symptoms (in months) before treatment and the presence of symptoms such as paresthesias, numbness, and pain in the hand or wrist were also recorded. Evaluation was aided considerably by employing a standardized hand evaluation form, in use at our institution since the late 1970s, which included data fields for all of the above information. Twelve patients had thenar atrophy and fifteen patients had no thenar atrophy in the hand included in the study; the presence or absence of thenar atrophy was not documented for the remainder of the hands. The mean body mass index was 25.8. The mean duration of symptoms was 28.1 months (range, one to 121.7 months). Nine patients had had an open carpal tunnel release, and twenty-one had had an endoscopic carpal tunnel release.

All patients had had diagnostic neurophysiological testing, including electromyography and nerve conduction studies performed according to the standards of the American Association of Electrodiagnostic Medicine[16,17], and the diagnosis of carpal tunnel syndrome was confirmed in each case. The severity of the carpal tunnel syndrome was determined with use of the Mayo Clinic scale for neurophysiologic severity of carpal tunnel syndrome[18]. According to these criteria, three patients had mild, fourteen had moderate, and thirteen had severe carpal tunnel syndrome.

The control group consisted of ten fresh-frozen cadavers from which subsynovial connective tissue of the ulnar bursa of the carpal tunnel was obtained. In order to have a close age match with the patient group, we selected the youngest cadavers available. A total of twenty-six charts were reviewed to select the cadaver control group. Five subjects were excluded because a history of carpal tunnel syndrome was noted in the medical record. Two were excluded because diabetes mellitus was noted in the record and five, because hypothyroidism was noted (the same exclusion criteria as used in the patient group). Four were excluded because of old age (mean, 89.5 years; range, eighty-four to ninety-five years). This left ten cadaver controls without a history of carpal tunnel syndrome.

The cadavers of six women and four men were included in the control group. The mean age at the time of death was 75.3 years (range, 67.0 to 82.2 years), and the mean body mass index was 23.8. The control tissue was obtained from four left hands and six right hands. Information regarding hand dominance was not available.

Roughly 1 cm^3 of synovial tissue was obtained from each cadaver.

Histological and Immunohistochemical Analysis

For all patients, an initial pathological analysis was performed on frozen sections at the time of surgery, and all remaining tissue was formalin-fixed and paraffin-embedded. For the purposes of this study, 5-µm-thick sections were made from these paraffin blocks. Standard hematoxylin and eosin staining methods were used. The distribution of collagen types I, II, III, and VI was investigated with use of monoclonal anti-collagen types I, II, and VI (mouse IgG1 isotype; Medicorp, Montreal, Quebec, Canada) and monoclonal anti-collagen type III (mouse IgG1 isotype; BioGenex, San Ramon, California). Anti-human and TGF- RI (rabbit IgG) and TGF- RII (rabbit IgG) and TGF- RIII (goat IgG) polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, California) were used to stain for TGF- receptor subtypes. Biogenex negative control sera/immunoglobulins were used with each specimen to evaluate nonspecific staining.

In each staining run, we used paraffin-embedded positive controls. For collagen type I, this control consisted of a human flexor digitorum profundus tendon of the long finger in the carpal tunnel from a cadaver; for collagen type II, human cartilage; for collagen type III, human tissue from a skin wound closed by secondary intention twenty-one days postoperatively; for collagen type VI, human skin tissue; and for TGF- RI, RII, and RIII, human skin tissue.

One of the authors, who was not involved in the treatment of any of these patients, measured the size of twenty randomly chosen transversely or longitudinally cut collagen fibers on each slide of subsynovial connective tissue stained with hematoxylin and eosin in the patient and control groups. The mean size of the collagen fibers was calculated from these measurements.

Fibroblast density was measured by counting the number of fibroblasts in five randomly chosen areas on each slide. A light microscope with a ruler, with the magnification set at 40×, was used to mark the areas by turning the ruler 360° so that all of the areas were the same size. The microscope was calibrated with a scale division of 1:0.0235 mm for the 40× objective. The total surface area in which we counted the number of fibroblasts was thus 0.0434 mm^2.

Three independent observers, blinded to the origin of the specimens, graded the intensity of immunostaining for the collagen types in both groups. We randomly chose eight different areas with the magnification set at 20×. In each of these areas, we classified the intensity of staining as not stained (grade 0), mildly stained (grade 1), moderately stained (grade 2), or intensely stained (grade 3).

Analysis of TGF- RI staining was done by measuring the percentage of fibroblasts with TGF- RI expression in eight different randomly chosen areas. The areas were marked by using the total area seen with the microscope with the 20x objective.

Statistical Methods

The information was entered into a database, and statistical data analysis was done with the SPSS software program, version 10.0 for Windows (SPSS, Chicago, Illinois).

Two-sample t tests were used to compare continuous variables between the groups[19]. The proportions of specimens exhibiting the various grades of collagen were compared between the groups with use of the exact Wilcoxon test for ordered categorical data[20]. The Spearman rank-order method[21] was used for analysis of correlation among patient age, duration of symptoms, severity of the carpal tunnel syndrome, and the histological and immunohistochemical data.

All statistical tests were two-sided, and p values of <0.05 were considered significant. Results are reported as the mean and standard deviation unless otherwise indicated.

Results

Histological Analysis

The tissue obtained during surgery included the subsynovial sheet of the ulnar bursa in all cases. All specimens had initially been given a pathological diagnosis of noninflammatory fibrous tissue. Compared with the tissue in the control group, all tissue specimens from the patients showed vascular proliferation and vascular hypertrophy with intimal thickening.

The mean fibroblast count in the measured area (0.0434 mm^2) was 51.9 ± 16.3 in the specimens from the patients with carpal tunnel syndrome and 31.3 ± 9.3 in the control specimens. The mean fibroblast density was 1195 ± 374/mm^2 in the specimens from the patients with idiopathic carpal tunnel syndrome and 721 ± 215/mm^2 in the control group ($p < 0.001$).

The mean size of the collagen fibers in the subsynovial connective tissue was estimated to be 6.59 ± 3.43 μm in the specimens from the patients with carpal tunnel syndrome and 3.80 ± 1.51 μm in the control specimens ($p < 0.001$) (Figs. 2-A and 2-B).

Collagen Typing

No specific collagen type-I staining, except for immunostaining localized within a few small blood vessels (Fig. 3-A), was seen in the subsynovial connective tissue from either the patients or the controls. There was also no specific collagen type-II staining of the subsynovial connective tissue from either group.

The results of the collagen type-III staining showed a mean grade of 1.41 ± 1.04 in the patient group, which was significantly higher than the grade in the control group (0.35 ± 0.62) ($p < 0.001$). One (3%) of the patients had grade-0 staining; eighteen (60%), grade-1; seven (23%), grade-2; and four (13%), grade-3. In the control group, eight specimens had grade-0 staining and two had grade-1. Collagen type III was randomly present within the tissue and was not concentrated around vessels. Figure 3-B shows the immunohistochemical staining for collagen type III in a flexor tendon within the subsynovial connective tissue of one of our patients at 4× magnification. The subsynovial connective tissue in this image shows specific staining for collagen type III in the area under the tendon.

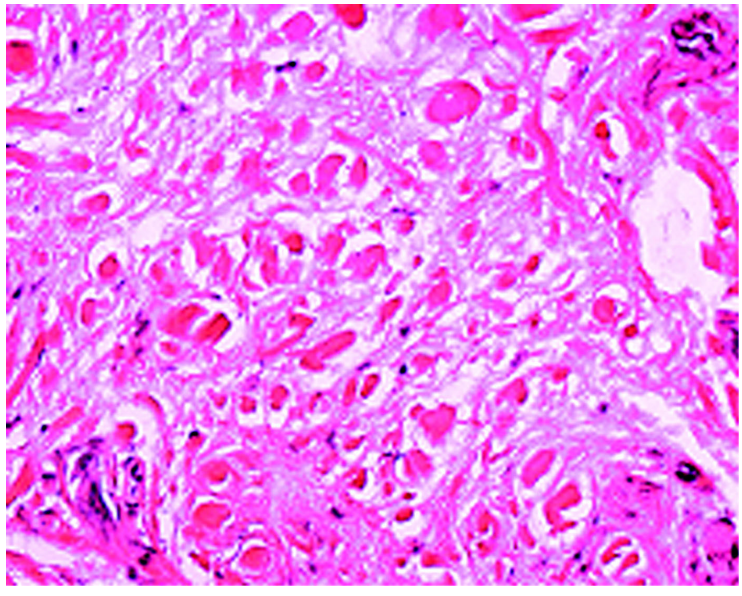

Fig. 2-A
Collagen fibers in a specimen from a patient with carpal tunnel syndrome (hematoxylin and eosin; original magnification, 40x).

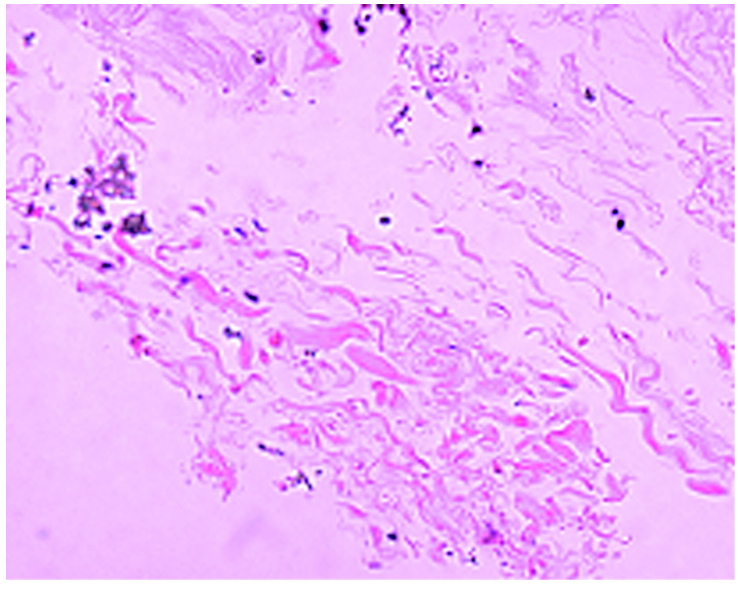

Fig. 2-B
Collagen fibers in a control specimen (hematoxylin and eosin; original magnification, ×40).

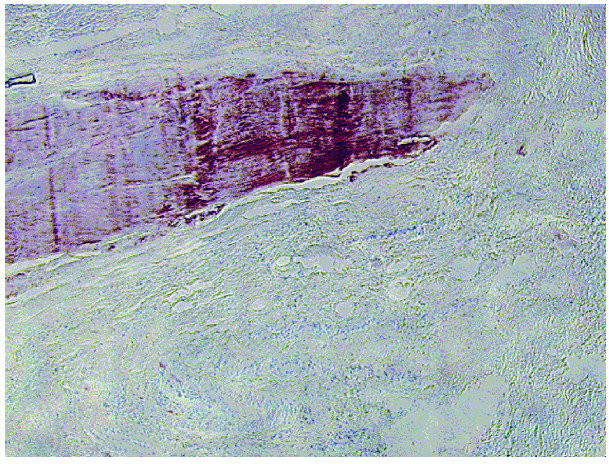

Fig. 3-A
Staining for collagen type I in a part of a tendon within the subsynovial connective tissue from a patient with carpal tunnel syndrome (original magnification, ×4).

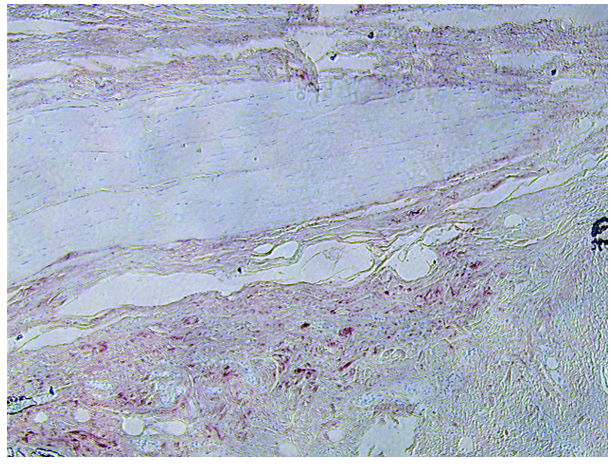

Fig. 3-B
Staining for collagen type III in the subsynovial connective tissue (red color) from a patient with carpal tunnel syndrome (original magnification, ×4).

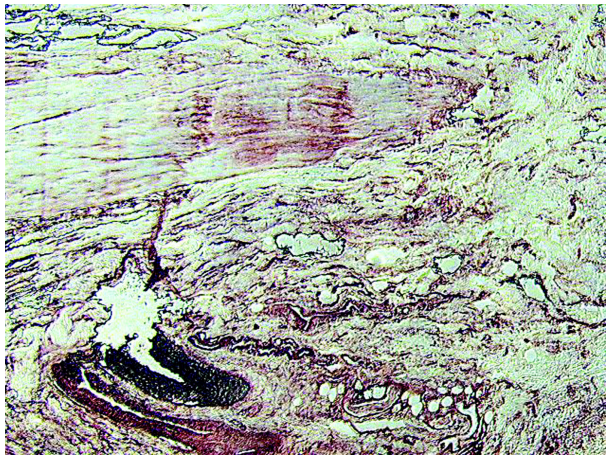

Fig. 3-C
Staining for collagen type VI in part of a tendon and the subsynovial connective tissue from a patient with carpal tunnel syndrome (original magnification, ×4).

The results of the collagen type-VI staining showed a mean grade of 2.16 ± 0.83 in the patient group compared with 2.52 ± 0.67 in the control group ($p = 0.213$). There were five patients (17%) with grade-1 staining, fifteen (50%) with grade-2, and ten (33%) with grade-3.

In the control group, there was one specimen with grade-1 staining, three with grade-2, and six with grade-3. Figure 3-C shows immunohistochemical staining for collagen type VI in a human flexor tendon within the sub-synovial connective tissue of one of our patients at 4× magnification.

The subsynovial connective tissue in this image shows specific staining for collagen type VI as marked by the red color.

Immunohistochemical staining for collagen type VI showed strong mural staining of small and intermediate-size muscular arteries within the adventitial and internal elastic lamina and was also seen throughout the subsynovial connective tissue.

TGF-β

The results of the TGF- RI staining showed expression in the endothelial cells and in the fibroblasts of all

specimens of subsynovial connective tissue from the patients and the controls; however, there was a markedly higher number of fibroblasts with expression of TGF- RI in the patient group than in the control group. The mean percentage of TGF- RI expression in eight randomly chosen areas in specimens from the patients was 62.7% ± 20.9%, whereas it was 19.5% ± 15.0% in the control group ($p < 0.001$). Figure 4 shows immunohistochemical staining of TGF- RI within the endothelial cells and fibroblasts in subsynovial connective tissue from a patient.

TGF- RII was seen in the endothelial cells, endovascular smooth muscle, and a few fibroblasts of the subsynovial connective tissue from the patients, but it was seen only within the endothelial cells of the control tissue. TGF- RIII was found in the endothelial cells within the subsynovial connective tissue of both the patients with carpal tunnel syndrome and the controls. We found it too difficult to use a grading system for the analysis of TGF- RII and TGF- RIII. Our positive control tissue showed staining for TGF- RI and RIII within the epidermis. We found staining for TGF- RII of the basement membrane of the skin.

Association of Pathological Findings with Severity of Carpal Tunnel Syndrome

With the numbers available, we found no correlation between the severity of the carpal tunnel syndrome and the duration of symptoms, fibroblast density, collagen size, or grades of collagen types I, II, III, and VI. There was also no correlation between the duration of symptoms and the fibroblast density, collagen size, or grades of collagen types I, II, III, and VI or between age (patient or control) and fibroblast density, collagen size, or grades of collagen types III and VI.

There was a significant positive correlation between patient age and the severity of the carpal tunnel syndrome (correlation coefficient, 0.591; $p < 0.01$). There was also a significant association between the presence of TGF- RI and collagen size ($p < 0.001$), fibroblast count ($p < 0.001$), fibroblast density ($p < 0.001$), and the presence of collagen type III ($p < 0.05$).

Discussion

We hypothesized that, if there were an insult to the subsynovial connective tissue in patients with carpal tunnel syndrome, there should be some histological and immunohistochemical evidence of it, and that these findings would not be present in unaffected individuals. This injury could be mechanical, as postulated by Guimberteau[11], or ischemia-reperfusion, as suggested by Freeland et al.[22]. The vascular proliferation and vascular hypertrophy with intimal thickening, the significant increase in fibroblast density and collagen fibril size, and the presence of collagen type III in the subsynovial connective tissue in the specimens from our patients with idiopathic carpal tunnel syndrome all support this hypothesis of injury.

The histological findings in the tissue from the patients compared with those in the control tissue support the findings of previous histological analyses of carpal tunnel syndrome[8,10,23-29]. The difference between our study and others is the analysis of collagen type, fiber size, and TGF-. The distribution of the different collagen types provides information about the structure of the subsynovial connective tissue and, perhaps, about the pathophysiology of carpal tunnel syndrome. For example, as collagen type III is characteristic of an injury response and is weaker than collagen type I[30], it could predispose to a vicious cycle of further injury.

Collagen type I is the major component of tendon, but there was no positive staining for collagen type I in the subsynovial connective tissue from either the patients or the controls. Instead, collagen type VI seems to be a major component of the subsynovial connective tissue. This is a new and potentially important finding as, again, collagen type VI does not have the strength of collagen type I and could be more susceptible to injury.

Additional immunohistochemical analysis should be performed to identify other components of the subsynovial connective tissue. Collagen types V and XI are also fibrillar collagens[31] and might be present in the subsynovial connective tissue. There was an increase in collagen fiber size in our patients relative to the size in the control group. These changes could affect the material properties or permeability of the synovium, as has been suggested by Freeland et al.[22]. Those changes may, in turn, contribute to the elevation in carpal tunnel pressure seen in patients with carpal tunnel syndrome.

Fibroblasts play important roles in granulation tissue formation, wound contraction, matrix synthesis, wound repair, and scar formation[32-34]. Our data confirmed that the fibroblasts express TGF- receptor isoforms and thus would be responsive to TGF- signaling within the subsynovial connective tissue. The finding of greater amounts of TGF- RI in the fibroblasts within the noninflammatory subsynovial connective tissue of the patients than in the control tissue supports our hypothesis that there is a wound-healing process in the subsynovial connective tissue of these patients[35-42]. The increase in TGF- RI expression in the fibroblasts within the subsynovial connective tissue in our patient group also supports our hypothesis that this process leads to scarring and fibrosis and may thus play a role in the etiology of carpal tunnel syndrome.

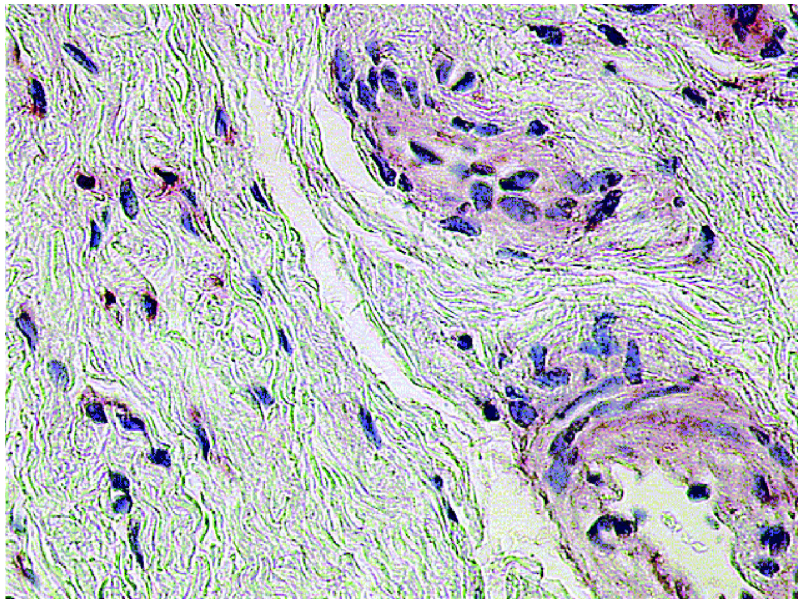

Fig. 4
Expression of a TGF- RI within the endothelial cells and fibroblasts of the subsynovial connective tissue in a patient with carpal tunnel syndrome.

The strength of this study lies in the systematic histological analysis of the subsynovial connective tissue from individuals with and without carpal tunnel syndrome. The weaknesses are those inherent in any retrospective study. Specifically, the synovial tissue that was available from patients with carpal tunnel syndrome was not collected routinely or randomly but from specific patients (thirty of more than 1000 treated with carpal tunnel release at our institution over the three-year study period) in whom a synovectomy was performed for reasons other than to treat a comorbid condition, such as rheumatoid arthritis. The specimens that were available to us may therefore not be representative. In addition, we did not have complete histories on the activity of these patients; thus, we cannot make any comment about whether the activity level of the patients with carpal tunnel syndrome was in any way different from that of the control individuals.

In conclusion, we found evidence of histological and immunohistochemical changes in the subsynovial connective tissue of patients with carpal tunnel syndrome that were different from those seen in individuals of similar age but without carpal tunnel syndrome. The findings were similar to those seen after injury in other tissues and therefore are consistent with the hypothesis that the subsynovial connective tissue had sustained some sort of injury. The exact nature of this injury and its relationship, if any, to carpal tunnel syndrome remain to be elucidated.

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2.2

Changes in the Functional Structure of the Tenosynovium in Carpal Tunnel Syndrome: a Scanning Electron Microscope Study.

Ettema AM, Amadio PC, Zhao C, Wold LE, O'Byrne MM, Moran SL, An KN.

Plastic and Reconstructive Surgery 2006 Nov;118(6):1413-1422

Abstract

Background: The subsynovial connective tissue (SSCT) lies between the flexor tendons and visceral synovium in the carpal tunnel. While tenosynovial fibrosis is nearly universally noted in patients with carpal tunnel syndrome (CTS), the relationship, if any, between the fibrosis and nerve pathology is unknown. In this observational study, we used light and scanning electron microscope (SEM) imaging of the SSCT to gather information about its organization.
Methods: Human SSCT was studied to determine its ultrastructural morphology. Human SSCT biopsies of 12 patients with idiopathic CTS, 14 cadaver controls and 2 cadavers with a history of CTS were obtained for SEM imaging and histopathology.
Results: The visceral synovial layer is an uninterrupted membrane, which defines the bursa dorsally. The SSCT consists of fibrous bundles, which run parallel to the tendon, interconnected by smaller fibrous fibers. The SSCT connects to the synovial membrane and the flexor tendons. During tendon motion the loose fibers between adjacent layers are stretched. Our control tissue showed interconnections between all the parallel layers, while in the patients with idiopathic CTS these interconnections were absent, replaced with thicker parallel fibrous bundles. We found similar changes in our cadaver CTS specimens. The pathologic changes in the patient and cadaver CTS specimens were most apparent close to the tendon, and became progressively less severe in more superficial layers.
Conclusions: Our observation that the most severe changes in the SSCT were found close to the tendon, suggest to us that these changes may be the result of a shearing injury.

Introduction

The modern understanding of carpal tunnel syndrome has evolved from a variety of clinical and pathologic observations[1-7]. While fibrosis of the tenosynovium is nearly universally noted in patients with carpal tunnel syndrome (CTS), the relationship, if any, between the fibrosis and the nerve pathology is unknown.

The carpal tunnel is a narrow (1-1.5 cm diameter) space at the base of the palm, bounded dorsally, medially and laterally by the carpal bones and palmarly by the flexor retinaculum. The contents of the carpal tunnel include the flexor digitorum profundus (FDP) tendons, the flexor digitorum superficialis (FDS) tendons, the flexor pollicis longus (FPL) tendon, the subsynovial connective tissue (SSCT) associated with these tendons, the radial bursa and the ulnar bursa and the median nerve. The SSCT loosely connects the finger flexor tendons and the synovial membrane, which in turn encloses the tendons within the ulnar tenosynovial bursa.

Gelberman et al. have identified two main classes of tendon, intrasynovial and extrasynovial, that differ significantly in their response to injury or loading[8]. The closed synovial fluid environment of intrasynovial tendons is enveloped by a layer of parietal synovium, which is attached to soft tissues such as pulleys, capsule, or the bone, and a visceral synovial layer which covers the tendon surface. These two layers are

continuous at the proximal cul-de-sac, the vincula origins, and the tendon insertions[9, 10]. The nutrition of the intrasynovial tendon is based on both the intrinsic blood supply and diffusion of synovial fluid, with synovial fluid playing the dominant role[11]. The extrasynovial tendons are surrounded by a loosely arranged matrix of collagen with abundant vasculature (paratenon) that supplies the tendon nutrition[8, 9].The sliding mechanism of the extrasynovial tendon relies on its paratenon[12], which limits tendon gliding. Guimberteau et al. have shown that the paratenon also plays an important role in maintaining the tendon physiologically[13]. A tendon can also be intrasynovial in one location and extrasynovial in another. For example, the extensor tendons of the fingers are intrasynovial at the wrist but extrasynovial more distally.

While the anatomical structure of the carpal tunnel has been well studied, the flexor tenosynovial organization in this area has not been examined in detail. The flexor tendons in this area are neither typically intrasynovial nor typically extrasynovial. The surface of the tenosynovium in the carpal tunnel is part of the visceral synovial layer of the ulnar wrist bursa. This bursa is connected to the flexor tendons and the median nerve[14] by the subsynovial connective tissue (SSCT). Guimberteau has hypothesized that, during tendon motion, the SSCT serves as a sliding unit to reduce the friction and protect the blood supply to the tendon and synovium (Figure 1)[13].

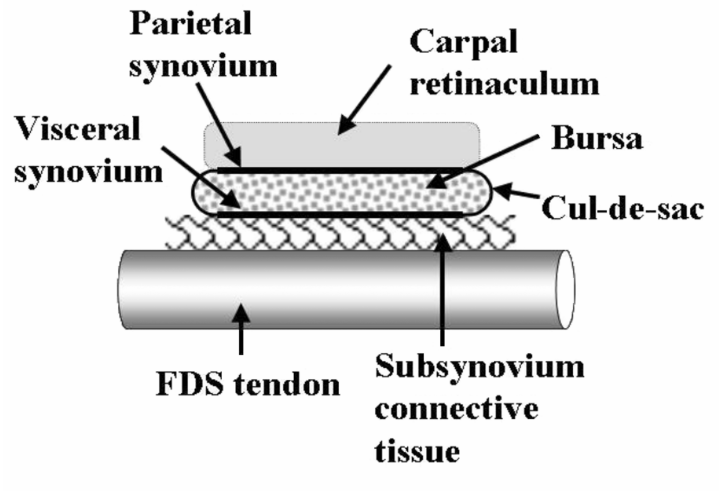

Figure 1. The structure of the sliding unit in the carpal tunnel region.
Schematic diagram (this picture was previously published by Ettema et al. in the J Bone Joint Surg (JBJS) 2004 Jul; 86-A(7): p1459[15]. Copyright owned by JBJS; used with permission).

Fibrosis of the SSCT is the most characteristic histopathological finding in patients with carpal tunnel syndrome[2, 4, 7, 15-17]. The question arises as to whether the changes in the SSCT are primary or secondary changes and, if the former, what the etiological mechanism might be. We hypothesize that carpal tunnel syndrome is the result of shearing injury to the SSCT with secondary changes in local vascularity[18],

leading to fibrosis and scarring[15] that subsequently result in nerve ischemia and compression. If this is the case, then individuals with carpal tunnel syndrome should have thickening and distortion of the SSCT, and such changes should be most apparent closest to the flexor tendons, where the shearing injury would most logically occur. To test this hypothesis, we evaluated the SSCT in individuals with and without carpal tunnel syndrome by scanning electron microscopy (SEM).

Materials and Methods

Human carpal tunnel tissue was studied to determine its microscopic and ultrastructural morphology. SSCT biopsies for SEM imaging were obtained from 12 patients with idiopathic carpal tunnel syndrome, 14 cadaver controls and 2 cadavers with a history of carpal tunnel syndrome. Before harvesting, the visceral synovium was marked with a marker pen, to orient the specimen with regard to superficial and deeper layers in the tissue.

In the 12 patients and two cadavers with an antemortem diagnosis of CTS we sent additional synovial tissue to our Department of Laboratory Medicine and Pathology for routine hematoxylin and eosin histopathology. The biopsies for SEM were fixed in Trump's fixative (1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2)[19], and dehydrated through a graded series of ethanol solutions in a critical point dryer. Tissue was then rinsed for 30 minutes in 2 changes of 0.1 phosphate buffer, pH 7.2. The tissue was dehydrated in progressive concentrations of ethanol. The specimens were then mounted on aluminum stubs and sputter coated with gold-palladium. Images were captured on a Hitachi S4700 cold field emission scanning electron microscope operating at 2KV (Hitachi S-4700, Hitachi High Technologies America, Inc., Pleasanton, CA, USA). Pictures were taken with the palmar side of the tissue up, and at different levels from the tendon through the SSCT to the synovium.

In a fifteenth cadaver control we obtained a larger sample, including the visceral synovium, SSCT and tendon, in order to image the tissue after simulation of movement of the tendon and SSCT in the carpal tunnel. This specimen was then prepared for SEM as described above.

In a sixteenth cadaver control, we obtained tissue containing a part of the flexor retinaculum, parietal and visceral synovium, SSCT and middle flexor digitorum superficialis tendon for standard hematoxylin and eosin (HE) staining. The tissue was formalin-fixed and paraffin-embedded. Sections of five μm thickness were made by our Laboratory Medicine and Pathology Department.

The changes in the SSCT by SEM (x1.0k) were documented as normal (0), mild (1), moderate (2) or severe (3). We defined the tissue with the presence of many interconnections as normal SSCT. The severe changes were defined as complete absence of the interconnecting fibrous fibers. Mild and moderate changes were classified to be in between normal and severe (see Figures 5A-D). Two independent observers, one trained in orthopedics and one trained in biochemistry and molecular biology, both blinded to the origin of the specimens, graded the changes in the SSCT

by SEM. Histopathological changes such as small vessel proliferation and fibroblast cellularity were measured by grading the presence of the small vessels and fibroblast of each slide (x200) for the patients with carpal tunnel syndrome. In each slide we classified the number of small vessels and fibroblast as normal (0), mild increase (+) and severe increase (++). The collagen bundle thickening was noted "+" and "++" when there was a visible increase in thickening with the use of the light microscope (x200). Two independent observers, one trained in pathology and one trained in orthopedics, both blinded to the origin of the specimens, graded the histopathological changes in the SSCT.

CTS patients and cadaver patients versus controls

We compared 12 SSCT biopsies in 12 hands from 11 patients with a mean age of 60 (range 38-76) with 12 biopsies in 12 hands from 11 cadaver controls with a mean age of 81 (range 70-98). Biopsies were taken from 7 right and 5 left hands for the patients and 8 right and 4 left hands in the cadaver control group.

There were 7 women and 4 men in the patient group, all with right hand dominance. In the patient group there were 2 right hands from male and 5 right hands from female patients. There were 11 patient hands with electrodiagnostic testing confirming carpal tunnel syndrome[20]. There were 2 mild, 6 moderate and 3 severe carpal tunnel syndrome hands and in one hand the EMG was normal, but the clinical symptoms were classic for carpal tunnel syndrome and this patient was considered to have mild carpal tunnel syndrome.

There were 3 females and 8 males in the cadaver control group. Specimens were taken from 6 right hands from the male and 2 right hands from the female cadavers.

There were 2 cadaver hands with an antemortem history of carpal tunnel syndrome. One female cadaver with an age of 92 years had had a carpal tunnel release of her right hand 32 years prior to death. One male cadaver with an age of 81 years had had carpal tunnel syndrome with an electrodiagnostic test result confirming mild carpal tunnel syndrome, and had had a single steroid injection 17 years prior to death. It was this cadaver hand in which we obtained 3 biopsies (palmarly and dorsally of the middle flexor digitorum superficialis tendon and dorsally of the middle flexor digitorum profundus tendon).

Patient tissue

We performed medical record reviews on patients who were scheduled for CTS release at our institution from March 2004 to August 2004. The medical history of each patient was abstracted to identify those with idiopathic carpal tunnel syndrome. Demographic data such as age, gender, hand dominance, side of involvement, and carpal tunnel syndrome severity were recorded.

Exclusion criteria included a history of diabetes, glucose intolerance, thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendinitis, gout, hemodialysis, BMI>30, sarcoidosis, amyloidosis, peripheral nerve disease or traumatic injuries to the ipsilateral arm.

Patients meeting these criteria were interviewed by one of us and requested to consent to synovial biopsy during their carpal tunnel release surgery, consisting of approximately 10x3mm of the SSCT of the middle flexor digitorum superficialis tendon (FDS III), including the visceral synovial layer and SSCT closest to the tendon. No effort was made to perform a complete synovectomy, or to estimate the total volume of synovium in these specimens. The biopsies were marked with a surgical marker (Skin Markers, Devon Industries, Inc, Buffalo, New York) on the distal-palmar side and put in the Trumps fixative.

Additionally, a specimen of the SSCT of the middle flexor digitorum superficialis tendon (FDSIII) measuring approximately 5x3mm was also obtained for each patient. These biopsies were formalin fixed and paraffin-embedded. Standard hematoxylin and eosin staining methods were used. This study was approved by our Institutional Review Board.

Cadaver tissue

Sixteen fresh frozen upper limb specimens from 10 male and 6 female cadavers were defrosted for the study. Ten right hands and 6 left hands were used.

A medical record review was performed on each member (mean age of 82 years) of the control group before harvesting the tissue, to be sure that all individuals met the same exclusion criteria as the patients, and that, in addition the control specimens, did not have an antemortem diagnosis of carpal tunnel syndrome. Twelve control specimens were used to compare with the clinical tissue. Two cadaver specimens with an antemortem history of idiopathic carpal tunnel syndrome were specifically sought as well, to compare the cadaver findings of individuals with known CTS with those of the surgical specimens and the cadaver control.

A palmar skin incision was made longitudinally at the carpal tunnel to expose the middle finger FDS tendon. A biopsy, approximately 10 mm in length and containing the visceral membrane, the SSCT, and a section of the middle flexor digitorum superficialis tendon (FDS III) was obtained. All biopsies were marked with a surgical marker on the distal-palmar side of the visceral synovium and put in the Trumps fixative. In one cadaver patient we harvested synovial tissue palmarly and dorsally of the middle flexor digitorum superficialis tendon and dorsally of the middle flexor digitorum profundus tendon.

In one biopsy (fifteenth cadaver control), the dissected tendon, 15mm in length, was fixed with 2 clamps and the visceral synovium was moved proximally (approximately 15 mm) as it would during flexion. We put a few drops of Trump's fixative on this specimen to prefix in this position and then suture the specimen in this position by 2 sutures, one on the proximal and one on the distal end of the biopsy. After fixation in this position the specimen was placed into the Trump's fixative container.

In another biopsy (sixteenth cadaver control) a part of the middle superficial flexor tendon, approximately 15mm in length, the attached SSCT, visceral synovium and flexor retinaculum was formalin fixed and brought to our Laboratory Medicine and Pathology Department for paraffin embedding and standard hematoxylin and eosin (HE) staining.

Statistical Methods

Fisher's exact tests were used to evaluate the association between the severity of the carpal tunnel syndrome and the scanning electron microscopy (SEM) and histopathology data. The SEM change scores were compared between the three layers (superficial, middle, and near tendon) using Friedman's test. Comparison of the SEM data between the patients and the controls was conducted using Fisher's exact tests. The analysis was carried out using SAS (SAS Institute Inc., Cary, NC). All statistical tests were two-sided, and p values less than 0.05 were considered significant.

Results

Gross inspection of the biopsy specimens showed few obvious differences between the patient and cadaver specimens. In general, the patient specimens had a more dense, fibrous appearance than the cadaver tissue, which had a more filmy appearance. The patient SSCT tended to be more adherent to tendon; the cadaver tissue less so.

The SSCT sliding unit in control specimens

In all cases, whether cadaver or patient, with or without CTS, the SSCT consisted of fibrous bundles parallel to the tendon, which were connected to one another by smaller fibers. Light microscopic and SEM imaging of the sliding unit in cadaveric SSCT clearly showed the different tissue layers (e.g. flexor retinaculum, parietal synovium, visceral synovium, SSCT and tendon) in the carpal tunnel (Figure 2).

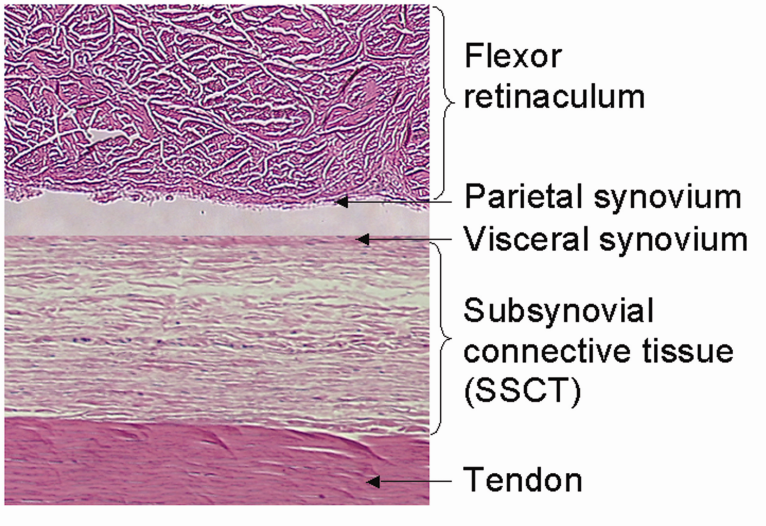

Figure 2. Sliding unit in the carpal tunnel region has both structures, i.e. paratenon and synovial sliding mechanism. All layers from the flexor retinaculum to the tendon are shown. The tendons in the carpal canal have a SSCT and a bursa, containing synovial fluid between the parietal and visceral synovium (H&E, original magnification x400).

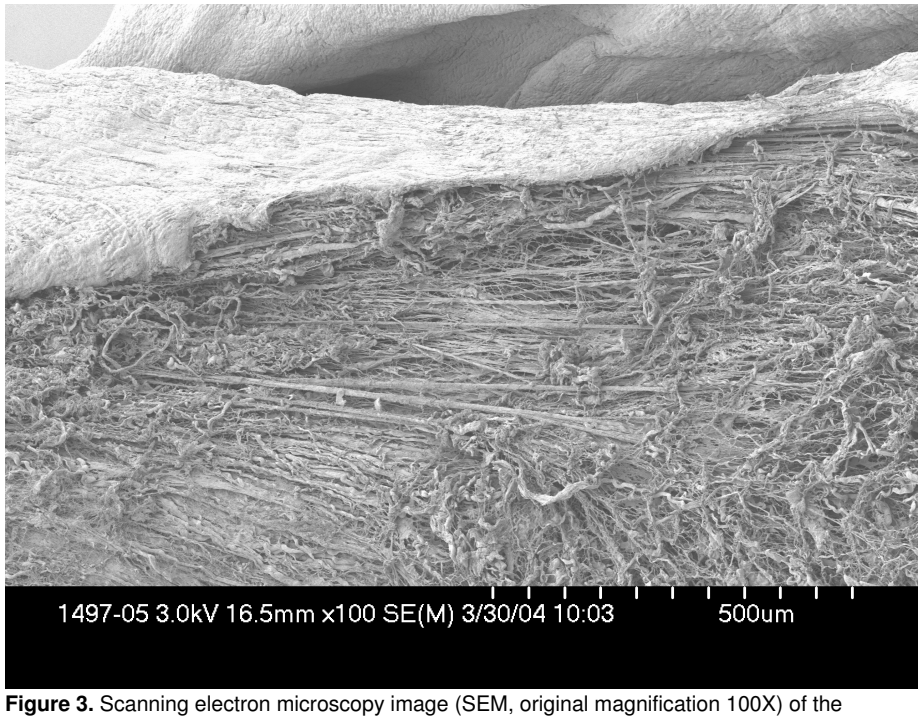

Figure 3. Scanning electron microscopy image (SEM, original magnification 100X) of the visceral synovium and SSCT in a patient. The collagen bundles are parallel to the visceral synovium and tendon. This picture was taken in a patient and shows that the interconnections are present in the superficial layers.

The visceral synovial layer was seen as an uninterrupted synovial membrane (Figure 3). The SSCT was loosely connected to the synovial membrane and finger flexor tendons. The SSCT consisted of a fibrous network, arranged in bundles parallel to the tendon, with spaces evident between the bundles.
Loose vertical fibers were noted between the parallel bundles (Figure 4A). Imaging of the SSCT after simulation of flexor tendon movement under the scanning electron microscope showed that the vertical fibers between adjacent bundles were stretched (Figure 4B).

CTS patients and cadaver patients versus controls
The parallel fibrous bundles in the patients tissue (including the two cadaver CTS specimens), were thicker than those in the control SSCT. In all patient SSCT specimens (including the two cadaver CTS specimens), we found that, closer to the tendon, the vertical interconnecting fibrils between fibrous layers were absent (Figure 5D). In the cadaver control SSCT tissue, these connecting fibrils were evenly present throughout the SSCT (Figure 5A). In the patients with idiopathic CTS the interconnections were replaced by thick parallel fibrous bundles (Figure 6).

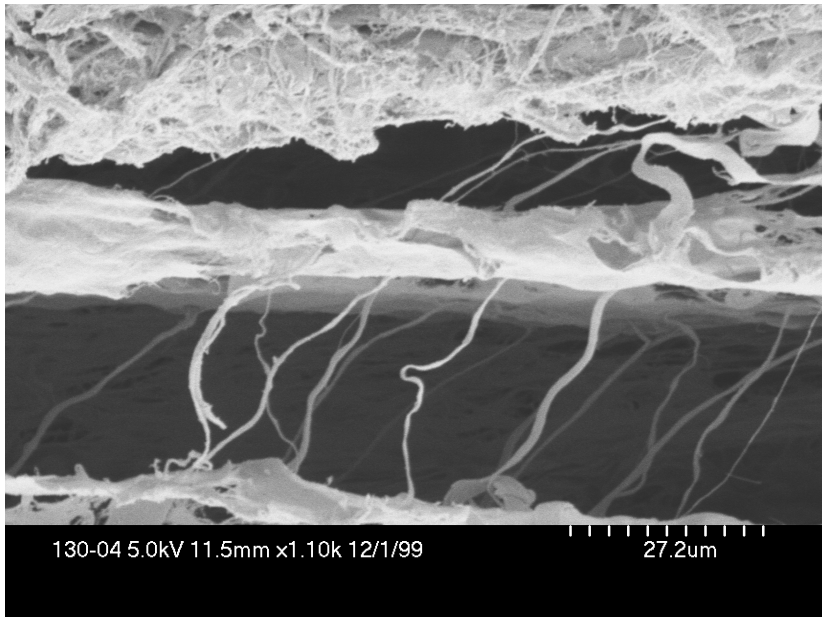

Figure 4A. Loose vertical fibers join adjacent layers in the SSCT (SEM, original magnification x1.10K).

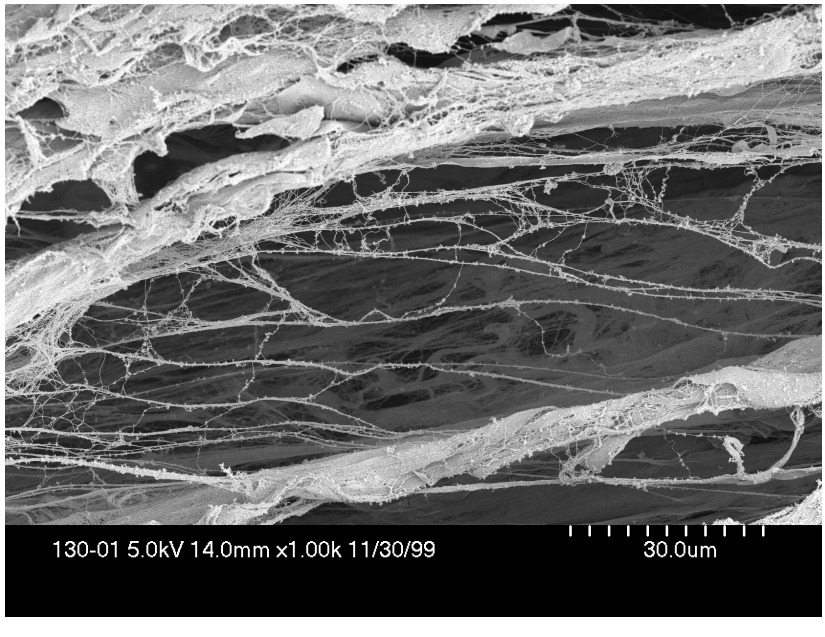

Figure 4B. The loose vertical fibers between adjacent layers are stretched during flexor tendon movements (SEM, original magnification x1.00K).

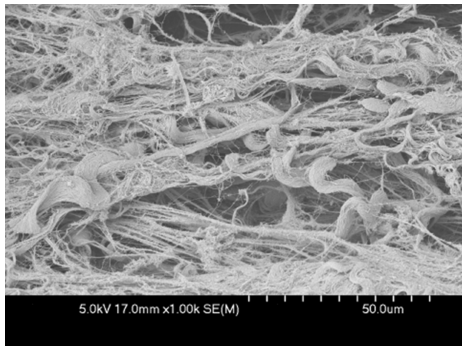

Fig 5a

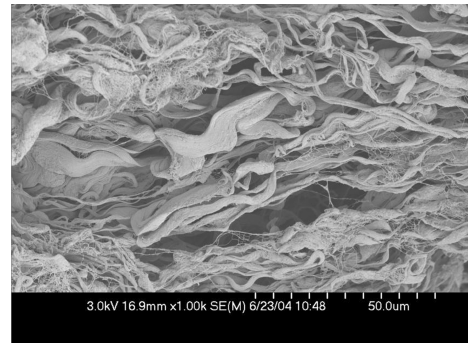

Fig 5b

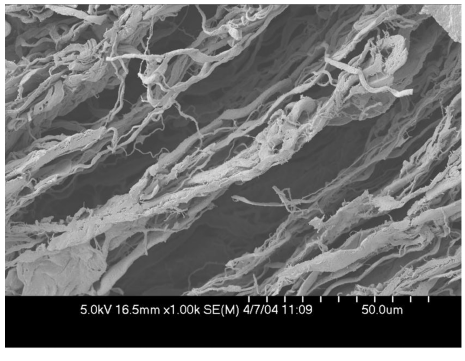

Fig 5c

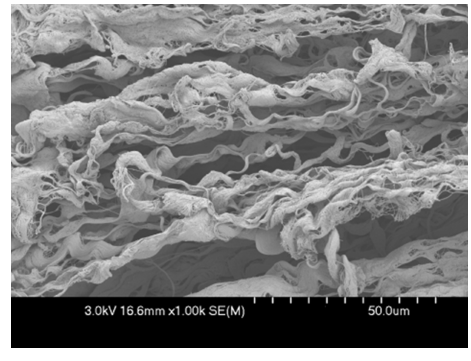

Fig 5d

Figure 5. 5A. Patient subsynovial connective tissue (SSCT) close to the tendon with the palmar side up (SEM, original magnification x 1.00K). Defined as "normal" SSCT by SEM. **5B.** Patient subsynovial connective tissue (SSCT) in the middle of the SSCT with the palmar side up(SEM, original magnification x 1.00K). Defined as "mild" change in the SSCT by SEM. **5C.** Patient subsynovial connective tissue (SSCT) in the middle of the SSCT with the palmar side up(SEM, original magnification x 1.00K). Defined as "moderate" change in the SSCT by SEM. **5D.** Cadaver control subsynovial connective tissue (SSCT) close to the tendon with the palmar side up (SEM, original magnification x 1.00K). Defined as "severe" change in the SSCT by SEM.

The pathological changes in the patient specimens were most apparent close to the tendon, and became progressively less severe in more superficial layers.

In the superficial layers of the SSCT, 50% of the patients had mild SEM changes, while 33% had moderate SEM changes; no severe changes were observed at this level. In the middle layers of the SSCT, the proportion of patients with a moderate score for SEM changes increased to 50%, and a severe score was observed in 8%. Finally, for the layers nearest the tendon in the SSCT, the proportion of patients with a moderate score for SEM changes was 50%, and the proportion with a severe score increased to 33%. When the SEM change scores were evaluated numerically using Friedman's test, we observed significant differences between the mean scores of the 3 levels ($p<0.001$).

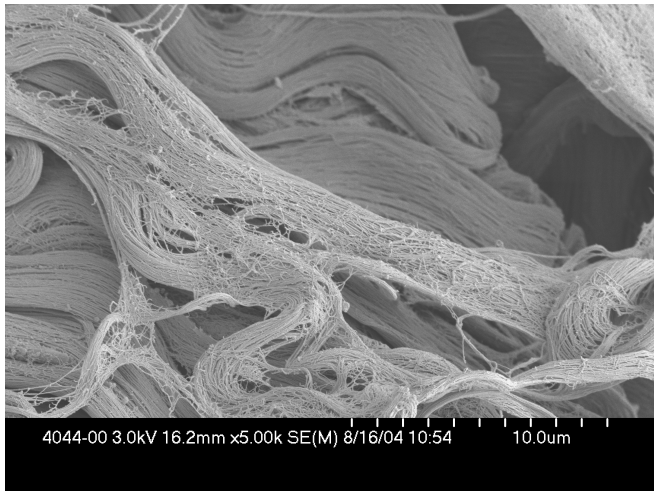

Figure 6. SEM image (original magnification x5.0K) of the subsynovial connective tissue (SSCT) in a patient with severe idiopathic carpal tunnel syndrome. The microfibers seem to attach to the parallel fibrous bundles.

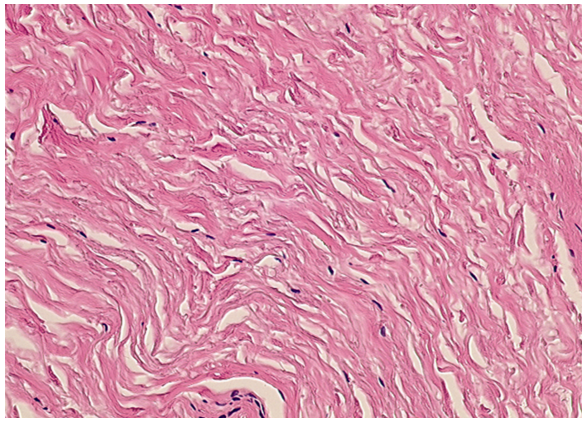

Fig. 7A. SSCT of control tissue (H&E, original magnification 200X).

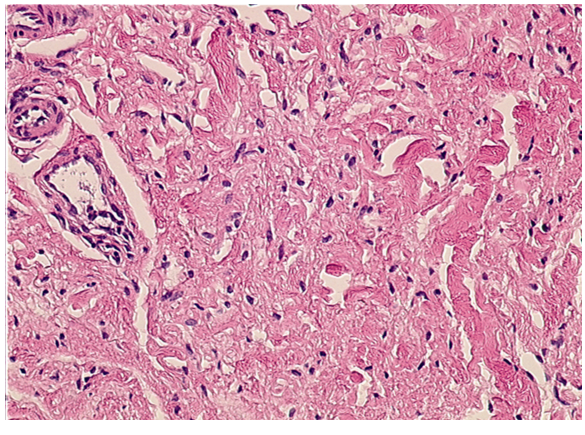

Fig. 7B. SSCT of a patient with carpal tunnel syndrome showing small vessel proliferation and hypertrophy with intimal thickening, thicker collagen bundle size and higher fibroblast density (hematoxylin and eosin; original magnification 200X).

The mean (±SD) scores were 1.17 (±0.72) in the superficial layer of the SSCT, 1.67 (±0.65) in the middle, and 2.17 (±0.72) close to the tendon (Table 1 and Figure 5).

The cadaver controls all had a severity score of zero (normal) for the changes in the SSCT by SEM.

The difference in the changes found with SEM between the patients and controls were significant ($p<0.001$) for the 3 levels in the SSCT. The statistical power to show a difference between the changes in the SSCT for the superficial layer, middle layer and layer close to the tendon between the patients and controls was respectively 96%, 99% and 99%.

The histopathological changes in our patient tissue were non-inflammatory with vascular proliferation and hypertrophy with intimal thickening and increase in fibroblast cellularity and collagen bundle thickening as described in Table 1 and Figure 7A and 7B.

Table 1. Electromyography severity[20] versus severity of SSCT changes by SEM in different layers to the tendon (i.e. superficial SSCT, middle of SSCT and close to the tendon) and histopathology of the biopsies. 0= no changes, 1= mild changes, 2= moderate changes, 3= severe changes.

		Scanning Electron Microscopy			Histopathology		
Patient	EMG Severity	Superficial SSCT	Middle of SSCT	Near Tendon	Small vessel proliferation	Fibroblast cellularity	Collagen bundle thickening
1	Mild	1	1	2	+	+	+
2	Severe	2	2	**3**	++	++	
3	Moderate	2	3	**3**	++	+	
4	Severe	0	1	2	++	++	
5	Moderate	0	1	1	+	+	
6	Mild	1	1	1	+	+	
7	Moderate	2	2	**3**	+	-	++
8	Moderate	1	1	2	++	+	++
9	no	1	2	2	+	+	
10	Moderate	2	2	**3**	+	++	
11	Moderate	1	2	2	++	+	
12	Severe	1	2	2	+	++	

The 3 biopsies from different levels in the carpal tunnel in the cadaver with a history of mild idiopathic carpal tunnel syndrome showed changes similar to that seen in the patient specimens, with fewer interconnections closer to the tendon. The changes were worse in the SSCT biopsy obtained palmar to the middle flexor digitorum superficialis tendon, but the SSCT biopsies obtained dorsal to the middle superficial flexor tendon and dorsal to the middle profundus flexor tendon also showed thicker bundles and less interconnections. The histopathology of these 3 biopsies showed vascular proliferation and increase in fibroblastic cellularity. In the biopsies obtained palmar and dorsal to the middle flexor digitorum superficialis tendon we also found an increase in collagen bundle thickness.

Severity of the Carpal Tunnel Syndrome versus Pathological Findings
The proportion of patients without EMG (clinically mild) and with mild EMG severity that had moderate SEM changes in the layer close to the tendon was 67%; no patients in this group had severe changes. In contrast, 33% of the patients with moderate EMG severity had moderate SEM changes, and 50% had severe SEM changes in the layer close to the tendon. In the group of patients with severe EMG severity, 67% had moderate SEM changes in the layer close to the tendon, and severe changes were seen in 33%.

With the numbers available, we found no significant association between the severity of the carpal tunnel syndrome and changes in the SSCT observed with SEM for the superficial layer (Fisher's exact test, $p=0.44$), middle of the SSCT (Fisher's exact test, $p>0.99$) nor near the tendon (Fisher's exact test, $p=0.79$). However, there was a trend towards more severe changes in the moderate and severe EMG groups. This trend was most evident in the SSCT nearest the tendon (Table 1). The statistical power to show associations between electrodiagnostic severity and changes in the SSCT severity by SEM was limited due to small sample size (<40% approximate power for all tests). In order to get an 80% power we would need observations from 50 specimens.

No significant association was observed between severity of the electrodiagnostic tests and small vessel proliferation, collagen bundle thickening and age of the patients at the moment of surgery.
However, there was a significant positive association between the severity of the carpal tunnel syndrome and the fibroblast cellularity ($p=0.049$).

Discussion

The SSCT is an anatomic feature which is unique to the tendons in the carpal tunnel. In this study, light and scanning electron microscopy confirmed the arrangement in layers (e.g. flexor retinaculum, parietal synovium, visceral synovium, SSCT and tendon) of the sliding unit in the wrist as described by Guimberteau[13]. The SSCT consists of bundles of collagen, which are interconnected by smaller vertical fibers. It seems that by stretching and relaxing of the SSCT the loose fibers between adjacent layers are stretched (Figure 4B), and the fibrous bundles move layer by layer and are pulled by the interconnections, more or less as an arm would move within layers of sleeves. When the tendon moves, the fibrils connected by the tendon are stretched first, followed by fibrils connected to the paratenon layers. In this way, the lengthening propagates layer by layer until finally the visceral synovium (VS) moves.

In the patients SSCT, each layer is separated from the adjacent layer by a wider space, and contains thicker bundles of collagen fibrils[15].

Stress on the SSCT may exceed its elastic threshold, which causes damage of this tissue. Our findings suggest that these interconnections break (Figure 7) and attach to the parallel fibrous bundles, which in turn become thicker and more coarse, consistent with literature describing collagen fibril diameter in carpal tunnel syndrome

SSCT by light and electron microscopy[15, 21]. These findings are consistent with those seen in a chronic healing process, with fibrosis and scarring[5, 15, 16, 22].

Although we did not study the material properties in our specimens, it is likely that these pathological changes also change the SSCT mechanical properties. The SSCT layered gliding function is also likely to be affected as more and more gliding layers become effaced by the progressive fibrosis. The stiffness of the damaged part of the SSCT may also increase, which would increase the risk of injury to remaining normal gliding layers, and possibly affect the ability of the SSCT to support differential tendon gliding, differential tendon and nerve, and even diffusion of nutrients through the SSCT, as has been observed by others[23, 24].

Our observation that the most severe changes in the patients were found close to the tendon, where motion would be the greatest, also suggest to us that these changes may be the result of a shearing injury. Based on these observations, we hypothesize that the characteristic findings in the tenosynovium of patients with CTS are the result of shearing forces between tendons, which exceed the elastic limit of the SSCT. We further hypothesize that the resulting thickening of the SSCT alters its gliding and nutritional function, and thereby affects the median nerve, resulting in the characteristic findings of carpal tunnel syndrome. Such shearing injuries could be the result of marked or repetitive differential motion of adjacent digits, and may support the hypothesis of a traumatic etiology for carpal tunnel syndrome. We plan to test these hypotheses in future studies.

If these hypotheses are confirmed, then new treatment options for carpal tunnel syndrome may appear. Empiric experience to date suggests that synovectomy does not improve the outcome of carpal tunnel release, but if the changes which we observe in the SSCT could be detected earlier in the course of carpal tunnel syndrome, then perhaps an intervention might be considered which could avoid the need for later surgery. Possibilities include injection of drugs or cytokines which might reverse or inhibit SSCT fibrosis, or therapy modalities. These observations also help explain the improvement reported by some for synovectomy without release of the flexor retinaculum.

In summary, we have observed changes in the subsynovial connective tissue of patients with carpal tunnel syndrome which suggest the possibility of injury to the subsynovial connective tissue as an etiological factor in carpal tunnel syndrome. Such findings, if confirmed, could support a trauma or repetitive trauma etiology for carpal tunnel syndrome, and may open new treatment possibilities for this common condition.

Acknowledgement

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CHAPTER 3

Motion Characteristics of the SSCT

3.1

Gliding Characteristics of Flexor Tendon and Tenosynovium in Carpal Tunnel Syndrome (a Pilot Study)

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Clinical Anatomy in press

Abstract

Purpose The characteristic pathological finding in carpal tunnel syndrome (CTS) is non-inflammatory fibrosis of the synovium. How this fibrosis might affect tendon function, if at all, is unknown. The subsynovial connective tissue (SSCT) lies between the flexor tendons and the visceral synovium (VS) of the ulnar tenosynovial bursa. Fibrosis of the SSCT may well affect its gliding characteristics. To investigate this possibility, the relative motion of the flexor tendon and VS was observed during finger flexion in patients undergoing carpal tunnel surgery, and for comparison in hands without carpal tunnel syndrome, in an in vitro cadaver model.
Methods We used a camera to document the gliding motion of the middle finger flexor digitorum superficialis (FDS III) tendon and SSCT in 3 patients with CTS during carpal tunnel release and compared this with simulated active flexion in 3 cadavers with no antemortem history of CTS. The data was digitized with the use of Analyze Software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN).
Results In the CTS patients, the SSCT moved en bloc with the tendon, whereas, in the controls the SSCT moved smoothly and separately from the tendon. The ratio of VS to tendon motion was higher for the patients than in the cadaver controls.
Conclusion These findings suggest that in patients with CTS the synovial fibrosis has altered the gliding characteristics of the SSCT**.** The alterations in the gliding characteristics of the SSCT may affect the ability of the tendons in the carpal tunnel to glide independently from each other, or from the nearby median nerve. These abnormal tendon mechanics may play a role in the etiology of carpal tunnel syndrome.

Introduction

Carpal tunnel syndrome (CTS), a compression neuropathy of the median nerve, is the most commonly reported nerve compression syndrome, affecting as much as 5% of the adult population in some studies[1, 2]. Despite its ubiquity, however, in most cases the etiology of carpal tunnel syndrome remains idiopathic.

Within the carpal tunnel are the flexor digitorum profundus (FDP) and superficialis (FDS) tendons to each finger; the flexor pollicis longus (FPL) tendon, the tenosynovium, two bursae, the radial one for the FPL and the ulnar one for the other tendons; and the median nerve. The subsynovial connective tissue (SSCT) lies between the flexor tendons and the visceral synovium of the ulnar tenosynovial bursa[3]. The SSCT is an anatomic feature which is unique to the tendons in the carpal tunnel (Figs. 1A and 1B).

The intrasynovial tendons of the hands and feet have parietal (PS) and visceral synovial (VS) sheets that form a closed space containing synovial fluid for lubrication. In extrasynovial tendons, such as the Achilles tendon, there is a peritendinous sheet of paratenon, composed of loose fibrillar tissue, which functions as an elastic sleeve, permitting free movement of the tendon against the surrounding tissue[4]. Guimberteau has stated [3], but without any peer-reviewed observations, that the structure of the

flexor tenosynovial organization within the carpal tunnel is a hybrid of these two mechanisms, involving both paratenon (the SSCT) and synovial mechanisms (Fig. 2).

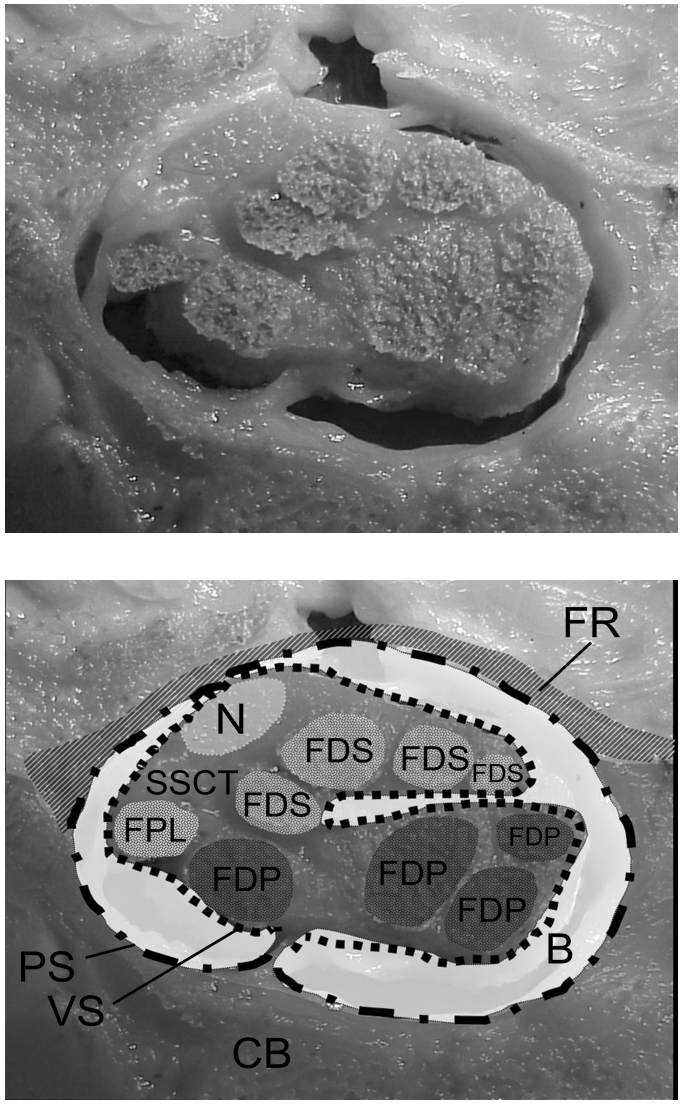

Figure1. 1A. Transverse cut section through a human carpal tunnel at the hamate level. **1B.** Scematic overview of figure 1A showing the structures within the carpal tunnel. The flexor retinaculum (FR) and carpal bones (CB) surrounding the carpal tunnel and within the carpal tunnel are the median nerve (N), the flexor pollicis longus (FPL) tendon, the flexor digitorum profundus (FDP) and superficialis (FDS) tendons, the bursa (B), the SSCT, the parietal (PS) and the visceral synovial (VS) layer.

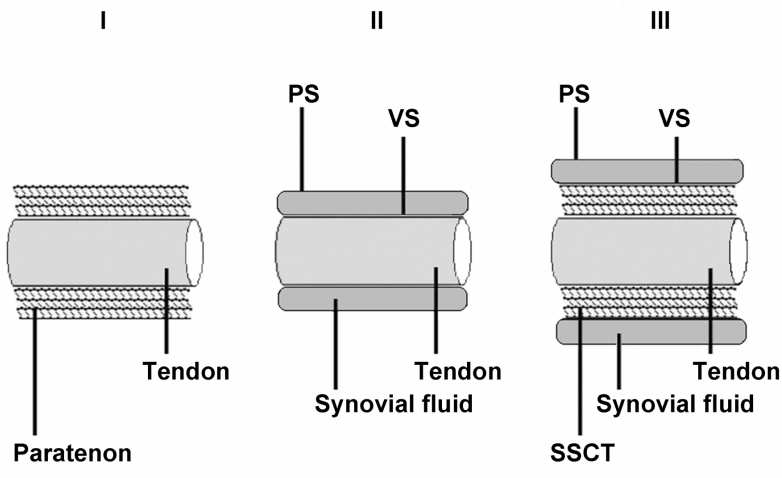

Figure 2. (I) Extrasynovial tendon with the paratenon. (II) Intrasynovial tendon with parietal synovium (PS) and visceral synovium (VS) sheets. (III) Flexor tendon in the carpal tunnel with SSCT and synovial fluid in the bursa.

The underlying disease mechanism for CTS is increased carpal tunnel pressure[5-11].

The carpal tunnel pressure can be increased, as a result of either a reduction in the size of the space in the carpal tunnel or an increase in the volume of its contents. The latter is thought to be a main factor as the most common pathological finding in CTS is non-inflammatory fibrosis and thickening of the synovium[12-17]. Any condition that increases the volume of the contents of the carpal tunnel tends to compress the median nerve[13]. Although there are many diseases that are associated with carpal tunnel syndrome, in most cases the etiology is idiopathic.

Similar fibrotic changes in the tenosynovium are also noted in such conditions as de Quervain's syndrome[18-20], trigger finger[21], lateral epicondylitis[22, 23], and tibialis posterior tendon dysfunction[24].

How this fibrosis might affect tendon function, if at all, is unknown. In the carpal tunnel, such changes may affect nerve function, as the median nerve is often found to be tethered to the thickened SSCT in patients operated on for carpal tunnel syndrome[25-30].

In this observational study we wished to obtain pilot/proof of concept data, specifically that the relative motion of tendon and synovium can be clearly measured,and that there are measurable differences in this relative motion between individuals affected with carpal tunnel syndrome, and those without sufficient to justify a larger clinical study. We also wished to determine whether cadaver controls, which

are ethically advantageous over but not identical to living age matched controls, would be different enough from the patients to be acceptable as controls in a larger study.

Materials and Methods

We monitored the active tendon and VS gliding motion in 3 patients with carpal tunnel syndrome during carpal tunnel release surgery and compared this with the corresponding simulated active tendon and VS motion in 3 cadaver controls. The motion of the middle superficial flexor tendon (FDS III) and its SSCT in the carpal tunnel, as compared to a reference point, the flexor retinaculum, was examined during finger movement with the wrist in neutral position and in neutral alignment. The flexion movement monitored was from 0° extension position to maximum individual flexion position.

The middle finger superficialis tendon was measured because it has the longest excursion of the finger flexor tendons; it is the most palmar tendon and thus moves most directly against the carpal flexor retinaculum during finger or wrist motion; it is adjacent to the median nerve within the carpal tunnel; and finally, because it is not encumbered by lumbrical muscle attachment or a common muscle belly with other tendons.

This protocol was approved by our Institutional Review.

Patient selection and preparation during surgery

We selected 3 patients scheduled for open carpal tunnel release for monitoring of gliding motion. The medical records were examined to obtain demographic data such as age, gender, hand dominance, side of involvement and relevant medical history of carpal tunnel syndrome-associated conditions. The surgery was preformed under local anesthesia, with an open surgical incision extending from 2 cm proximal to the wrist crease to the mid-palm. After the flexor retinaculum was transected, the carpal tunnel was exposed by a self-retaining Weitlander retractor. A small window (approximately 3mm diameter) was made in the visceral synovium and subsynovial connective tissue to expose the middle finger FDS tendon. With the wrist in neutral position and the fingers passively extended to 0°, a mark was made on the middle finger FDS tendon surface with a surgical marker (Skin Markers, Devon Industries, Inc, Buffalo, New York). The visceral synovium surface was marked at a level 5mm proximal to the tendon mark. A third mark was made on the cut edge of the flexor retinaculum (parietal synovium) to serve as a reference point (Fig. 3).

The wrist was supported on the operating table in neutral position for testing. The patients were then asked to make a fist, while a video camera (Sony Digital 8® Camcorder DCR-TRV350, Sony Corporation, Japan) recorded the motion (Fig. 4). The camera was set up perpendicular to the operating table, using a tripod with a spirit level. After the motion was recorded, the carpal tunnel operation proceeded as normally. The experimental portion of the procedure took less than five minutes per patient. A millimeter ruler was included in the camera field, so that the data measured

with the camcorder could be converted into a distance figure. The data was digitized with the use of Analyze Software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN) to determine the motion characteristics of the three marks. Any changes in the x and y axis of the digitized values of the reference point were passed on in the calculations of the other 2 markers.

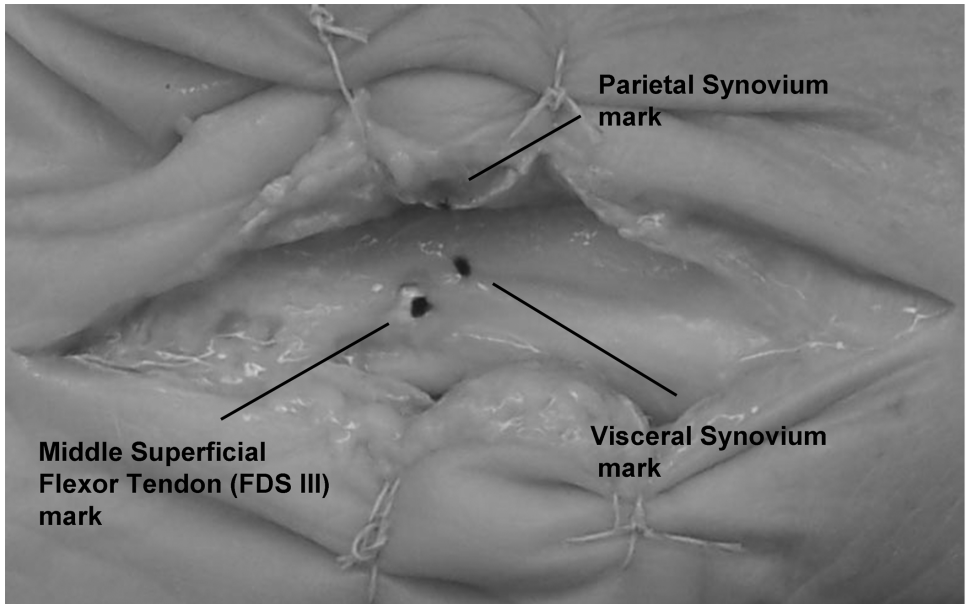

Figure 3. Markers on the middle superficial flexor tendon (FDSIII), visceral synovium and flexor retinaculum (parietal synovium) in a cadaver hand.

Cadaver specimen selection and preparation

Three fresh frozen human cadaver upper extremities, amputated approximately 15 cm proximal to the wrist joint, were thawed at room temperature immediately prior to testing. A medical record review was performed on each cadaver donor, to obtain the same demographic data and to be sure the individual did not have a recorded antemortem diagnosis of CTS.

A longitudinal skin incision approximately 8 cm in length was made and the flexor retinaculum was transected to open the carpal tunnel. The flexor retinaculum and skin were fixed with stay sutures laterally and medially to expose the carpal tunnel.

A window approximately 3mm in diameter was made in the visceral synovium and subsynovial connective tissue to expose the middle superficial flexor tendon FDS tendon. The middle superficial flexor tendon, the visceral synovium and the flexor retinaculum (parietal synovium) were marked with a marker pen similar to the patients during surgery (Fig. 3).

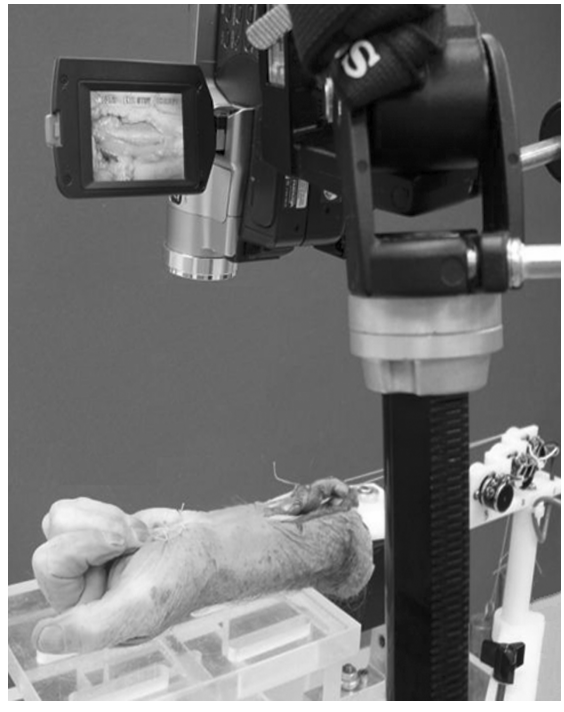

Figure 4. Digital camera set-up with a cadaver hand. The tendons are attached to the pulley with a cord for simulating active motion. Figure 2 is the image of the digital camera.

The specimen was then fixed in a custom-made mounting device, holding the wrist in the neutral position, by clamping the proximal end of the radius and ulna.

The four FDS and four FDP tendons were sutured together at the proximal end of the tendons in the maximum individual flexion position of the fingers and attached to a Dacron cord. The cord controlling the flexor tendons was then actively pulled proximally by one investigator to maximum flexion of the fingers, while the motion of the three markers (from 0º extension to maximum individual flexion) was detected by anteroposterior recording with a digital camcorder (Fig. 4). A millimeter ruler was included in the camera field, so that the data measured with the camcorder could be converted into a distance figure. The data was digitized with the use of Analyze Software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN) to determine the motion characteristics of the three markers. Any changes in the x and y axis of the digitized values of the reference point were passed on in the calculations of the other 2 markers.

After the testing of each cadaver specimen, SSCT biopsies were taken and sent to our Department of Laboratory Medicine and Pathology for routine hematoxylin and eosin histopathology. Light microscopy was used to evaluate the SSCT.

Accuracy of the testing equipment

For motion analysis accuracy testing we applied two marks to a Dacron cord, attached one end of the cord to a pulley with a 200 gram weight and the other end to

an electro-potentiometer. The electro-potentiometer was set up to move the cord 40 mm. We measured the distance between the 2 marks on the cord with a ruler, marked in 1 mm increments to use as a reference for the translation of the distance in the camera pictures.

The excursion of the 2 markers was calculated from the start point and compared with the actual movement given by the actuator. This was repeated 5 times, with the camera in renewed setup positions and also new marks on the string, giving a total of 10 sets of marks for validation testing. With this method we measured a mean excursion of 40.6mm (range 39.5-42.0), with 80% of all repeated measurements within 1mm of each other, and 90% within 2mm.

Statistical Methods

The relationship between the motion of the tendon and motion of the synovium was estimated by the slope of the simple linear regression line through the series of measurements taken for each patient and control subject. Specifically, a regression analysis of the movement of the synovium on the movement of the tendon was done individually for each subject.

The mean slope of the regression lines was calculated for each group (patients or cadavers), and reported with 95% confidence intervals. In addition to reporting these parameter estimates, these results were used to calculate a sample size for future studies. While the focus of the analysis was on parameter estimation, to be complete we also compared the mean slopes between the two groups using a Wilcoxon rank sum test. The analysis was conducted using SAS (SAS Institute Inc., Cary, NC).

All results are reported as mean and 95% confidence interval unless otherwise indicated.

Results

There were two female patients and one male. The females were 61 years and 70 years of age and the male patient was 61 years of age. Both women had their left hand involved, and the man the right hand. All three patients were right hand dominant. The man had idiopathic carpal tunnel syndrome, one woman had hypothyroidism and one woman had diabetes mellitus type I. They all had severe electrodiagnostic test results.

The cadaver controls included two females and one male. The age of death of the female cadavers was 86 years and 97 years of age and that of the male cadaver was 74 years of age. None of the cadavers showed any documented carpal tunnel syndrome or carpal tunnel syndrome associated disease in their history. The synovial biopsies taken after testing of the cadaver controls were normal.

Figure 5 shows the movement of the tendon and the visceral synovium. We observed a relative difference in this motion, when comparing carpal tunnel syndrome and non-carpal tunnel syndrome individuals. In the cadaver specimens, the visceral synovium moved noticeably less in comparison with the tendon motion, than did the visceral synovium in the carpal tunnel patients. This suggests that the visceral synovium is

more tightly tethered to the tendon in the patients than in the cadavers. This could be the result of fibrosis in the SSCT, which is the characteristic histological finding in the SSCT of individuals with carpal tunnel syndrome[12, 31].
Figure 5 shows that the total movement of the VS layer is greater than that of the cadaver controls.
The focus of this study was on parameter estimation, rather than on comparison. But, to be complete we compared the mean slopes of the regression lines from the two groups.

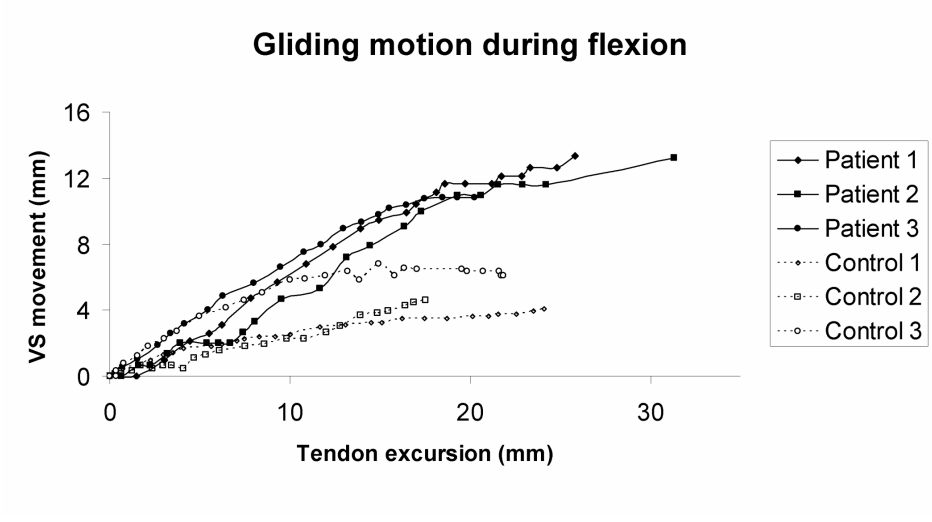

Figure 5. The motion of the FDS III tendon and visceral synovium (VS) during flexion of all the digits in 3 patients and 3 cadaver controls. As the tendon moves, it indirectly pulls the visceral synovium along, mediated by the SSCT. Normally, the SSCT is a filmly layer, so that one should expect that the tendon will move more than the VS,as indeed was observed. All VS curves have a slope less than 1; i.e., the VS moves less than the tendon. In the patients, though, the relative VS motion tends to be more than that seen in the cadavers. This suggests that in the patients, the SSCT is somehow more adherent to tendon and VS than it is in the cadavers.

While we were unable to show a statistically significant difference in the relative SSCT motion between the groups (p=0.10), with such a small sample size, the power to detect differences was low. The mean slope of the regression lines for the patients with carpal tunnel syndrome was 0.56, 95% CI (0.39-0.73), while the control subjects had a mean slope of 0.27, 95% CI (0.05-0.49). The difference in means was 0.29 with a 95% confidence interval of (0.11- 0.47). Based on this data, we estimated that in a future study, a sample of 8 patients and 8 controls would provide 90% power to detect a difference in mean slopes equal to 0.29, which we would consider to be potentially clinically significant. We plan to conduct such a study in the future.

Discussion

The SSCT is an anatomic feature which is unique to the tendons in the carpal tunnel. Based on Guimberteau's initial description [3] and our own observations here, as well as by scanning electron microscopy and histology[12, 31], the gliding mechanism of the flexor tendons in the carpal tunnel region appears to be a hybrid of the intrasynovial and extrasynovial mechanisms.

In accordance with this mechanism, when a tendon within the SSCT moves, the fibrils connected to the tendon are stretched first, followed by fibrils connected to the paratenon layers. In this way, the lengthening propagates layer by layer until finally the visceral synovium (VS) moves (Fig. 6). In this study, since only the tendon was moved, any motion of the visceral synovium must be mediated through the SSCT, which connects the tendon to the visceral synovium. If the tendon, SSCT and visceral synovium were rigidly linked, tendon motion and synovial motion would be simultaneous. If they were completely separated, there would be no visceral synovial motion with tendon motion. Some delay after tendon motion before synovial motion begins suggests some sort of less direct or "differential" link via the SSCT.

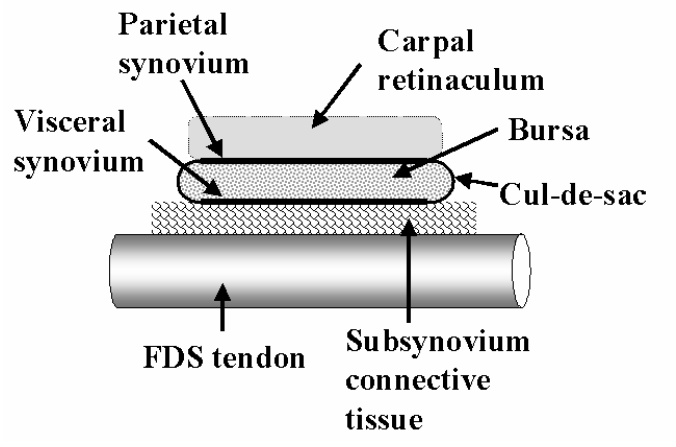

Figure 6. The structure of the sliding unit in the carpal tunnel region.
First the tendon moves, then the subsynovial connective tissue and then the visceral synovium starts to move (this picture was previously published: A Histological and Immunohistochemical Study of the Subsynovial Connective Tissue in Idiopathic Carpal Tunnel Syndrome. AM Ettema, PC. Amadio, C Zhao, LE. Wold, KN An. J. Bone Joint Surg. Am., Jul 2004; 86: 1458 - 1466. Permission to reuse figure granted.)

Two factors theoretically will affect the relative motion between VS and flexor tendon. One factor is the mechanical properties of the SSCT. The other is the gliding resistance (friction) of the VS. In our cadaver specimens, the visceral synovium moved noticeably less, in comparison with the tendon motion, than did the visceral synovium in the carpal tunnel patients. This suggests that the visceral synovium is more tightly thethered to the tendon in the patients than in the cadavers. This could

be the result of fibrosis in the SSCT, which is the characteristic histological finding in the SSCT of individuals with carpal tunnel syndrome[12, 31]. Our patients findings also suggest an increase in the stiffness and friction of the SSCT.

Increased carpal tunnel pressure may come about from either decreased size of the canal; increase in canal contents; decreased compliance of canal contents; abnormal intracanal mechanics, such as tethering, associated with tendon motion, which limits the normal dispersal of canal contents with loading; or alteration in the normal fluid flows which distribute the pressure within the canal. The phenomenon that we have observed could affect all but the first mechanism.

The moving resistance of the VS includes the surface gliding resistance between VS and PS and the mechanical resistance of moving the synovial bursa. The VS will start to move when the force applied to the VS (the force transferred from tendon through the SSCT layer by layer) is greater than the VS moving resistance. As the stiffness of the synovium increases, a greater force will be needed to move the VS, which may lead to earlier muscle fatigue or, if the force is great enough, further damage to the SSCT, establishing a vicious cycle. In the current study, there was a trend for greater VS motion in CTS patients than in controls, which may indicate greater adherence of the SSCT to the tendons in carpal tunnel syndrome patients. Such adherence may limit or increase the tendon force needed for independent, differential tendon motion; could result in tethering of the adjacent median nerve; and could also predispose the SSCT to shearing injury with differential tendon movement. This is a potential mechanism to explain how repetitive use might alter tendon mechanics in the carpal tunnel and predispose an individual to carpal tunnel syndrome.

The limitations in this study lie in the small sample size and the use of cadaver controls. However, we were reluctant to embark upon a larger study without first confirming Guimberteau's observations more precisely, and testing the feasibility of the measurement method. We chose to use cadavers as controls, because of ethical concerns regarding the use of surgery to release the carpal tunnels of healthy volunteers without carpal tunnel syndrome. Previously studies have shown that the carpal tunnel synovial histology in such specimens is normal[12, 31]. We have been unable to identify any studies where others compared the carpal tunnel synovium of living healthy volunteers either to pathological specimens or to cadaver tissue. Histologically our cadavers, although older, had guite normal appearing synovium, easily distinguishable from that of the patients. This has been noted in other studies as well[12, 31]. Unfortunately it is difficult to get case matched cadavers, as the peak ages for death and CTS are roughly 30 years apart. The fact that there were notable differences, even in cadavers which might if anything be expected to be not completely normal, we believe reinforces our initial decision that cadavers could be an acceptable control in this case, and justifies a larger study comparing patients with cadaver controls.

We believe that the basic features of normal SSCT mechanics can be obtained from fresh frozen cadaver specimens. The fact that the histology of our cadaver SSCT was normal, while that in carpal tunnel syndrome is not, also provides evidence that these cadaver specimens can indeed provide adequate control for this

sort of study. In addition, this data will be invaluable in establishing a power estimate for future studies. For example, we have been able to estimate the difference in mean slope between the CTS patients and cadaver specimens. It is clinically relevant in that it is the difference between individuals with known pathology and what one might consider either normal, or perhaps even slightly abnormal, if one considers normal aging to be a pathological process. If this difference is substantiated in future studies, then consideration might be given to development of non-invasive methods, such as ultrasound, to measure tendon and VS motion in vivo. The magnitude of difference in VS and tendon motion above the normal range might be usefull as a diagnostic test for the presence of some SSCT abnormality.

The strength of this study is that it shows for the first time that tendon and synovial gliding may be affected in patients with carpal tunnel syndrome. For the motion analysis we used a relatively simple technique, without damaging the visceral synovium, easily adaptable to open carpal tunnel release, using a digital video camera. In addition, we have provided a power estimate that should be extremely valuable in planning future studies.

These findings suggest that in patients with CTS the synovial fibrosis has altered the gliding characteristics of the SSCT. The alterations in the gliding characteristics of the SSCT may affect the ability of the tendons in the carpal tunnel to glide independently from each other or from the nearby median nerve. These abnormal tendon mechanics may play a role in the etiology of carpal tunnel syndrome. Based on these data, we plan to pursue a larger study to investigate this possibility further, and to define more specifically the gliding characteristics of normal and abnormal SSCT.

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3.2

Flexor Tendon and Synovial Gliding During Simultaneous and Single Digit Flexion in Idiopathic Carpal Tunnel Syndrome.

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Abstract

Background: The characteristic pathological finding in carpal tunnel syndrome (CTS) is non-inflammatory fibrosis of the subsynovial connective tissue (SSCT), which lies between the flexor tendons and the visceral synovium (VS). How this fibrosis might affect tendon function, if at all, is unknown. To better understand the normal function of the SSCT, the relative motion of the middle finger flexor digitorum superficialis (FDS III) tendon and VS was observed during finger flexion in normal cadavers, and compared with that in patients with CTS, and cadavers with a history of CTS.
Methods: A digital camcorder was used to monitor gliding motion of the middle finger flexor digitorum superficialis (FDSIII) tendon and SSCT in 8 patients with idiopathic CTS undergoing carpal tunnel surgery (CTR) and in 8 cadavers with an antemortem history of CTS, and compared these with 8 cadaver controls. Living normal controls were not used because of ethical concerns relating to the need for a surgical exposure to obtain the measurements. The data was digitized with the use of Analyze™ Software.
Results: There were no significant differences noted in total movement. However, the pattern of movement in the CTS patients and cadavers with an antemortem history of CTS, differed from the controls in one of two patterns, reflecting either increased adherence or increased dissociation of the VS and tendon.
Conclusions: In CTS the gliding characteristics of the SSCT are qualitatively altered. These changes may be the result of increased fibrosis within the SSCT, which in some cases has ruptured, resulting in SSCT-tendon dissociation. Similar changes are also identified post mortem in the CTS patient, suggesting to us that the fresh cadaver controls may likewise be similar to normal living controls.
Clinical Relevance: The abnormal kinematics of the SSCT in CTS may be identifiable by non-invasive means. If so, new diagnostic tests for CTS, as well as for SSCT movement patterns which might predict CTS, may be possible.
Keywords: kinematics, human cadaver, tenosynovium, carpal tunnel syndrome, flexor tendon, fibrosis, SSCT.

Introduction

The subsynovial connective tissue (SSCT) is an important and unique structure surrounding the tendons in the carpal tunnel[1-5]. It lies between the median nerve, the flexor tendons and the visceral synovium of the ulnar bursa[5, 6]. Originally described by Guimberteau[5], the SSCT consists of layered bundles of collagen which run parallel to the tendon, and which are interconnected by smaller vertical fibers. By stretching and relaxing the SSCT during finger movement, the loose fibers between adjacent layers are stretched, and the fibrous bundles move layer by layer and are pulled by the interconnections, more or less as an arm would move within layers of sleeves[2]. In this way, the lengthening propagates layer by layer until finally the visceral synovium (VS) moves[2]. When the VS moves with the tendon, an intrasynovial type synovial sliding occurs between the VS and the parietal synovium (PS).

Carpal tunnel syndrome (CTS) is the most common peripheral nerve entrapment syndrome. It is often described as an occupational disease among persons who perform repetitive work with their hands [7-12]. The most commonly reported pathological finding is non-inflammatory fibrosis and thickening of the SSCT[1, 12-17].

The median nerve is often found to be adherent to the thickened SSCT in cases of CTS [18-23] but it is unclear whether these changes in the SSCT are primary or secondary, and it is also unknown whether these changes affect tendon gliding within the carpal tunnel.

To investigate this possibility, the relative motion of the flexor tendon and visceral synovium were observed during carpal tunnel release surgery, and compared to the same observations in cadaver hands from individuals with and without an antemortem history of CTS. Normal living controls were not used, because of ethical concerns regarding the need for surgical exposure, including release of the carpal tunnel, to perform the measurements.

Materials and Methods

We monitored the active gliding motion of the middle superficial flexor tendon (FDS III) and SSCT in 8 patients with CTS undergoing carpal tunnel release surgery (CTR) and compared these with simulated flexion in 8 cadavers with an antemortem history of CTS and in 8 cadaver controls without an antemortem history of CTS.
The motion of the FDS III tendon and its SSCT in the carpal tunnel, as compared to a reference point, the flexor retinaculum, was examined during finger movement with the wrist in neutral position and in neutral alignment. The flexion movement monitored was from 0° extension position to the maximum individual flexion position.

The middle finger superficialis tendon was measured because it has the longest excursion of the finger flexor tendons; it is the most palmar tendon and thus moves most directly against the carpal flexor retinaculum during finger or wrist motion; it is adjacent to the median nerve within the carpal tunnel; and finally, because it is not encumbered by lumbrical muscle attachment or a common muscle belly with other tendons.

This protocol was approved by our Institutional Review Board, and informed consent was obtained from each patient.

Patient selection and preparation during surgery

The medical records of each patient were examined to obtain demographic data such as age, gender, hand dominance, side of involvement, severity of CTS[24] and relevant medical history of carpal tunnel syndrome-associated conditions. Patients with specific etiologies or risk factors of carpal tunnel syndrome, such as diabetes, glucose intolerance, thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendinitis, gout, hemodialysis, obesity, sarcoidosis, amyloidosis, peripheral neuropathy, traumatic injuries to the ipsilateral arm, and wheelchair use, were excluded.

The surgery was performed under local anesthesia without sedation (another exclusion factor, as it would affect cooperation), with an open surgical incision extending from 1 cm proximal to the wrist crease to the mid-palm. After the flexor retinaculum was transected, the carpal tunnel was exposed by a self-retaining Weitlander retractor. A small window (approximately 3mm diameter) was made in the visceral synovium and subsynovial connective tissue to expose the middle finger FDS tendon. With the wrist in neutral position and the fingers passively extended to 0°, a mark was made on the middle finger FDS tendon surface with a surgical marker (Skin Markers, Devon Industries, Inc, Buffalo, New York). The visceral synovium surface was marked at a level 5mm proximal to the tendon mark. A third mark was made on the cut edge of the flexor retinaculum to serve as a reference point (Figure 1). The wrist was supported on the operating table in neutral position for testing. The patients were then asked to make a fist and subsequently to flex and extend the middle finger individually, while a video camera (Sony Digital 8® Camcorder DCR-TRV350, Sony Corporation, Japan) recorded the motion. The camera was set up perpendicular to the operating table, using a tripod with a spirit level. After the motion was recorded, the carpal tunnel operation proceeded as normal.

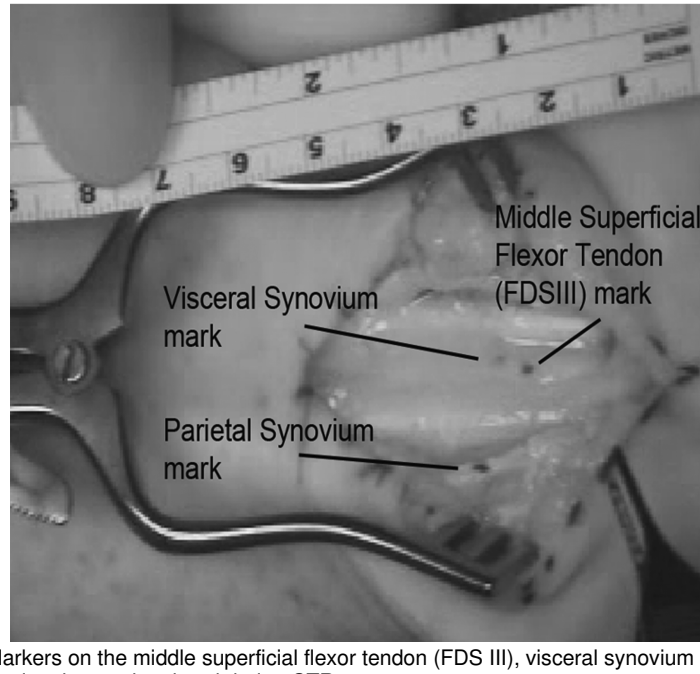

Figure 1. Markers on the middle superficial flexor tendon (FDS III), visceral synovium and parietal synovium in a patient hand during CTR surgery.

The experimental portion of the procedure took less than five minutes per patient. A millimeter ruler was included in the camera field, so that the data measured with the camcorder could be converted into a distance figure. The data was digitized with the use of Analyze™ Software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN) to determine the motion characteristics of the three marks.

Cadaver control specimen selection and preparation

A postmortem medical record review was performed on all donors to our institution's Willed Body Program, to obtain the same demographic data as for the patients described above, and to identify eight individuals with an antemortem diagnosis of CTS (cadaver CTS) and eight individuals of similar age and gender who did not have a recorded antemortem diagnosis of CTS or any carpal tunnel syndrome-associated conditions (cadaver control), such as diabetes, glucose intolerance, thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendinitis, gout, hemodialysis, obesity, sarcoidosis, amyloidosis, peripheral neuropathy, traumatic injuries to the ipsilateral arm, and wheelchair use. The sixteen fresh frozen human cadaver upper extremities, so identified were amputated approximately 15 cm proximal to the wrist joint, and thawed at room temperature immediately prior to testing.

A longitudinal skin incision approximately 8 cm in length was made in the palm and distal forearm and the flexor retinaculum was transected to open the carpal tunnel. The flexor retinaculum and skin were fixed with stay sutures laterally and medially to expose the carpal tunnel.

A window approximately 3mm in diameter was made in the visceral synovium and subsynovial connective tissue to expose the middle finger flexor digitorum superficialis (FDS) tendon. The FDS tendon, the visceral synovium and the flexor retinaculum were each marked with a marker pen similar to the patients undergoing surgery (Figure 1).

The specimen was then fixed in a custom-made mounting device, holding the wrist in the neutral position, by clamping the proximal end of the radius and ulna.

The four FDS and four FDP tendons were sutured together at the proximal end of the tendons in the maximum individual flexion position of the fingers and attached to a Dacron cord (cord A) for simultaneous finger motion simulation. The middle finger FDS (cord B) and FDP tendons (cord C) were also separately sutured with a Dacron cord for differential finger movement testing. In this construct cord B, controlling the FDS tendon, which passed around a pulley, was actively pulled by one of the investigators while cord C, controlling the FDP tendon, passed around a pulley with a 200g weight attached to the proximal end of the cord.

Cord A was then actively pulled proximally by one investigator to maximum flexion of the fingers, while the motion of the three markers (from 0º extension to maximum individual flexion) was detected by anteroposterior recording with a digital camcorder. After cord A was cut and the second, fourth and fifth fingers were extended and fixed in 0° extension position, cord B was then actively pulled proximally by one investigator to maximum flexion of the middle finger FDS tendon, and the motion of the markers again recorded. A millimeter ruler was included in the camera field, so that the data measured with the camcorder could be converted into a distance figure. The data was digitized with the use of Analyze Software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN) to determine the motion characteristics of the three markers.

Statistical Methods

The relationship between the motion of the tendon and motion of the visceral synovium was estimated by the slope of the simple linear regression line through the series of measurements taken for each patient, cadaver CTS and cadaver control specimen, and the movement of the visceral synovium (VS) was regressed on the movement of the tendon in each case.

The slopes were calculated two ways. First, the slopes were calculated using the entire series of measurements of VS and tendon. Second, the presence or absence of an initial delay in the start of movement of the VS as compared to the start of tendon movement was noted for each subject. For subjects with a delay, the length of the delay was calculated and presented as the percentage of tendon movement in which the VS started to move. The slopes were then calculated a second time at the start of movement for each subject. Due to skewed distribution of the slopes, non-parametric tests were used to compare the median slopes of the groups. Kruskal-Wallis or Wilcoxon rank sum tests were used accordingly, and the medians and ranges are reported, unless otherwise noted.

The severity of carpal tunnel syndrome in the 8 patients was recorded as moderate, moderate-severe, or severe. The slopes were compared between severities using a Kruskal-Wallis test.

Because the slopes of the patients was presented either an increase or a decrease in the slopes as compared to the range of the slopes of the cadaver control group, we removed the 5 subjects (2 patients and 3 cadaver CTS hands) with an increased slope to perform a subanalysis on the remaining subjects.

All analyses were conducted using SAS software (SAS institute Inc., Cary, NC).

Results

Patients

There were eight idiopathic carpal tunnel syndrome patients, with four left and four right hands operated on in 5 females and 3 males. There were six right-hand dominant and two left-hand dominant patients. The dominant hand was involved in six patients. The mean age was 55 years (range 34-73). All patients had the diagnosis of carpal tunnel syndrome confirmed by electrodiagnostic testing, which conformed to the standards established by the American Academy of Electrodiagnostic Medicine (AAEM)[25, 26]. There were four patients with moderate, two patients with moderate-severe and two patients with severe electrophysiological changes[24].

Cadaver specimens with an antemortem diagnosis of CTS (cadaver CTS)

Approximately 90 cadavers are received each year by our institution's Willed Body Program. All were patients of our institution, and medical records were available for all specimens. These were reviewed for demographic and medical data, and for evidence of an antemortem diagnosis of carpal tunnel syndrome. Within one year we identified eight fresh frozen cadavers with an antemortem history of CTS (cadaver

CTS). There were three left and five right affected hands from six females and two males with an average age of 77 years (range 58-92). The dominant hand was right in six and left in two of these individuals. In seven cases the affected hand was the dominant one.

There were five cadaver hands with an antemortem diagnosis of idiopathic carpal tunnel syndrome. All of the other three cadavers had a BMI > 30, and one of these also had a history of gout and glucose intolerance. There were five cadaver hands in which a carpal tunnel release had been done; two of these also had a biopsy of synovium taken during surgery and one also had an internal neurolysis. Of the other three hands (which also did not show a scar at the carpal tunnel), one had a history of having been given a single steroid injection and there were two hands in which the diagnosis had been noted by treating physicians but no treatment had been documented. The three non-idiopathic cadaver CTS donors all had had surgery for their CTS and two of these also had had a biopsy of the synovium taken. In each of the cadaver CTS donors who had not had carpal tunnel surgery, there was an

Table 1. The cadavers with an antemortem diagnosis of CTS (cadaver CTS) and their specifics (numbered 1-8) as recorded in their medical charts. These numbers correspond to the numbers given in Figure 3 which show the slopes.

Cadaver CTS	Treatment	EMG	CTS related conditions
1	CTR + biopsy		BMI>30, gout, glucose intollerance
2	CTR + biopsy		BMI>30
3	CTR + neurolysis		BMI>30
4	CTR		idiopathic
5	CTR		idiopathic
6	Steroid injection	Mild	idiopathic
7	No treatment	Mild	idiopathic
8	No treatment	Mild	idiopathic

antemortem electrodiagnostic test which confirmed the diagnosis. The severity was mild in each case. The other five cadaver CTS donors without documented electrophysiological data all had had carpal tunnel surgery (Table 1).

Cadaver control specimens

Eight fresh frozen cadavers were selected as the control group. They did not have an antemortem history of CTS recorded in available medical records, nor evidence of any diseases associated with carpal tunnel syndrome. There were 3 females and 5 males with 6 right and two left hands. Hand dominance was noted only in two right-hand dominant males. The mean age was 86 years (range 78- 98).

Simultaneous digit motion (full extension to fist)

The mean total excursion of the FDSIII tendons which was required for full flexion of the middle finger while making a fist was 26.1 ± 4.9mm for the patients, 25.2 ± 3.4mm for the cadaver CTS hands and 24.4 ± 5.1mm for the cadaver control hands.

The mean total excursion of the visceral synovial layer was 6.9 ± 7.0mm for the patients, 7.8 ± 7.2mm for the cadaver CTS hands and 9.0 ± 4.2mm for the cadaver control hands.
The minimum and maximum movement of the visceral synovial layer as a percentage of the individual FDSIII tendon excursion at the point of full flexion of the middle finger while making a fist was between 1.1%-75.9% for the patients, 7.9%-96.0 % for the cadaver CTS hands and between 24.6%-52.4 % for the cadaver control hands.

We found a median slope of 0.145 (range 0.015- 0.752) in all patients, 0.227 (range 0.076- 0.974) in all cadaver CTS hands and 0.329 (range 0.207- 0.585) in all cadaver control hands (Table 2). These were not significantly different (p=0.24).
In the CTS patients and cadavers with an antemortem history of CTS, however, we found that the displacement of the visceral synovial (VS) layer and surrounding soft tissue was behaving qualitatively differently from the controls (Figure 2 and 3). One of two patterns was noted: adherence of the SSCT to the tendon, so that simultaneous or near simultaneous and synchronous motion occurred between the tendon and visceral synovium (1 patients and 1 cadaver CTS hand), or dissociation (5 patients and 4 cadaver CTS hands), so that the visceral synovial layer showed a decrease in movement and less synchronous effect on VS layer motion (Figure 2 and 3).

The slopes of the 5 patients who exhibited a pattern of dissociation had a significant lower median slope (0.133, range 0.015- 0.149) than the 8 cadaver controls (0.329, range 0.207- 0.585), ($p<0.01$). The 4 cadaver hands with an antemortem CTS history and a pattern of dissociation showed a significantly lower median slope (0.101, range 0.076- 0.158) than the 8 cadaver controls (p=0.018). There was no significant difference between the median slopes of the patients and cadaver CTS hands (p=0.45).

The slopes of the patient (0.752) and the slope of the 1 cadaver CTS hand with an antemortem CTS history (0.974) were observed to be higher than the median of the 8 controls, but due to small numbers, a formal statistical comparison was not performed. The distribution of the slopes for the different groups are shown in Table 2.

Isolated FDS motion

The mean maximum excursion of the FDSIII tendons which was required for full flexion of the middle finger while keeping the other fingers in 0° extension position was 16.9 ± 6.8mm for the patients, 18.6 ± 4.0mm for the cadaver CTS hands and 22.0 ± 4.6mm for the cadaver controls. The mean maximum excursion of the visceral synovial layer was 2.9 ± 2.7mm for the patients, 3.4 ± 5.3mm for the cadaver CTS hands and 3.1 ± 1.6mm for the cadaver control hands during isolated FDSIII motion.

The range of movement of the visceral synovial layer as a percentage of the individual FDSIII tendon excursion at the point of full flexion of the middle finger while keeping the other fingers in 0° extension position was 3.4- 49.6 % for the patients, 2.9- 98.9% for the cadaver CTS hands and 6.7-24.1% for the cadaver control hands (Figure 4).

We found a median slope of 0.122 (range 0.025- 0.503) in all patients, 0.065 (range 0.023- 0.990) in all cadaver CTS hands and 0.120 (range 0.057- 0.233) in all

Table 2. The distributions of the slopes for simultaneous and single digit movement (differential). P values are from the Wilcoxon signed rank test for comparisons between simultaneous and differential finger movement within each groups.

			Slope from simultaneous movement		Slope from differential movement		
		N	Mean (SD)	Median (range)	Mean (SD)	Median (range)	p-value
All		24	0.300 (0.226)	0.253 (0.015-0.974)	0.164 (0.208)	0.106 (0.023-0.990)	<0.01
Patient		8	0.248 (0.243)	0.145 (0.015-0.752)	0.181 (0.168)	0.122 (0.025-0.503)	0.15
Cadaver CTS		8	0.300 (0.295)	0.227 (0.076-0.974)	0.183 (0.328)	0.065 (0.023-0.990)	0.08
Cadaver control		8	0.353 (0.125)	0.329 (0.207-0.585)	0.127 (0.061)	0.120 (0.057-0.233)	0.01
Patient	**decreased slope**	5	0.110 (0.055)	0.133 (0.015-0.149)	0.083 (0.042)	0.078 (0.025-0.136)	0.08
	increased slope	1	0.752 (*)	0.752 (0.752-0.752)	0.379 (*)	0.379 (0.379-0.379)	**
Cadaver CTS	**decreased slope**	4	0.109 (0.381)	0.101 (0.076-0.158)	0.056 (0.039)	0.048 (0.023-0.107)	0.27
	increased slope	1	0.974 (*)	0.974 (0.974-0.974)	0.990 (*)	0.990 (0.990-0.990)	**

* Not applicable

** N too small to compare

cadaver control hands. There was no significant difference between these groups (p=0.31). The distribution of the slopes for the different groups is shown in Table 2.

With isolated FDS movement there was no significant difference found between the 5 patients with decreased slope for simultaneous motion, the 4 cadaver CTS hands who had a decreased slope for simultaneous motion, and the 8 cadaver control hands (p=0.12).

Simultaneous versus single digit flexion

We compared the motion of the SSCT as a percentage of the FDS tendon of the middle finger between making a fist and isolated flexion of the middle finger. For moving the fingers simultaneously we found a median slope of 0.253 (range 0.015-0.0974) and for moving the middle finger only there was a median slope of 0.106 (range 0.023-0.990). Comparison between simultaneous and single digit motion with the inclusion of all the patients, cadaver CTS hands and cadaver control hands (N=24) showed a statistical significant difference of the slopes ($p< 0.01$). The mean, standard deviation, median, and range of the slopes with the p values for the Wilcoxon signed rank test of comparison between simultaneous and single digit motion within the different groups are presented in the Table 2.

The slopes of the patients and the cadaver CTS hands were not observed to be significantly different between simultaneous and single digit motion. It is interesting to note that while the patients and cadaver CTS hands had smaller slopes than the controls in simultaneous motion (although not significantly so), their slopes were larger (although again not significantly so) during isolated motion.

Severity of CTS and motion

The median slope during simultaneous finger movement was 0.126 (range 0.015-0.218) for the four patients with moderate electrophysiological changes, 0.450 (range 0.149-0.752) for the two patients with moderate-severe changes, and 0.302 (range 0.133-0.470) for the two patients with severe changes. There was no statistically significant difference between the slopes during simultaneous finger motion. (p=0.29).

Delay until movement

Five patients (63%) and three cadaver CTS hands (37%) showed a pronounced initial delay of movement of the VS. One cadaver control hand also showed this initial delay. In the five patients and three cadaver CTS hands the tendon would move 5.9 $\pm$ 5.2 mm (23% of total tendon movement) before the VS would start to move. In the control the delay was 1.5 mm (7% of total tendon movement). All other patients and cadavers did not show a delay and the tendon and VS started to move at the same time.

Discussion

There has recently been increased attention paid to the structure and potential function of the SSCT[1, 2, 5]. The histopathology of this tissue in patients with CTS is well

documented[1, 12-16]. Its role as a potential etiology of CTS has been discussed[16]. Here we have investigated the kinematics of the SSCT in patients with carpal tunnel syndrome, and compared it to that to cadavers with a history of carpal tunnel syndrome, and cadaver controls.

In our study we compared the motion of markers on the visceral synovium (VS) and tendon, and inferred from the in differences the behavior of the intervening SSCT.

While no statistically significant differences in SSCT motion were noted, we observed three qualitatively different patterns of SSCT motion in the 24 carpal tunnels we studied.

In one of the patients and in one of the cadaver CTS hands, the SSCT moved almost simultaneously with the tendon, suggesting that the SSCT was adherent to the tendon.

This is consistent with the SSCT fibrosis which is commonly seen in patients with idiopathic CTS[1, 12-17]. While our observation allows only speculation as to the etiology, a partial rupture of the SSCT, such as might occur with adjacent fingers moving in opposite directions, stretching the SSCT beyond its elastic limit, healing with subsequent fibrosis, might produce such a picture. In five of the patients and three of the cadaver CTS hands, there was a distinct lag in the initiation of SSCT motion after tendon motion began, suggestive of a physical dissociation of the SSCT and tendon. We believe that this may represent the rupture of the normal tendon-SSCT connections, consistent with a possible traumatic event, such as might occur with adjacent fingers moving in opposite directions, stretching the SSCT to failure. In two of the CTS patients and three of the CTS cadaver hands, the pattern of SSCT motion was similar to that seen in the normal hands.

If the stiffness of the SSCT increases, a greater force will be needed to move the VS, which may lead to earlier muscle fatigue or, if the force is great enough, further damage to the SSCT, establishing a vicious cycle. In the current study, there was a trend for greater VS motion in CTS patients than in controls, which may indicate greater adherence of the SSCT to the tendons in carpal tunnel syndrome patients. Such adherence may limit or increase the tendon force needed for independent, differential tendon motion; could result in tethering of the adjacent median nerve; and could also predispose the SSCT to shearing injury with differential tendon movement. We did not find any correlation between clinical severity of the CTS and changes seen in the gliding motion of the SSCT. Power analysis showed that we would need to monitor fifty patients with varying severity of CTS in order to show a correlation with the pattern of changes in gliding motion of the SSCT compared to the tendon. Our findings do, though, suggest that patients with carpal tunnel syndrome have changes in the functioning of the SSCT which could well explain the known pathophysiology of this disorder. If fibrosis of the SSCT does indeed result in adherence between the SSCT and the underlying tendons, then the normal delayed recruitment of SSCT motion will not occur, and this in turn may predispose to shear injury of the SSCT, further adherence, further shear, until either the tendon becomes totally fixed to the SSCT or pulls completely free. These are the two scenarios observed in our CTS patients. The resulting SSCT fibrosis could also be expected to

disrupt fluid flows [27-29] and raise hydrostatic pressure in the carpal canal[30-35], consistent again with observations of the fine microvasculature of the SSCT in CTS patients[1, 3]. Our observations that isolated motion of the FDS have a greater effect on SSCT motion than does group tendon action suggest that such motions may particularly predispose to SSCT shear injury, and indeed there is other recent evidence to support this concept[36].

We believe that the strength of this paper lies in the detailed information provided on the motion of the SSCT in patients with carpal tunnel syndrome. This abnormal motion may be an early marker for carpal tunnel syndrome, and, we believe, warrants further study, as it may prove helpful in both the diagnosis and possibly even treatment of CTS.

The limitations in this study lie in the lack of normal living controls (discussed above), and the relatively small sample size. However, we were reluctant to embark upon a larger study without first testing the feasibility of the measurement method. The fact that we included 3 cadaver CTS hands with non-idiopathic CTS, and five which had had surgery, might also have skewed the results of this study. We used these specimens simply to see if the findings in cadaver hands with a history of CTS were similar to those in patients with CTS, and different from those in normal cadaver hands, since we believe strongly that the opening of carpal tunnels in normal volunteers would be unethical. The fact that the postoperative findings in the cadaver CTS hands were similar to the intraoperative findings in the patients suggests to us that the changes seen in the SSCT gliding are irreversible.

In summary, we have analyzed the relative movement of the visceral synovium and underlying tendon in hands with and without a diagnosis of CTS, and inferred from those observations the behavior of the intervening SSCT. While the differences in motion did not reach statistical significance, qualitative differences in motion in the hands affected with CTS suggest that the SSCT fibrosis known to exist in patients with idiopathic CTS may affect tendon mechanics, and increase the work of flexion in the hands of individuals with CTS. Such changes could create a vicious cycle of repetitive injury, resulting in progressively more fibrosis, until complete rupture of the SSCT occurs. We plan further studies to investigate these phenomena in our patients with CTS, and plan to develop an animal model of SSCT fibrosis to study the evolution of these phenomena more closely.

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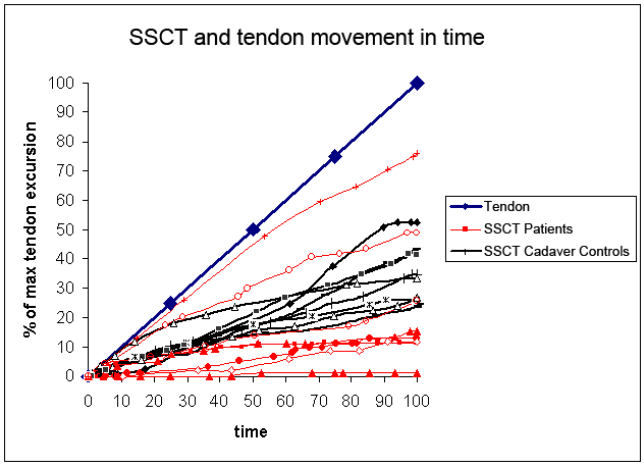
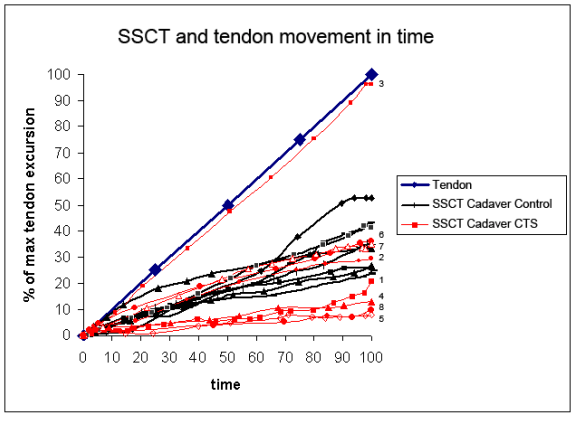


Figure 2. Movement of the tendon (blue) and SSCT of the 8 patients (red) versus the 8 cadaver controls (black) as a percentage of each maximum tendon movement. Six out of the eight patients show a decreased displacement as compared to the controls. In two patients the SSCT moves en bloc with the tendon.

Figure 3. Movement of the tendon (blue) and SSCT of the 8 cadaver CTS hands (red) versus the 8 cadaver control hands (black) as a percentage of each maximum tendon movement. Four out of the eight cadaver CTS hands show a decreased displacement as compared to the controls and one moved synchrone with the tendon. Three cadaver patients moved similar to the controls. Number 1-8 represent each cadaver patient as described in Table 1.



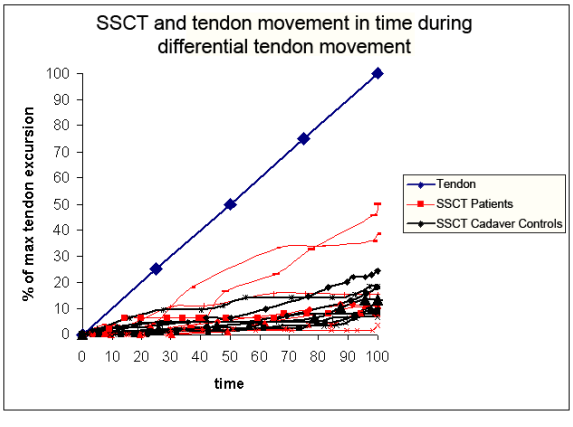


Figure 4. Movement of the tendon (blue) and SSCT of 8 patients and 8 controls during single digit (differential) motion as a percentage of each maximum tendon movement.

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3.3

High-Resolution Ultrasound Analysis of Subsynovial Connective Tissue in Human Cadaver Carpal Tunnel

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Abstract

Background: The carpal tunnel contains the median nerve, nine flexor tendons, two synovial bursae, and peritendinous subsynovial connective tissue (SSCT). Fibrosis of the SSCT is the most consistent pathologic finding in patients with carpal tunnel syndrome. We investigated the anatomy and gliding characteristics of the flexor digitorum superficialis tendon and its adjacent SSCT with high-resolution ultrasound (15MHz). Our hypothesis was that tendon and SSCT are distinguishable by ultrasound and that their velocities during tendon excursion are different.
Methods: Qualitative ultrasound analysis of a flexor tendon and its SSCT was performed on five cadaver wrists and correlated to respective findings after anatomic study of the same cadavers. Quantitative Doppler velocity analysis of eight cadaver wrists was done to asses the sliding movement of the tendon and its SSCT within the carpal tunnel.
Results: There was no significant difference between the thickness of SSCT measured by ultrasound and that measured directly after dissection. The SSCT moves slower than its flexor tendon. The SSCT velocities were statistically different from the tendon velocities (T-test, $p>0.001$).
Conclusions: High-resolution ultrasound is a very precise method to display the anatomy of the tendon and SSCT within the carpal tunnel and it is possible to detect their different velocities with Doppler.
Clinical Relevance: Non-invasive assessment of the thickness and velocity of the tenosynovium in carpal tunnel syndrome by high-resolution sonography might potentially be a new diagnostic tool for disorders affecting the SSCT, especially carpal tunnel syndrome.

Introduction

The use of diagnostic ultrasonography has greatly enhanced our ability to diagnose injuries of tendons and tendon sheaths that were previously either unrecognized or poorly understood. The usefulness of ultrasonography in monitoring carpal tunnel syndrome has also been investigated by many authors [1-7].

Carpal tunnel syndrome (CTS) is the most common form of peripheral nerve entrapment neuropathy and is caused by compression of the median nerve in the carpal tunnel. Within the carpal tunnel, there are the flexor digitorum profundus (FDP) and superficialis (FDS) tendons to each finger; the flexor pollicis longus (FPL) tendon, the tenosynovium of these tendons, two bursae, and the median nerve. The subsynovial connective tissue (SSCT) lies between the flexor tendons and the visceral synovium of the ulnar tenosynovial bursa. The characteristic pathological finding in CTS is non-inflammatory fibrosis and thickening of the SSCT. As the size and shape of the tunnel is uncommonly altered, it is assumed that the increased pressure results from some change in the volume or material properties of the contents of the carpal tunnel [8, 9], of which the most likely is the subsynovial connective

tissue (SSCT), which is known to be fibrotic in patients with carpal tunnel syndrome [8, 10-12].

Ultrasound imaging has been described to detect pathologies such as thickening of the flexor tendons [13] and transverse carpal ligament[14], shape and echogenicity alterations, restricted median nerve sliding in the carpal tunnel [15, 16], synovial proliferation, soft tissue infection and joint effusion, tissue calcification and tumors [17], persistent median artery [18], tendinous and ligamentous injuries and swelling of the median nerve in the proximal part of the carpal tunnel, and flattening of the median nerve in the distal part of the carpal tunnel [1, 14, 19-21].

High-resolution ultrasonography with ≈7 MHz transducers has been shown suitable for the assessment of dynamic changes in the carpal tunnel. Ultrasonography is comparable in image quality to magnetic resonance imaging and computed tomography [22]. The utility of ultrasonography in the evaluation of carpal tunnel syndrome is favorably compared to electromyography [23].

Color Doppler imaging systems are used mainly for blood flow measurements, but have also been used to assess tendon velocity and excursion in the hand and wrist region [24-26]. Thus, ultrasound offers the possibility of investigating synovial structure and function non-invasively. This offers an intriguing possibility for patients with CTS, as some investigators [8,9,11] have suggested that fibrosis of the SSCT may be a cause, and not merely an effect, of carpal tunnel syndrome. If this were so, then a method to detect changes in SSCT morphology or function might be a useful adjunct to the ultrasonic evaluation of patients with CTS. For example, changes in SSCT morphology or function could be correlated with the known ultrasonographic changes in median nerve morphology, such as nerve enlargement and/or flattening, seen in patients with carpal tunnel syndrome.

The aim of this study was two-fold. Firstly, we explored the ability of high-resolution grayscale ultrasound to visualize the SSCT in relation to the middle finger FDS tendon, to qualitatively characterize local anatomy (including carpal ligament, median nerve, SSCT and tendon), and to analyze the SSCT thickness. The middle finger FDS tendon was chosen as representative because it is the most superficial of the flexor tendons in the carpal tunnel, and therefore most accessible to ultrasound; it moves most directly against the carpal flexor retinaculum during finger or wrist motion; it is adjacent to the median nerve within the carpal tunnel; and finally, because it is not encumbered by lumbrical muscle attachment or a common muscle belly with other tendons. Secondly, we quantitatively analyzed SSCT motion with Doppler ultrasound by measuring its peak flexion and extension velocity in relation to the middle finger FDS tendon velocity. All grayscale and Doppler ultrasound scans were done on cadaver hands. Our hypothesis was that the SSCT can be qualitatively and quantitatively distinguishable by ultrasound from the middle finger FDS tendon.

Methods

The use of human cadavers was approved by our Institutional Review Board.

Cadaver selection and preparation

Ten upper extremities of 9 fresh frozen human cadavers, amputated approximately 15 cm proximal to the wrist joint, were thawed at room temperature immediately prior to testing.

A medical record review was performed on all cadaver donors before testing, to be sure that all individuals met the same exclusion criteria, and that the individuals did not have a reported antemortem diagnosis of carpal tunnel syndrome. Exclusion criteria included a history of diabetes, glucose intolerance, thyroid disease, rheumatoid arthritis, osteoarthrosis, degenerative joint disease, flexor tendonitis, gout, hemodialysis, BMI>30, sarcoidosis, peripheral nerve disease, amyloidosis or traumatic injuries to the ipsilateral arm.

We used 2 cadaver hands to identify the anatomy of the different structural layers in the carpal tunnel (i.e. carpal ligament, ulnar bursa, SSCT and superficial flexor tendon) and also to optimize our methods for recording the velocity of the different layers within the carpal tunnel by using Doppler ultrasound. Then, we tested these methods on 8 cadaver hands without carpal tunnel syndrome.

The specimens were fixed in a custom-made mounting device, holding the wrist in the neutral position, by clamping the proximal end of the radius and ulna and with support to the dorsum of the hand. The middle finger FDS and FDP tendons were attached to a Dacron cord at the proximal end of the tendons. A 200-mg weight was attached to both cords controlling the middle finger flexor tendons. Both the cords passed around a pulley which contained an electro-potentiometer connected to a computer, for measuring the excursion (and time) of the flexor tendons during testing. The motion of the middle finger superficial flexor tendon (FDS III) and its SSCT in the carpal tunnel was examined during finger movement with the wrist in neutral position. Motion within the carpal tunnel was induced by one investigator moving the second, third, fourth and fifth fingers of the cadaver hand together from neutral extension position to approximately 90 degrees flexion of the MCP joints.

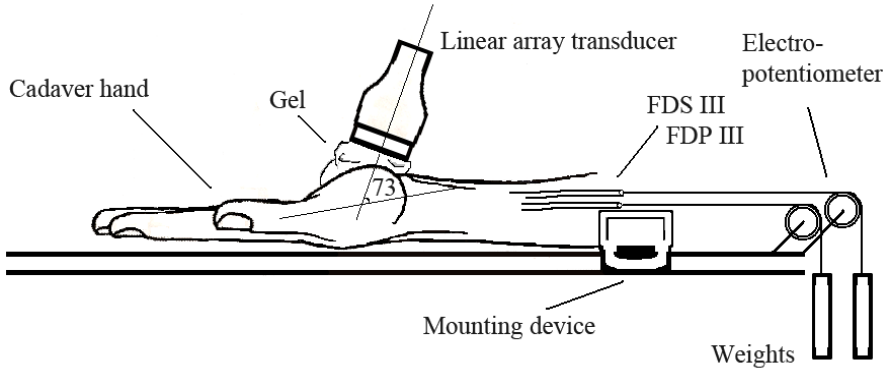

Figure 1. Schematic of the scan apparatus.

Ultrasound imaging system and experimental setting

We performed this study using the Acuson Sequoia 512® ultrasound system (Acuson Sequoia 512®, Siemens Medical Solutions, Malvern, PA, USA), equipped with the 15L8 linear array transducer set to depth of 5 mm, and 15-MHz acquisition frequency for anatomical imaging and 8-MHz frequency during Doppler measurements. Doppler gain was typically 12 dB, dynamic range 68 dB. Velocity range was set to avoid aliasing. The transducer was manually placed on the palmar wrist surface of the cadaver hand, with the wrist in neutral anatomic position. A bulk of transmission gel between transducer and wrist surface assured acoustic coupling. Scans were set to optimal depth, focus, and pulse repetition frequency (PRF). To minimize compression of the SSCT and thus its motion, the scan head was applied to the skin without additional pressure (Figure 1).

For analysis of local anatomy and assessment of SSCT thickness, we obtained longitudinal ultrasonograms of the middle finger superficial flexor tendon and the SSCT at three different anatomic levels; at the wrist crease (proximal tunnel); at the hook of the hamate (mid-tunnel); and at the distal edge of the flexor retinaculum (distal carpal tunnel).

For motion analysis we obtained longitudinal ultrasonograms of the middle finger superficial flexor tendon and the SSCT at the wrist crease level (proximal tunnel). This location avoided the undesirable physical contact of the flexing cadaver fingers with the transducer and allowed us to better control the angle between the ultrasound beam and the structures of interest.

Proper positioning of the transducer was assured by identifying specific anatomical structures as follows. While flexing and extending the middle finger, we first detected the middle superficial flexor tendon; because tendons are fibrillar in morphology, we recognized the flexor tendon tendon as a moving structure with a multitude of parallel striations. Then, more palmarly, the surrounding soft tissue and the transverse ligament were identified as non-moving structures. The SSCT appeared as a thin, typically low-echogenicity layer located between the flexor tendon and transverse ligament.

Localization of SSCT and measurements of its thickness by ultrasound

The localization of the SSCT was analyzed in 2 cadaver wrists with conventional grayscale ultrasound. A needle was inserted into the SSCT under ultrasound guidance. Then we dissected the specimen and opened the carpal tunnel to verify that the target structure was indeed the SSCT.

For measuring the SSCT thickness we used 5 cadaver wrists. The thickness was measured by placing two digital calipers on both the edges of the displayed SSCT. The ultrasound machine then calculated the distance between these two calipers. The measurements were obtained by two investigators independently, five times at each level.

After the examination with ultrasound, the 5 cadaver wrists were frozen (-80°C). The wrists were then transversely cut at the 3 testing levels (i.e., wrist crease, hamate, and distal edge) and digitally photographed after thawing of the slices. A millimeter ruler was included for calibration. Analyze™ Software (Biomedical Imaging

Resource, Mayo Clinic, Rochester, MN) was used to determine the thickness of the SSCT; the mean of 10 thickness measurements was obtained for each level.

Measurements of SSCT motion by ultrasound and reference electro-potentiometer system

The cadaver fingers were flexed and extended manually to achieve continuous motion of the middle finger FDS tendon. To minimize the subjectivity of the continuous manually-driven motion, we asked two individuals for assistance and blinded them from all data acquisition. Although different absolute velocities of finger motion were generated in this way and the velocities were not perfectly constant, the purpose of the study was the comparison of the velocity the SSCT with respect to that of the tendon rather than an analysis of absolute velocities.

Excursions of tendon motion were measured with the electro-potentiometer simultaneously with Doppler data acquisition by ultrasound. An event marker (electrical spike) was used to delimit, in the ultrasound machine and the electro-potentiometer system, an interval from the beginning to the end of a randomly selected series of flexions and extensions. The marked interval typically lasted for 10 to 12 flexions/extension cycles. During this interval, acquisition of velocities started by placing a Doppler gate (ie, Doppler sampling window; approximately 1 mm long) at the SSCT level. Then, after 5-6 flexion/extension cycles, the sample was moved along the scan line onto the tendon. In this way, the same Doppler angle was maintained, and a similar number of flexion/extension cycles for the two structures was obtained. For the purposes of this analysis, we assumed that the tendon velocity was relatively constant between runs, and that the Doppler shift of SSCT and tendon are similar for similar velocities, even though these tissues may differ in anisotropy. Doppler velocity spectra corresponding to the tendon and SSCT were then obtained from 3 randomly selected flexions and extensions. Motion analysis was done directly with the ultrasound system: Doppler velocity spectra were interactively outlined and the machine calculated the peak velocity, excursion (by integrating the velocities), and duration of the movement.

At this point, we used tendon excursions measured with the electro-potentiometer as the reference and adjusted the Doppler angle cursor until the values of the Doppler-measured excursions matched the reference. We used this indirect method in which excursions of the tendon measured by the electro-potentiometer were used to calibrate excursions obtained by ultrasound because the angle-correction cursor on the ultrasound system screen was virtually invisible on the complex patterns of an echogenic soft tissue background. In conventional applications of the used cardiac ultrasound system the cursor is placed on a low-echogenicity background of a blood pool.
This corrected angle was then used when measuring peak velocities of the tendon and SSCT.

Statistical analysis

Data are presented as mean ± standard deviation (SD). A two-sided paired t-test was used in all analyses. We compared SSCT thickness by ultrasound to anatomical

measurements. We also compared velocity ratios of the tendon and the corresponding SSCT during both flexion and extension cycles. Differences with p values < 0.05 were considered significant.

Results

SSCT localization

In this phase the ultrasound characteristics of the SSCT were elucidated and compared to the cadaver dissections and correlated with characteristics of normal cadaver wrists. Two fresh frozen cadaver wrists were defrosted for the testing. Figure 2 shows a photograph after dissection of one of the cadaver specimens in which a needle was inserted under ultrasound guidance to mark the SSCT in a cadaver carpal tunnel. After dissection, we verified that the correct structure, i.e., SSCT was visualized by ultrasound and the needle correctly guided.

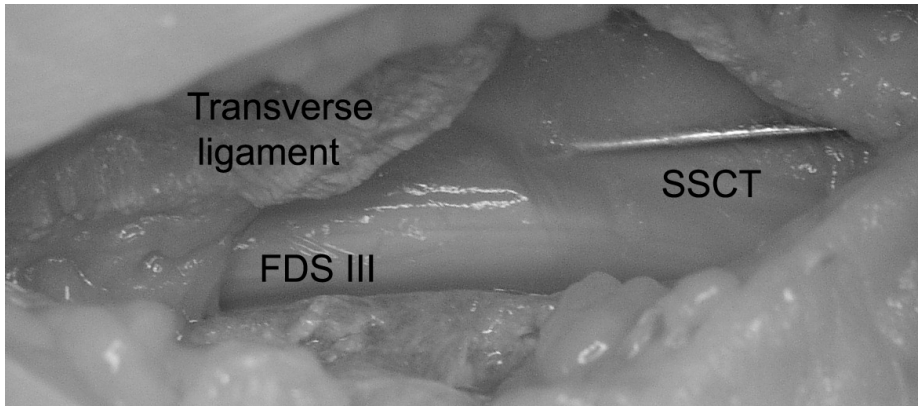

Figure 2. Cadaver carpal tunnel after opening the canal. The needle which is located in the SSCT was placed in the SSCT palmar to the flexor digitorum profundus tendon of the middle finger (FDP III) under ultrasonic guidance.

Figure 3 demonstrates an ultrasound longitudinal view of the carpal tunnel in a cadaveric hand. Different layers are visible in this view as follows. The transverse carpal ligament appears as a gray line and the middle finger flexor tendons generate horizontal striation patterns. Between the superficial flexor tendon and the transverse ligament is the SSCT, the structure of interest. The SSCT appears as a thin layer attached parallel to the FDS III tendon. All structures, and the SSCT with its corresponding tendon in particular, become much more recognizable during motion, as they move with visibly different velocities. The bursae are not visualized in this projection. The external cortical surface of the carpal bones was well visualized at approximately 15 mm depth as a smooth bright reflection.

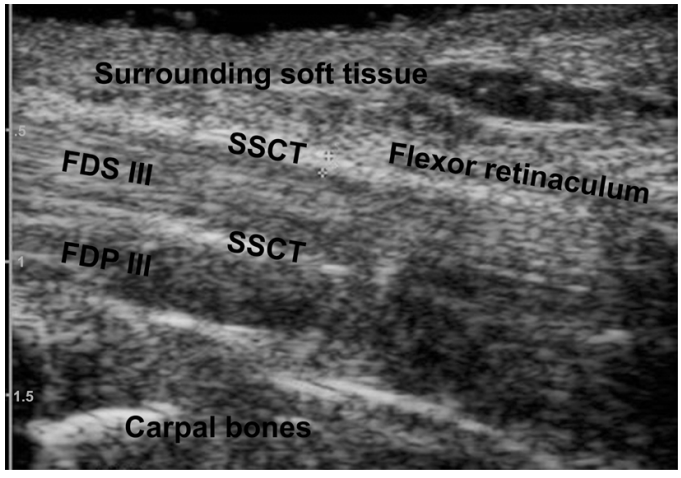

Figure 3. Longitudinal gray scale image of the middle finger flexor digitorum superficialis (FDS III) and flexor digitorum profundus tendon (FDP III) in a cadaver with the palmar side up. The white thin layers parallel to the tendons represent subsynovial connective tissue (SSCT). The 2 markers in the middle of the picture are placed on both the edges of the SSCT of our interest. The scale represents the depth is in cm.

SSCT thickness

For measurements of the SSCT thickness, we used 5 cadaver wrists (3 right and 2 left) from 4 cadavers (2 male, 2 female) with a mean age of death of 75.3 years (range 49-89 years).

The comparison of the thickness of the SSCT measured with ultrasound and after anatomical dissection is shown in Figure 4. At the wrist crease level we found a mean thickness of 0.62 mm (range 0.41-0.85 mm), at the hamate level 0.66 mm (range 0.39-1.08 mm) and at the distal edge this was 0.66 mm (range 0.41-0.89 mm) with ultrasound. After digitizing the transverse anatomical images with the ruler, we

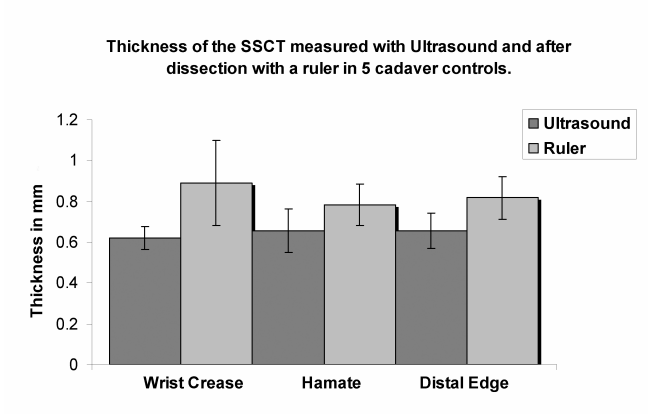

Figure 4. The mean thickness (±SD) of the SSCT measured with ultrasound and after dissection with a ruler in five cadaver controls.

found at the wrist crease level a mean thickness of 0.89 mm (range 0.60-1.12 mm), at the hamate level 0.78 mm (range 0.51-1.02 mm) and at the distal edge this was 0.82 mm (range 0.64-0.96 mm). Although there appeared to be a small trend towards obtaining lower values of thickness with ultrasound, we did not find any statistical difference between the anatomic and ultrasound thickness measurements at these three testing levels (wrist crease $p = 0.12$, hamate $p = 0.06$, distal edge $p = 0.13$). Neither did we find any statistical difference among the ultrasound measurements or among anatomical measurements between each testing level ($p>0.05$). Finally, measurements between the two investigators were not statistically different either ($p=0.49$).

SSCT motion

We used 8 cadaver wrists (4 right and 4 left) from 7 cadavers (3 male, 4 female) with a mean age of death of 72.4 years (range 49-89).

The angle between the transducer and the tendon or SSCT was 73 ± 8.7°. The ratio of peak flexion and extension velocities of the middle finger FDS tendon and the SSCT measured with ultrasound are shown in Figure 5.

The mean velocity of the middle FDS tendon from the 8 cadaver wrists was 14.7 ± 7.5 cm/sec and for the SSCT this was 9.5 ± 5.5 cm/sec during flexion movement. The mean velocity during extension movement for the middle finger FDS tendon was 14.6 ± 7.9 and for the SSCT this was 9.3 ± 5.0 cm/sec.

We found a significant difference in peak velocities for both flexion and extension motion between the tendon and the SSCT ($p=0.007$); the velocity of the SSCT was consistently lower (Figure 6A and 6B).

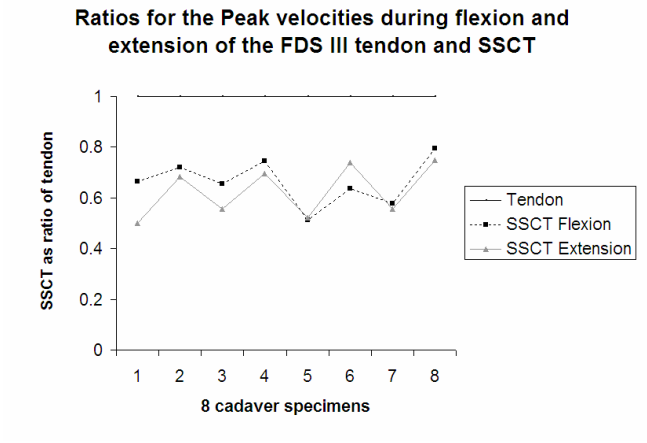

Figure 5. Mean peak velocities of the SSCT as a ratio of the mean peak velocities of the FDS III tendon measured with ultrasound and represented in 8 cadaver specimens (number 1-8) during flexion and extension.

Discussion

To our knowledge, this study demonstrates for the first time that ultrasound can distinguish the SSCT from the middle finger FDS tendon. In addition, we have shown that high-frequency ultrasound not only can measure SSCT thickness but also can

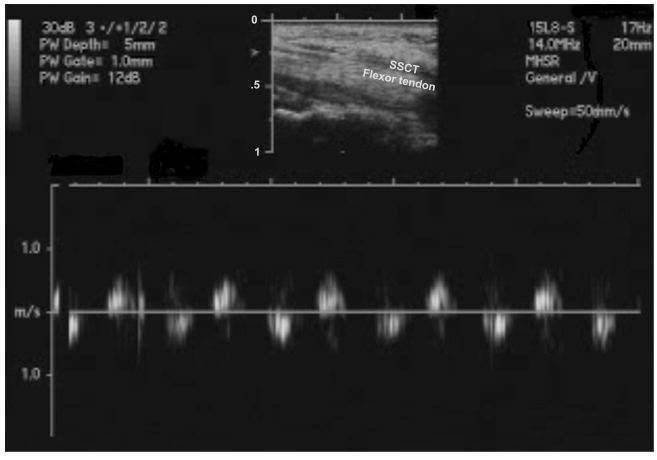

Fig 6A

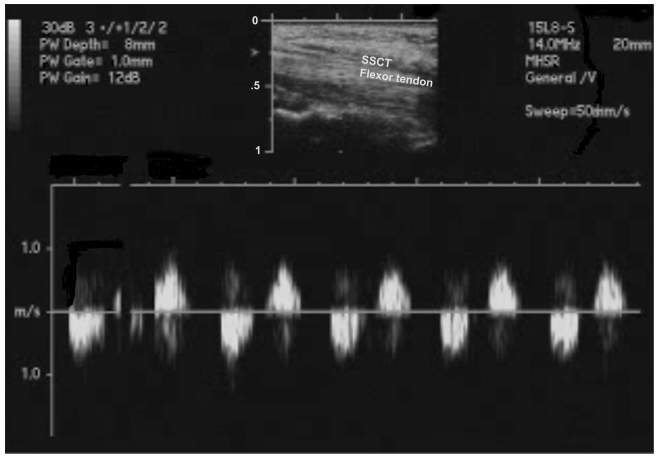

Fig 6B

Figure 6. **A**. Doppler ultrasound image with the sample window (gate) set at the SSCT. **B**. Doppler ultrasound image with the sample window (gate) set at the tendon.

functionally differentiate the SSCT from the tendon by comparing peak velocities during flexion and extension cycles.

Ultrasound imaging has been described as comparable to electrodiagnostic studies, with high sensitivity[1] and high diagnostic accuracy of the correlation between cross-sectional median nerve area and electrophysiologic severity with electromyography [23, 27], and considered as an initial test choice in patients with suspected carpal tunnel syndrome [1]. Qualitatively, ultrasound has been compared to anatomical sections and MR images and it has been shown that this imaging technique provides depictions comparable to dissections of the carpal tunnel [3, 23] and MR imaging [20]. Quantitatively, ultrasound has demonstrated high precision of median nerve thickness measurements compared to direct anatomical analyses [3]. Speckle

tracking of ultrasound images has been employed to assess longitudinal sliding of the median nerve [15, 28]. Color Doppler ultrasound has been used successfully for measurements of flexor tendon excursion [29].

In our study, we used spectral Doppler to measure the relative velocities of the tendon and SSCT. The reason for using spectral Doppler was its ability to identify the anatomical structures interrogated by the scan plane in motion and, at the same time, to steer the beam and place the Doppler gate at the desired location in the tendon or SSCT. In this way, we were able to sample the tendon or SSCT throughout the entire flexion and extension cycle. Of necessity, however, we were obliged to make two assumptions. Because electromechanical motors created artifactual ultrasound signals, we were obliged to use manual finger motion. Thus, we were obliged to assume that the velocities were relatively constant between the runs used for tendon motion analysis and those used for SSCT analysis. More importantly, we did not independently measure SSCT motion within the intact carpal tunnel, and thus have no way of validating whether the actual SSCT motion correlated to the measured SSCT motion, except for the assumption that the Doppler spectra would be similar for similar velocities, when comparing tendon and SSCT motion. However, as we did not independently measure the anisotropy of these structures, this remains an assumption, and a limitation of this study.

Using the electro-potentiometer as the reference, we have shown that, following appropriate angle correction, the Doppler method provides accurate peak flexion and extension velocities of the tendon in this cadaver model. We have also demonstrated that a combination of a high-frequency transducer and an ultrasound machine capable of measuring velocities within a small Doppler gate allow estimation of SSCT motion. There is presently no reference standard for velocity analysis of the SSCT. We base our findings about SSCT motion on initial careful qualitative validation of ultrasound detection of SSCT and on consistency with motion of the corresponding tendon.

Diagnosis of carpal tunnel syndrome is presently based on the presentation of classic clinical symptoms and confirmed by electrodiagnostic testing. In patients with carpal tunnel syndrome the tenosynovium becomes affected by fibrosis [8, 11, 30]. Indeed, some investigators hypothesize that the tenosynovial pathology may be the underlying source of the nerve compression in CTS, due to altered fluid flows, or by a simple bulk effect [8,9,11]. A better understanding of the relationship of synovial pathology with changes in nerve morphology and function could shed further light on this hypothesis. In addition, should the hypothesis be further supported, then measuring of thickness and velocity of the SSCT in these patients with ultrasound could provide an alternative or additional diagnostic method for patients with CTS.

Analysis of CTS by ultrasound so far has focused on cross-sectional area or flattening of the medial nerve in relation to CTS [1-3, 6, 20, 23, 27], but a universally standardized method for diagnosis of CTS with ultrasound has not been established. Erel et al.[15] used ultrasound to examine longitudinal median nerve sliding in patients with CTS and compared the results to controls. These authors used a custom frame-by frame cross-correlation algorithm and took advantage of the relatively wide cross section of the median nerve to track its motion.

While measurement of the median nerve or a tendon is relatively easy with current ultrasonographic techniques, measuring the relatively thin SSCT is more challenging. Cigali et al.[26], Buyruk et al.[29] and Soeters et al.[25] used color Doppler imaging to measure excursions and velocities of tendons. We also employed Doppler analysis for our studies of the FDS tendon and SSCT. We could not identify or create custom software capable of tracking the tendon and the SSCT simultaneously. For this reason, we explored the use of a readily available Doppler method, which also had the advantage of being widely available and, therefore, capable of supporting translation to clinical practice.

The ultrasound system we used during these initial studies was designed for cardiovascular applications, specifically for Doppler velocity analyses within structures much thicker than that of the tendon and SSCT. This machine represented at the time of our study the best compromise between a small-footprint linear-array transducer for scans at the carpal tunnel level and a high frequency of ultrasound signal. In addition, the range of velocities measurable by this particular machine was compatible with our testing velocities. We have shown that the SSCT produces a measureable Doppler shift, which may have implications in cases of peritendinous fibrosis. This motion can be quantified, assuming the validity of their calibration scheme, which requires further validation (Figure 7).

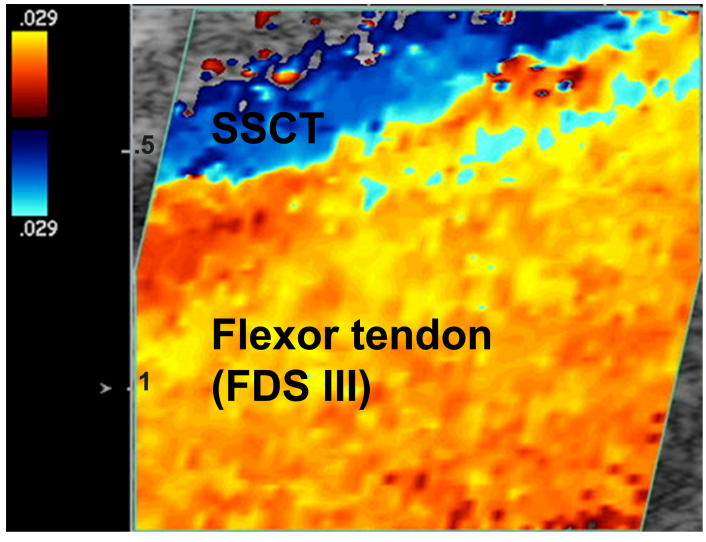

Figure 7. Color Doppler image showing the different velocities for the tendon and SSCT. Note that there is no signal of the soft tissue above the SSCT and that the SSCT has faster movement closer to the tendon.

Several difficulties resulted from using a machine intended for a different purpose. For example, the Doppler gate, represented by two thin line-cursors which were relatively long compared to SSCT thickness, was difficult to fit within the SSCT structure, unless the scanning angle was close to 90°. But Doppler spectra measure

motion toward and away from the sensor, and are incapable of measuring velocity when the scanning angle is perpendicular to the plane of motion. When we attempted to reduce the angle below approximately 70°, the Doppler window overlapped into the surrounding tissue, which could confound velocity
measurements. Fortunately, there appeared to be very distinct differences in velocity between the three adjacent structures, FDS, SSCT, and flexor retinaculum, so that accidental overlap into the tendon was immediately signaled by prompt increase of peak velocities of about 50 %, from approximately 10 cm/s to 15 cm/s), while accidental overlap with the immobile retinaculum was heralded by a corresponding sudden decrease in velocity.

Another practical issue related to the small size of the SSCT, with a thickness ranging from 0.4 mm to 1.1 mm, is that the resulting small Doppler gate had a high signal to noise ratio. While we have shown that our ultrasound measurements are accurate within a fraction of a millimeter, as compared to actual physical measurement for tendon motion, we can only assume that our SSCT measurements are similarly accurate. However, we believe that it is likely that such differences from actual measurements are likely to be small. Thus, we believe that this is a viable technique for measurements of the SSCT in distinction from the FDS tendon in cadavers.

We did not study cadaver wrists available with confirmed CTS. We would expect that in such wrists the SSCT would be thicker, due to the SSCT fibrosis that is characteristic of CTS. This should make detection and measurement easier than in normal individuals. In addition, in persons with CTS the SSCT might move differently,

either with velocities similar to those of the tendon (because of fibrosis and adherence to the tendon) or much lower (because of fibrosis and adherence to the flexor retinaculum). Ideally, simultaneous measurements of SSCT and tendon velocities are required. We are currently working to devise a method for simultaneous tendon and SSCT data acquisition, and plan to use such a system to study patients with CTS, and normal volunteers.

Conclusions

Ultrasound is a fast and non-invasive technique with relatively low cost. It has already proven to be of use in the evaluation of patients with CTS. High-resolution ultrasound is a very precise method to display the anatomy of the tendon and SSCT within the carpal tunnel and it is possible to detect their different velocities with Doppler. We believe that the data so obtained may prove useful in the evaluation of patients with CTS.

Acknowledgement

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CHAPTER 4

Gliding Characteristics of the SSCT

4.1

Gliding Characteristics between Flexor Tendons and Surrounding Tissues in the Carpal Tunnel: A Biomechanical Cadaver Study

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Abstract

The purpose of this study was to investigate the gliding characteristics of flexor tendons within the carpal tunnel with varied wrist positions and tendon motion styles, which may help us to understand the relationship between carpal tunnel syndrome and repetitive hand motion. Eight fresh human cadaveric wrists and hands were used. The peak (PGR) and mean (MGR) gliding resistance of the middle finger flexor digitorum superficialis tendon was measured with the wrist in 0, 30 and 60 degrees of flexion and extension. While moving all three fingers together, the PGR at 60 degrees flexion was significantly higher than that at 0 degrees, 30 degrees extension, or 60 degrees extension. While moving the middle finger alone, the PGR in at 60 degrees flexion and 30 degrees flexion was significantly higher than the PGR at 60 degrees extension. The PGR moving the middle finger FDS alone was significantly greater than that for all three digits moving together in 0 degrees, 30 degrees flexion, and 60 degrees flexion. Differential finger motion with wrist flexion elevated the tendon gliding resistance in the carpal tunnel, which may be relevant in considering the possible role of wrist position and activity in the etiology of CTS.

Introduction

Carpal tunnel syndrome (CTS), a compression neuropathy of the median nerve at the wrist, is a common diagnosis, with an estimated lifetime risk of 10% [1] and a prevalence of about 3% [2, 3]. It is generally accepted that repetitive, forceful hand or wrist motion, often associated with awkward wrist posture, is a risk factor for carpal tunnel syndrome (CTS), [4-12] but how these mechanical factors relate to the pathological changes of non-inflammatory synovial thickening seen typically in cases of carpal tunnel syndrome is unknown.

The anatomic structure of the carpal tunnel has been well studied [13-15]. Flexor tendons occupy roughly 90% of the carpal tunnel cross sectional area. The flexor tendons in the carpal tunnel are surrounded by a multi-layered matrix of collagen with abundant vasculature [16] commonly termed the subsynovial connective tissue (SSCT). The SSCT is histologically similar to paratenon [17-19]. Uniquely, the carpal tunnel also contains two synovially lined bursae, so that tendon movement is associated with both synovial and paratenon-related (extrasynovial) sources of friction.

During hand and wrist motion, the flexor tendons glide against the more or less fixed neighboring bone and ligament, and the median nerve. In addition to motion at the bursal surface, tendon motion results in sequential sliding of the layers of the SSCT and stretching of the connective tissue between SSCT layers. The resulting gliding resistance includes bursal friction, the surface friction of the SSCT layers, and any stretch of the collagen fibers which connect the SSCT layers [20].

Flexor tendon gliding characteristics have been well studied in the synovially lined spaces in the digits [21,22]. However, the gliding characteristics in the mixed environment within the carpal tunnel have not been reported. The purpose of the current study was to investigate the gliding resistance of a representative tendon, the

middle flexor digitorum superficialis (FDS) within the carpal tunnel during simultaneous (making a fist) and single finger movement of the middle finger.

Material and Methods

Eight fresh frozen human cadaver upper extremities, amputated approximately 15 cm proximal to the wrist joint, were thawed at room temperature immediately prior to testing. A medical record review was performed on each cadaver donor, to obtain demographic data and to be sure the individual did not have a recorded antemortem diagnosis of CTS. The flexor digitorum superficialis (FDS) tendons of the second, third and fourth digits were exposed proximal and distal to the flexor retinaculum, maintaining the carpal tunnel region intact. A 2 N load was attached to the proximal ends of each of the three FDS tendons by a cable, to maintain the tension on these three tendons. Marks were placed on the tendons and on the flexor retinaculum, a fixed reference point. Then the tendon excursion was measured from full flexion to full extension of all three fingers, both together and while moving the middle finger alone, holding the index and ring fingers in neutral position (0º extension), and the wrist fixed in the position of interest for that testing cycle. The wrist positions of interest were 60 º and 30º of extension, neutral (0º) and 30º and 60º of flexion. After the tendon excursions of all three fingers together and the middle finger alone were recorded for each wrist position, the tendons were dissected from their distal attachments, and the index, middle, ring, and small fingers were amputated at the MCP joint level, leaving the flexor retinaculum intact. A custom-designed external fixator was used to position the wrist in the desired position.

The specimen was then mounted on the testing apparatus by clamping the proximal end of the radius and ulna in a custom made clamping device (Figure 1). Load transducers were connected to the distal (F1) and proximal (F2) ends of the middle finger FDS tendon using a nylon cord. The proximal ends of all three FDS tendons (index, middle and ring fingers) were connected to a mechanical actuator. Three 2-Newton loads were attached, one to each of the distal ends of the index, middle and ring finger FDS tendons. Three 1 N loads were attached, one to each of the distal ends of the index, middle and ring finger FDP tendons, to maintain a minimal level of tension.

The FDS tendons were pulled proximally by the actuator against the weight at a rate of 2.0 mm/sec. This movement of the tendon toward the actuator was regarded as flexion. The actuator movement was then reversed, causing the tendons to be pulled distally by the distal 2N load. This movement of the tendon toward the load was regarded as extension. In simulating making a fist motion, the excursion of the FDS tendon with the least excursion was used as the excursion for all three tendons. Therefore, during testing, all FDS tendons moved within a physiological range.

For the testing of the middle finger FDS tendon motion alone, the proximal tendon ends of the second and fourth FDS tendons were disconnected from the actuator and fixed in one position, simulating 0º flexion of these digits, while tendon tension was maintained by the 2N load at their distal ends. Then the middle finger

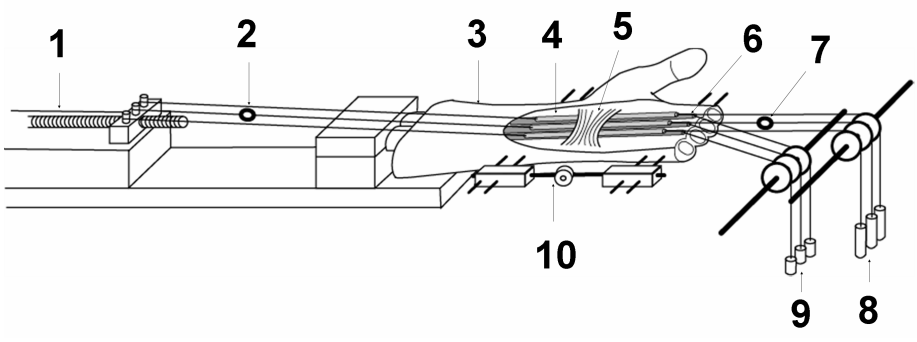

Figure 1. Testing apparatus. 1. Actuator with potentiometer; 2. Load transducer (F2) connected to the proximal end of the III FDS tendon; 3. Specimen; 4. Three (index, middle, and ring fingers); 5. Retinaculum; 6. Three (index, middle, and ring fingers) FDP tendons; 7. Load transducer (F1) connected to the distal end of the III FDS tendon; 8. Three 2-Newton weights attached to the FDS tendons; 9. Three 1-Newton weights attached to the FDP tendons; 10. External fixator to control the wrist angle.

FDS tendon was moved by the actuator to simulate a flexion/extension motion cycle of this digit alone, based on the tendon's previously measured excursion with fixation of the index and ring finger in the neutral position. The readings from the load transducers (F1, F2) and the corresponding excursions were recorded by a computer workstation at a sampling rate of 20 Hz. All specimens were kept moist throughout testing by frequently spraying a saline mist on them.
Eight specimens were tested for five constructs, representing the five different wrist positions (neutral position (0º), 30º and 60º flexion, and 30º and 60º extension) and two motion styles (all three FDS tendons together or the middle finger FDS tendon motion alone). Thus a total of ten conditions were tested for each specimen. The testing sequences were randomized. After the first testing for a motion style (all or middle finger FDS tendon alone), the motion style was alternated to the other motion style in the same wrist position. Then, the motion style from the second test was maintained for the first test in the next wrist position, and so on, until all conditions were tested.

Data Analysis

In each construct tested, the gliding resistance of the middle finger tendon was measured. The data was analyzed in two sets of data, mean gliding resistance (MGR) and peak gliding resistance (PGR). Mean gliding resistance was calculated based on the force values measured throughout the range of excursion by the following formula: (F2f-F1f) + (F1e-F2e) / 2. As F1f = F1e = 2 Newton (the applied load), the MGR formula simplifies to (F2f-F2e)/2. The PGR was the measured peak force during flexion (F2f-F1f). A typical curve of F1 and F2 during flexion/extension cycles testing is shown in Figure 2.

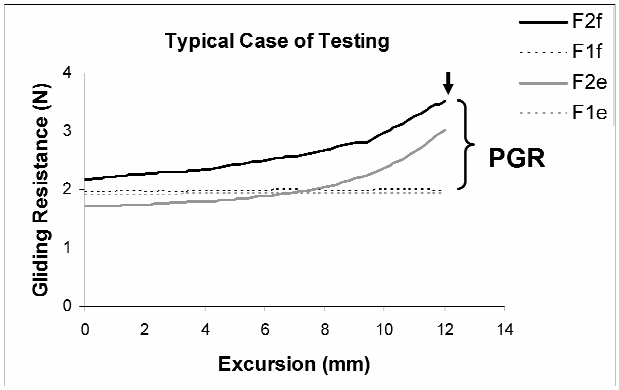

Figure 2. Representative curves of F1 and F2 during a flexion/extension cycle. The difference between F2f and F1f represented the gliding resistance during flexion motion, which included friction and tensile force by stretching the SSCT with increased tendon excursion. The maximal difference, i.e. PGR was calculated. The mean gliding resistance (F2f-F2e/2) mainly presented the surface friction as the elasticity of the SSCT was counteracted during cycle motion.

Our sample size requirements were determined by a power calculation. Prior studies of gliding resistance of the extensor digitorum communis of the index finger at the wrist level [23] reported a normal gliding resistance of 0.06 +/- 0.02 N in the wrist neutral position, while in 60° of wrist flexion , the friction increased to 0.17 +/-0.06 [23]. Assuming similar variability in our data, a sample of 8 specimens would provide 80% power to detect a difference of 0.07 N between any two of the ten testing conditions (alpha = 0.05, beta = 0.20). This level of detection appeared to be reasonable from a clinical point of view.

Data obtained from the gliding tests were analyzed using one factor repeated ANOVA measurement analysis of variance test to assess whether there were measured differences among the different groups, followed by a Tukey-Kramer post-hoc test for individual comparisons. A $p<0.05$ significance level was used in all cases.

Results

The excursion of the second, third and fourth FDS tendons in the different wrist positions were 29.6 (± 5.5) mm, 31.9 (± 5.7) mm, and 30 (± 5.7) mm, respectively while making a fist. The excursion of the middle finger FDS tendon while moving the middle finger alone was 23 (± 5.9) mm, somewhat less than that of the middle finger FDS tendon while moving all three fingers together.

While moving all three fingers together, the MGR of the middle FDS tendon with the wrist in 60 degrees of flexion was significantly higher than the MGR with the wrist in the neutral position ($p < 0.05$).

There was no significant difference among the MGR values for the other wrist positions (Figure 3). While moving the middle finger FDS tendon alone, the MGR in 60 degrees flexion was significantly higher than the MGR at 0 degrees and 30 degrees extension ($p < 0.05$). There was no significant difference among the MGR values for the other wrist positions (Figure 4).

While moving all three fingers together, the PGR at 60 degrees flexion was significantly higher than that at 0 degrees, 30 degrees extension, or 60 degrees extension ($p < 0.05$). There was no significant difference among the PGR values for the other wrist positions (Figure 5). While moving the middle finger alone, the PGR at 60 degrees flexion and 30 degrees flexion was significantly higher than the PGR in 60 degrees extension ($p < 0.05$). There was no significant difference among the PGR values for the other wrist positions (Figure 6).

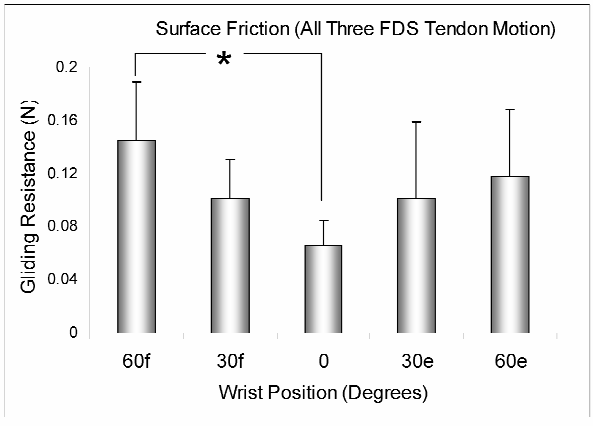

Figure 3. Mean gliding resistance with motion of all three FDS tendons. 60f: wrist in 60 degrees flexion; 30f: wrist in 30 degrees flexion; 0: wrist in 0 degrees position; 30e: wrist in 30 degrees extension; 60e: wrist in 60 degrees extension.
"*": indicates significant difference ($p < 0.05$)

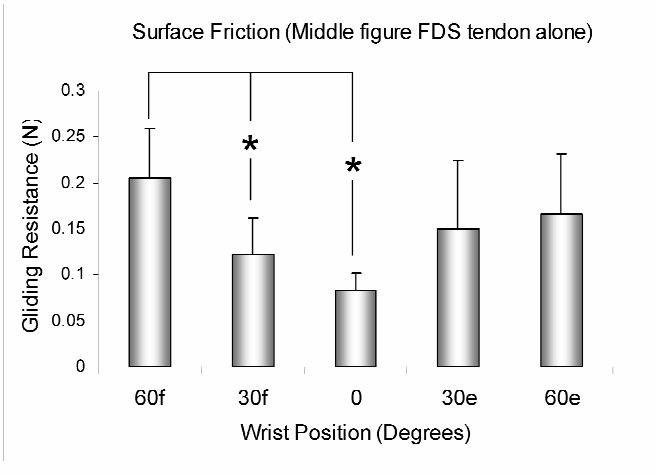

Figure 4. Mean gliding resistance of middle finger FDS tendon motion. 60f: wrist in 60 degrees flexion; 30f: wrist in 30 degrees flexion; 0: wrist in 0 degrees position; 30e: wrist in 30 degrees extension; 60e: wrist in 60 degrees extension.
"*": indicates significant difference ($p < 0.05$)

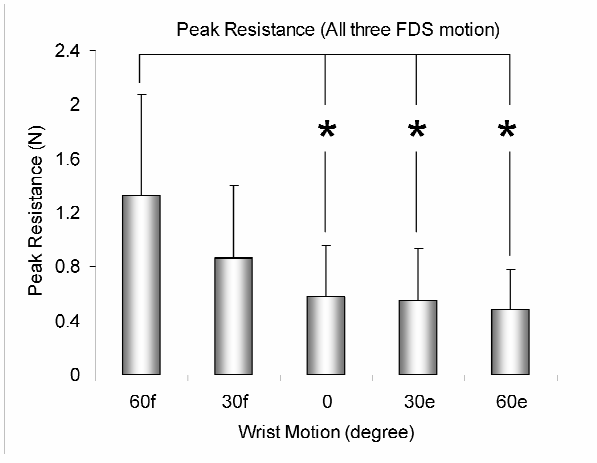

Figure 5. Mean peak gliding resistance of all three finger FDS tendons motion. 60f: wrist in 60 degrees flexion; 30f: wrist in 30 degrees flexion; 0: wrist in 0 degrees position; 30e: wrist in 30 degrees extension; 60e: wrist in 60 degrees extension.
"*": indicate significant difference ($p < 0.05$)

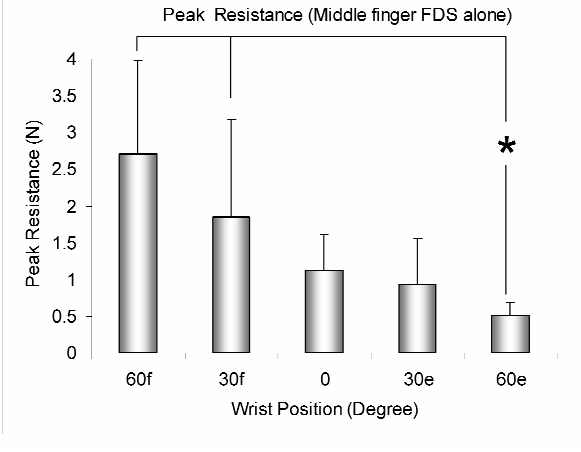

Figure 6. Mean peak gliding resistance of middle finger FDS tendon motion. 60f: wrist in 60 degrees flexion; 30f: wrist in 30 degrees flexion; 0: wrist in 0 degrees position; 30e: wrist in 30 degrees extension; 60e: wrist in 60 degrees extension.
"*": indicate significant difference ($p < 0.05$)

The difference between the two motion styles, i.e. moving all three FDS tendons versus moving the middle finger FDS tendon alone, was analyzed for each wrist angle. There was a significant difference in MGR between the two motion styles at 60 degrees flexion ($p < 0.05$). The PGR while moving the middle finger FDS alone was significantly greater than that for all three digits moving together, for the wrist positioned at 0 degrees, 30 degrees flexion, and 60 degrees flexion ($p < 0.05$).

Discussion

Carpal tunnel syndrome is a compression neuropathy of the median nerve. The most commonly noted pathological feature of carpal tunnel syndrome is fibrosis of the SSCT [24-27]. The most commonly noted epidemiological association with carpal tunnel syndrome is high force, high repetition activity [4-12] . The connection, if any, between these two observations is unknown, although some studies [25, 28] have shown that the SSCT of patients with carpal tunnel syndrome is less permeable than normal SSCT, and might therefore predispose to pressure elevation. Although the tensile strength and viscoelastic behavior of tendon has been well studied [29-35], to date there have been no studies assessing the motion characteristics of the flexor tendons within the carpal tunnel, or even demonstrating a method to do so. In this study we present both a method to measure gliding resistance within the carpal tunnel, and reference values from cadaver hands with no recorded antemortem diagnosis of carpal tunnel syndrome.

The gliding mechanism in the carpal tunnel is unique, due to in the special flexor tenosynovial organization [36]. The multiple layers of the SSCT permit motion by stretching of interconnecting collagen fibers, providing a viscoelastic component [37], while the carpal tunnel bursae provide a synovial fluid lubrication mechanism as well. We believe that our measurements capture both elements, with the peak resistance reflecting primarily the viscoelastic component. This allows us then a potential mechanism to study the normal viscoelastic limits of SSCT motion, and compare them with loads which might be applied by simulated activities in our cadaver model. This might in turn shed light on the relationship, if any, between specific activities and SSCT damage. As there is already a demonstrated relationship between fibrotic SSCT and impaired permeability of the SSCT, establishing a connection between activity and SSCT damage would bring us one step closer to connecting activity and carpal tunnel syndrome in an etiological chain.

Wrist position affected the flexor tendon gliding resistance in our study. Both the mean and peak gliding resistance were highest with the wrist in 60 degrees of flexion, while positioning the wrist in 0 degrees and 30 degree extension had the least gliding resistance. This is consistent with clinical studies which have shown that carpal tunnel pressure is increased with wrist flexion 38; our data provides further evidence that the wrist flexion position is adverse for tendon mechanics within the carpal tunnel.

We noted that gliding resistance was higher while moving the middle finger FDS tendon alone. This is logical, as the SSCT is shared by all three FDS tendons that we tested. Moving any single tendon would lead to stretching of the neighboring SSCT and thus would increase gliding resistance. This observation also suggests that differential finger motion could create a risk of SSCT shear injury. It is not obvious why single digit motion with wrist flexion should result in higher gliding resistance than single digit motion with an equivalent degree of wrist flexion, as in each case the arc of contact is the same. We believe that our data suggest a viscoelastic component to the PGR, which would be consistent with previous descriptions of the SSCT [16, 27]. Specifically, the lower PGR in wrist extension may indicate that the SSCT is stretched

somewhat in an extension direction in that starting position; then when the tendon is moved proximally in our model, the SSCT first relaxes from its extended position and is then slightly stretched into a flexed position, yielding a modest PGR. In contrast, with the wrist flexed the SSCT starting position may be already slightly stretched in a flexion direction, to which is then added the additional proximal tendon motion, resulting in a much higher PGR.

There are several limitations to our study. A cadaver model cannot perfectly mimic in vivo conditions. We did not move the three fingers independently, for technical reasons, relating to the complexity of the set up. Although the middle finger FDS tendon was chosen for the assessment based on several rationales (the superficialis tendons have the most independence, the middle finger superficialis is closest to the median nerve), the other FDS tendons and FDP tendons may have different gliding characteristics. Finally, the gliding resistance was measured with 2 N tension on the tendon. Other loads were not tested. We selected the a 2 N load as it is similar to the load recorded in vivo during unrestricted active finger motion [39]. Increased loading (which may simulate the different level of forceful hand work) may present different gliding characteristics.

In conclusion, this study assessed for the first time the flexion tendon gliding resistance within the carpal tunnel. We demonstrated that the middle finger FDS gliding resistance was elevated when the wrist was in 60 degrees of flexion. Differential finger motion increased the peak gliding resistance, especially with the wrist in flexion. Based on this data, future studies can be designed to study in more detail the viscoelastic properties of the SSCT, and the role that SSCT mechanics and injury may play in the etiology of carpal tunnel syndrome.

Acknowledgement

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CHAPTER 5

Search for an Animal Model

5.1

Comparative Anatomy of the SSCT in the Carpal Tunnel Syndrome in the Rat, Rabbit, Dog, Baboon and Human.

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Hand (2006) 1:78-84

Abstract

The tenosynovium in the human carpal tunnel is connected to the flexor tendons and the median nerve by the subsynovial connective tissue (SSCT). The most common histological finding in carpal tunnel syndrome (CTS), a compression neuropathy of the median nerve, is noninflammatory fibrosis of the SSCT. The relationship, if any, between the fibrosis and nerve pathology is unknown, although some have speculated that a change in the SSCT volume or stiffness might be the source of the compression. Yet, while animal models have been used to study the physiology of nerve compression, but none have been used to study the relationship of the SSCT pathology to the neurophysiological abnormalities. The purpose of this study was to identify animal models that might be appropriate to study the interaction of SSCT and nerve function in the development of CTS. The front paws of a rat, rabbit, dog and baboon were dissected. The carpal tunnel anatomy and subsynovial connective tissue of these animals were also examined by light and scanning microscopy and compared to the relevant human anatomy and ultrastructure.
The carpal tunnel anatomy and contents of the baboon and rabbit are similar to humans. The canine carpal tunnel lacks the superficial flexor tendons and the rat carpal tunnel is very small. The human, baboon and rabbit specimens had very similar organization of the SSCT and content of the carpal canal. We conclude that, while both the baboon and rabbit would be good animal models to study the relationship of the SSCT to CTS, the rabbit is likely to be more practical, in terms of cost and animal care concerns.

Introduction

Carpal tunnel syndrome (CTS), a compression neuropathy of the median nerve, occurs frequently, and has been studied by many investigators[1-5]. Despite the prevalence and economic impact of carpal tunnel syndrome[1,6], though, it is remarkable how little is known concerning its etiology. The majority of carpal tunnel syndrome cases are still described as being idiopathic[3,5,7,8], and the most common histological finding in carpal tunnel syndrome is non-inflammatory synovial fibrosis[3,4,9]. Many animal models have been used for carpal tunnel syndrome research[10]. In these models, carpal tunnel syndrome is induced by tightening the flexor retinaculum[4], nerve banding with a silastic tube[11-13], inserting an inflatable device[5] or fluid into the tunnel[14-16] or placing a tourniquet around the limb[17,18]. Most carpal tunnel studies focus on histomorphologic changes of the median nerve[11,13,19-23]. These animal studies may be more appropriately characterized as compression neuropathy models, rather than models designed to test hypotheses related to the specific etiology of carpal tunnel syndrome.
The tenosynovium in the human carpal tunnel is connected to the flexor tendons and the medial nerve by the subsynovial connective tissue (SSCT). The SSCT serves as a sliding unit to reduce the friction and protect the blood supply to the tendon and synovium[24]. In previous studies, histological and biological changes have been noted

within the subsynovial connective tissue of patients with CTS[3,25]. Several investigators have suggested that the nerve compression may actually be secondary to an initial change in SSCT stiffness, volume, or permeability[4,8]. Recently, a scanning electron microscopy study has shown that the most severe changes in the SSCT in patients with CTS [26] were found close to the tendon, suggesting that these changes may be due to a shearing injury.

Based on this evidence, we also believe that the etiology of carpal tunnel syndrome might be related to an injury of the subsynovial connective tissue. To study this possibility, an animal model with a similar anatomy and structure to the human carpal tunnel, including a similar SSCT organization, is essential, yet no studies to date have compared these features systematically between human and putative animal models. The objective of this study was, therefore, to identify a potential in-vivo animal model with similar anatomic features to the human carpal tunnel, including, for the first time, consideration of the structure of the SSCT. In order to accomplish this objective, we investigated the anatomy of the carpal tunnel contents in five different species (human, rat, rabbit, dog and baboon) and compared the morphology of the subsynovial connective tissue in these species by light and scanning electron microscopy.

Materials and Methods

The front limbs from fresh cadaver rat, rabbit, dog, and baboon specimens were obtained from our institutional Section of Veterinary Medicine. The animals had all been sacrificed in the course of other experiments. In each case the animals were euthanized by anesthetic overdose and the front paws were harvested from the elbow.

An upper extremity from a human female cadaver (age 38 years) and one upper extremity from a human male cadaver (age 75 years) were also used for this study. A medical record review was performed on the human cadavers before the study, to be sure that there had been no antemortem diagnosis of carpal tunnel syndrome. Exclusion criteria also included a history of diabetes, glucose intolerance, thyroid disease, rheumatoid arthritis, osteoarthrosis, flexor tendinitis, gout, hemodialysis, BMI>30, sarcoidosis, amyloidosis, peripheral nerve disease or traumatic injuries to the ipsilateral arm.

In addition to the anatomic specimens we used veterinary anatomy texts[27-31] to assist in the dissection and to help identify the anatomy of the carpal tunnel. In each of the five species studied, in addition to the anatomic dissections we harvested tendon and tenosynovial tissue for histology and scanning electron microscopy. In addition, one upper limb of each species was deep frozen (-20 degrees Celsius) and transverse sections were made through the carpal tunnel.

This study was approved by our Institutional Review Board and Institutional Animal Care and Use Committee (IACUC).

Light microscopy
In the rat, rabbit, baboon and human specimens the middle digit flexor digitorum superficialis tendon and its tenosynovium was dissected. This tendon was chosen because it is adjacent to the median nerve and connected to the SSCT and visceral synovium within the carpal tunnel. In the dog, the flexor digitorum profundus tendon was used, because the superficial flexor tendons do not pass within the carpal tunnel. The SSCT biopsies were formalin fixed and paraffin embedded. Five μm sections were made and standard hematoxylin and eosin (HE) staining procedures were done by our Department of Laboratory Medicine and Pathology.

Scanning electron microscopy
Scanning electron microscope (SEM) imaging was used to determine the ultrastructrural morphology of the tenosynovium in all five species. The contents of the carpal tunnel were excised en bloc in the rat and rabbit, and the middle digit flexor digitorum superficialis tendon was marked with a marker pen, to mount the dried tissue with the superficial layer up. In the dog, we collected the tenosynovium and approximately 2 cm of the middle digit flexor digitorum profundus tendon within the carpal tunnel. In the baboon and human cadaver we collected the middle digit flexor digitorum superficialis tendon and its tenosynovium.
The SSCT tissue was fixed in Trump's fixative (1% gluteraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2[32]. The biopsies were dehydrated through a graded series of ethanol solutions in a critical point dryer. Tissue was then rinsed for 30 minutes in 2 changes of 0.1 phosphate buffer, pH 7.2. The tissue was dehydrated in progressive concentrations of ethanol to 100% and either critical point dried. The specimens were then mounted on aluminum stubs and sputter coated with gold-palladium. Images were captured on a cold field emission scanning electron microscope operating at 2KV (Hitachi S-4700, Hitachi High Technologies America, Inc., Pleasanton, CA, USA).

Results

Human
The cross section of the carpal canal in the human is shown in Figure 1A. The human carpal canal forms a rigid passageway, bounded dorsally, medially and laterally by the carpal bones and palmarly by the flexor retinaculum. The contents of the carpal tunnel include the flexor digitorum profundus (FDP) tendons, the flexor digitorum superficialis (FDS) tendons, the flexor pollicis longus (FPL) tendon, the subsynovial connective tissue (SSCT), the radial and ulnar bursa and the median nerve.
The SSCT loosely connects the finger flexor tendons and the synovial membrane, which in turn encloses the tendons within the ulnar tenosynovial bursa. The SSCT consists of fibrous bundles parallel to the tendon, interconnected by smaller microfibrillar fibers (Figs. 2 and 3).

Fig 1A

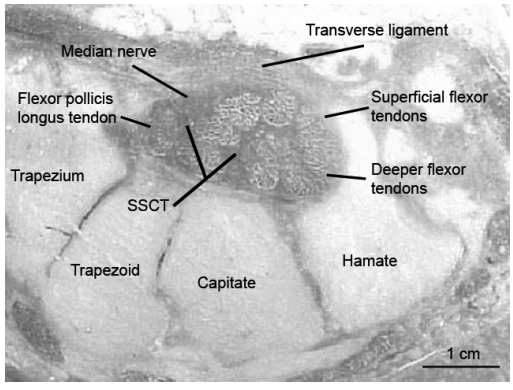


Fig 1B

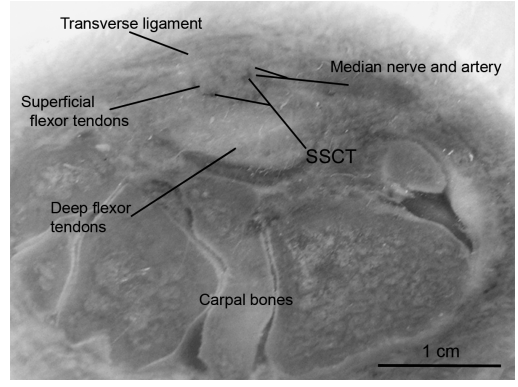


Fig 1C

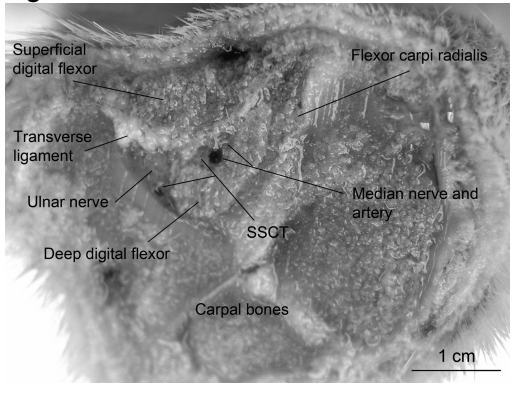


Fig 1D

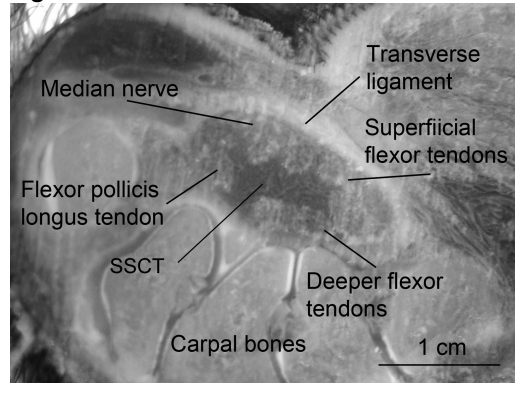


Figure 1. A. The cross section of the carpal canal in the human.**B.** The cross section of the carpal canal in the rabbit. **C.** The cross section of the carpal canal in the dog. **D.** The cross section of the carpal canal in the baboon.

Rat

The front paws of five Sprague Dawley male rats (0.35-0.5 kg) were used. Six front paws were used for dissection and four front paws were used to make cross sections. After making a longitudinal incision in the palmar skin, the median nerve, and flexor tendons were exposed. A flexor retinaculum was present. The superficial and deeper flexor tendons were separated structures within the carpal tunnel. A subsynovial connective tissue was present. Light and scanning electron microscopic images of the rat subsynovial connective tissue are shown in Figures 2 and 3. The SSCT of the rat does not show the typical construction of parallel fibrous cables with interconnections as seen in the human, but rather shows a meshwork of fine fibers which tend to form braids but lack the interconnecting fibrous structures. Cross sectional illustrations of the rat carpal tunnel did not provide sufficient information for presentation.

Rabbit

Both front paws from five New Zealand White rabbits (weight 4.0- 4.5 kg) were used for dissection of the anatomy. Two additional front paws were used for frozen and transverse sections. The cross section of the carpal canal in the rabbit is shown in Figure 1B.

In the New Zealand rabbit the carpal bones and the transverse carpal ligament form a rigid passageway at the wrist through which the flexor tendons and the median nerve travel. The carpal bones consist of three proximal bones, and a small accessory carpal bone and a distal carpal series (I-IV), which is attached to each metacarpal except the fifth. The transverse ligament contains pencil shaped or triangular shaped cartilage discs, which can be palpated and used as a marker for surgery or injections. The rabbit's median nerve, median artery, flexor digitorum profundus tendons and flexor digitorum superficialis tendons all lay inside the carpal tunnel (Figure 1B). The superficial flexor tendons are separate tendons. The profundus flexor tendons of the 4 digits are fused in the carpal tunnel. The flexor pollicis longus of the rabbit is also within the carpal tunnel. The median nerve originates from the 6^{th} and 7^{th} cervical nerves in the brachial plexus and on the caudal side of the humerus and continues across the elbow to the lateral surface of the forearm. In 4 rabbits (both front paws), we found that the median nerve passed through the carpal tunnel with the median artery on the radial volar side, while in both paws of one rabbit, the median nerve split about 2 cm proximal to the flexor retinaculum into a ramus medialis and ramus ulnaris, both of which went through the carpal tunnel. The small medial ramus of the median nerve ran superficial to the flexor tendons on the radial side adjacent to the medial artery. The ulnar ramus of the median nerve ran at the ulnar side in the carpal tunnel, also superficial to the tendons.

Standard hematoxylin and eosin staining of the subsynovial connective tissue shows that rabbit SSCT is similar to human SSCT (Fig. 2). By scanning electron microscopy we found that the subsynovial connective tissue of the rabbit consisted of fibrous bundles that run parallel to the tendon interconnected by smaller microfibrillar collagenous fibers, similar in structure to that of humans (Fig. 3).

Canine

The front paws from 5 mongrel dogs (approximately 30 kg in weight) were used for dissecting the anatomy and biopsies, and one for transverse sections.

In the forepaw, the carpus includes seven small, irregular bones arranged into two rows. On the palmar side of the wrist there is a fat pad, which is covered with the skin typically seen on the volar side of the digits. The carpal canal is formed by the accessory carpal bone laterally, the palmar carpal ligament and the carpal bones dorsally, and the flexor retinaculum on the palmar surface (Fig. 1C). All the metacarpal bones are fused.

The superficial flexor tendon lies beneath the skin and antebrachial fascia on the caudomedial side of the forearm. The tendon is at first single, then crosses the palmar surface of the carpus medial to the accessory carpal bone in the carpal canal, and finally divides into four tendons of nearly equal size. The deep digital flexor has three heads of origin which fuse at the carpus to form a single tendon. This tendon is

held in place in the carpal canal by the thick, deep part of the fibrous flexor retinaculum. The flexor retinaculum lies between the superficial and the deeper flexor tendon. The median nerve and median artery pass under the ligament (Fig. 1C). The light and scanning electron microscopic investigation of the SSCT showed that canine SSCT is similar in structure to the human SSCT (Figs. 2 and 3), and that the SSCT of the canine consists of fibrous bundles that run parallel to the tendon interconnected by smaller microfibrillar collagenous fibers.

Baboon

Both upper extremities from a female baboon, age approximately 4 years, and with a weight of 11.3 kg were used. One upper extremity was used for dissection of the anatomy and biopsy and one for the transverse section. The cross section of the carpal canal in the baboon is shown in Figure 1D. The baboon's hand is similar to that of the human. On the proximal palmar side, the baboon has a large fat pad covered with the skin typically seen on the palm of the hand. When dissecting the skin we found the transverse ligament going diagonally across the palm. It was approximately 2.5 cm long. Under the ligament, the median nerve was present and was approximately 2mm thick. The 4 superficial flexor tendons and the 4 profundus flexor tendons are separate structures within the carpal tunnel. We also found a flexor pollicis longus tendon within the carpal canal.

The subsynovial connective tissue lies between the carpal tunnel structures and consists of fibrous bundles parallel to the tendon, interconnected by smaller microfibrillar fibers which appear similar to human tissue (Figs. 2 and 3).

Discussion

The choice of an animal model to study CTS depends upon the purpose of the study design. For example, to study the sequence of structural alterations in the median nerve due to mechanical compression, any animal with myelinated mixed nerves could be used. The study of the etiology of CTS is a more complex endeavor, and requires the ability to observe the interaction of the median nerve in situ with the surrounding tendons and tenosynovium, in an arrangement similar to that in the target human population.

To assess the utility of various animals to study the etiology of the carpal tunnel syndrome, we therefore focused on the comparability of the anatomy of the animal carpal tunnel and the morphology of the SSCT.

The criterion considered most essential for proposing an appropriate animal model of CTS etiology was the morphology of the SSCT and in particular its relationship with the superficial flexor tendons and the course of the median nerve, since disruption of this structure and relationship is the principal non-neuropathologic finding in CTS. We also considered the relative independence of the superficial flexor tendons in these animals as compared to the human as this may be of special interest when it comes to simulating shearing injury to the SSCT, a possible repetitive use mechanism for the etiology of CTS.

Figure 2. Microscope images of the subsynovial connective tissue (SSCT) of the carpal tunnel in a human, rat, rabbit, dog and baboon (H&E; original magnification, 200x). Note SSCT (arrow) and tendon (star)

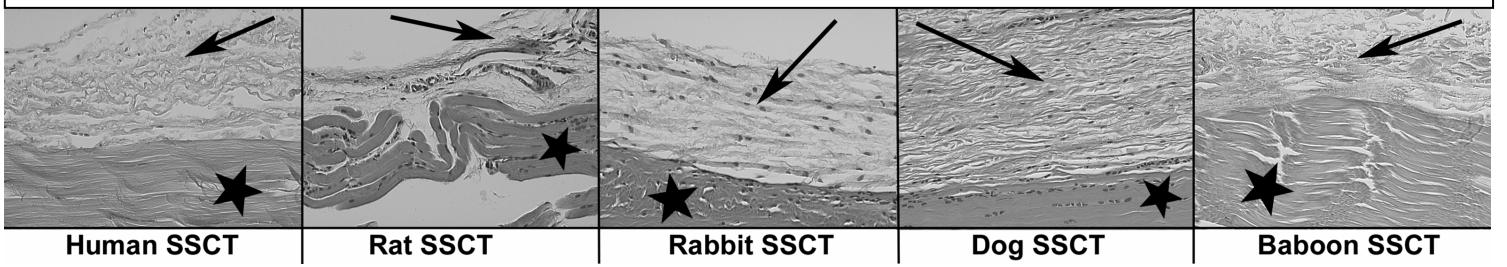
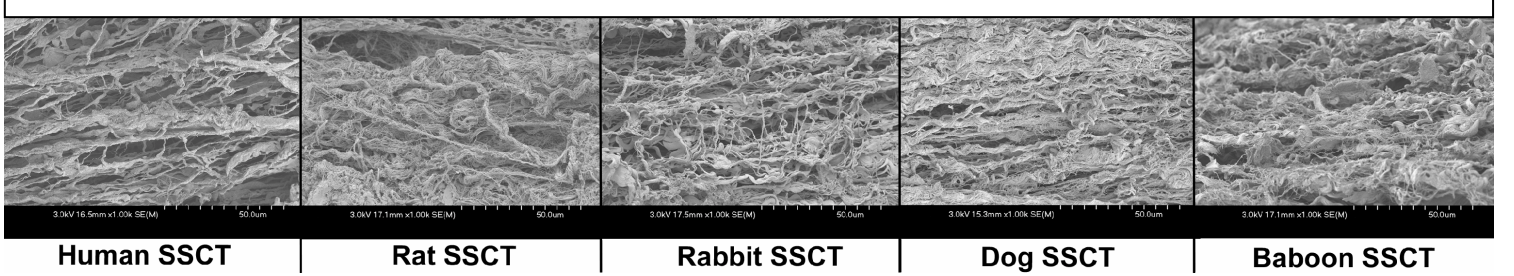


Figure 3. Scanning electron microscope images of the subsynovial connective tissue (SSCT) of the carpal tunnel in a human, rat, rabbit, dog (original magnification 1.0k).



In all of these animals, except partially in the baboon, the front paw is a weight bearing extremity. This means that the position of the wrist is in hyperextension. Neither rat, rabbit nor dog have an opposable thumb. Food-holding motions are accomplished by pressing material between the forepaws in the rat and rabbit, while the dog does not typically use this behavior. All three of these species can perform a unified flexing of the digits e.g. the dog can flex the digits when digging a hole in the ground, but the baboon is the only animal in this investigation which can perform grasping and differential finger movement. Yet, ultimately, none of the animals use their forelimbs as humans do. Thus, even an analogous anatomy does not imply a perfect model, and any conclusions in the model must still be validated clinically.

Rat

The rat has been used for a model of carpal tunnel syndrome[22] and has been shown to be a good model to investigate the neuropathological changes induced by nerve compression[21,23], especially the sciatic nerve. However, the small size of the front paw and the difference of the SSCT structure make this animal less appropriate for the study of the etiology of carpal tunnel syndrome.

Rabbit

The New Zealand rabbit has been commonly used as an animal model for CTS, so that there is extensive data available on the histopathology of the rabbit median nerve[5,14,20,33]. We used New Zealand White rabbits weighing 4.0- 4.5 kg, because the median nerve is better visualized when the rabbit is bigger and also there is more tenosynovium present. The rabbit has a nearly identical situation as the human regarding the osseous and connective tissue formations in the carpal canal[27,29,34], and there is extensive data available describing median nerve compression in the rabbit carpal tunnel[5,14,20,33]. We found an anatomical variation in the course of the median nerve, but this high bifurcation is similar to variations found also in humans[35,36]. The tenosynovium in the rabbit carpal tunnel is similar to human SSCT tissue, and is of sufficient volume to be examined for the purpose of etiologic studies. The lower costs and availability as compared to the primate makes this animal model attractive for study.

Canine

The canine model has been widely used for flexor tendon research[37-41], since it has a flexor tendon anatomy and functional structure similar to human and it is adaptable for surgery and therapy. However, the canine model for carpal tunnel syndrome has only been described in a few recent studies[42,43]. While the dog has an SSCT structure similar to that of humans, and the median nerve passes under the flexor retinaculum[28], there is no FDS tendon in the carpal tunnel in the canine model. This is an important drawback in etiological studies.

Baboon

The primate hand is closely related to the human hand, in terms of anatomy and function[44,45], and the carpal tunnel is identical to the human. The primate has been

used in a few studies of carpal tunnel syndrome, by directly compressing the median nerve with the use of a silastic tube[11,13] or by supplying fluid into the carpal canal[16].We found that the baboon's SSCT consisted of fibrous bundles parallel to the tendon, interconnected by smaller microfibrillar fibers, and looks similar to the human SSCT. The primate would be the obvious first choice for studies regarding repetitive movement and carpal tunnel syndrome. The limitations are mainly those of costs and regulatory requirements associated with primate studies.

Of all four animals investigated, the baboon and rabbit have osseous and connective tissue formations in the carpal canal that are closest to those of the human situation. Of these, the rabbit carpal canal is recommended as the most practical model for most studies, with the baboon or similar primate could be reserved for more complex studies involving the effect of specific tasks on the SSCT and median nerve function.

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5.2

Dextrose Induced Subsynovial Connective Tissue Fibrosis in the Rabbit Carpal Tunnel

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Submitted to Muscle and Nerve

Abstract

In this pilot study we used hypertonic dextrose solution to induce fibrosis of the subsynovial connective tissue (SSCT) and create an animal model of carpal tunnel syndrome (CTS). The SSCT of the carpal tunnel in 15 New Zealand white rabbits was injected with 0.05 ml of 10% dextrose solution in one paw and 0.05 ml of saline in the contralateral paw, to serve as a control. Animals were sacrificed at 1,2,4,8 or 12 weeks. While the saline side showed minimal changes at any time period, the hypertonic dextrose side showed progressive noninflammatory SSCT fibrosis, with vascular proliferation and thickening of collagen bundles. Demyelination of the median nerve developed at 12 weeks after the injection on the dextrose side. These findings are similar to the progression of pathology noted in humans with CTS.

Introduction

Carpal tunnel syndrome (CTS), compression neuropathy of the median nerve at the wrist, is the most common and best known of the compression neuropathies of the upper extremity. More than 200,000 carpal tunnel releases are performed each year in the United States, which makes it the most common surgical procedures performed on the hand. Each year close to 1,000,000 people require medical care, or are temporarily disabled by CTS[1].

Increased pressure within the carpal tunnel is the presumed immediate cause of the neuropathy[2,3]. In some cases, the cause of this pressure elevation is clear, as with fractures which deform the carpal canal [4-7], or localized inflammation which results in synovial hypertrophy[8,9], as in rheumatoid arthritis or infection. In most cases, though, the cause of this localized pressure elevation is itself unknown. For such cases of idiopathic CTS, clinical studies[3] and clinical observation suggest that the pressure elevations are first intermittent, resulting in transient symptoms, only later becoming continuous and resulting in neurophysiological changes consistent with demyelination. To explain this clinical picture, micro-trauma has been commonly implicated as an etiological factor[10], as well physiological abnormalities, especially diabetes mellitus[11-13].

Non-inflammatory fibrosis of the subsynovial connective tissue (SSCT) within the carpal tunnel is the most characteristic histopathological finding in patients with idiopathic carpal tunnel syndrome[14,15]. While it is reasonable to presume that a progressive non-inflammatory fibrosis of the SSCT might lead to an increased volume within the carpal tunnel and thus increased pressure on the nerve[16], an experimental model which can replicate this progression has yet to be established.

Previous experimental studies of CTS using animal models have focused on the pathology of the median nerve compression, induced by direct balloon catheter compression[17-19], surgically tightening the flexor retinaculum[10], or application of a tourniquet[3]. Although progressive demyelination of the median nerve has been observed in those experiments, such models only replicate those cases of CTS caused by acute space occupying lesions within the carpal tunnel, such as hematoma

or abscess, or acute alterations in carpal canal anatomy or perfusion, as may occur after wrist fracture or dislocation[5]. In essence, these are excellent models to study the intraneural pathology of compression neuropathy, while being less well suited to investigate the chain of events preceding and leading up to the neuropathy in patients with idiopathic CTS.

Experimental methods of inducing gradual, noninflammatory fibrosis are poorly defined. Recently, the concept of prolotherapy has been put forth in the alternative medicine literature. Prolotherapy, or proliferation therapy, is based on the premise that damaged soft tissues, such as tendons and ligaments can be treated by injecting into them a solution that stimulates cellular proliferation and neovascularization. Phenol, sodium morrhuate, glycerin, or hypertonic glucose are most commonly used. While some clinical studies have shown conflicting conclusions about the effectiveness of prolotherapy in treating musculoskeletal pain[20,21], other clinical studies have demonstrated more promising effects, especially in osteoarthritis and chronic tendon injuries[22-25]. There are also experimental data to support the effectiveness of common prolotherapy agents for inducing cellular proliferation and vascular changes[26].

The rabbit has been a commonly used animal model of CTS, because the rabbit's carpal tunnel anatomy is similar to that of the human[27]. In the rabbit, the carpal bones and the flexor retinaculum form a rigid passageway at the wrist through which the flexor tendons and the median nerve travel[27]. The rabbit's median nerve, flexor digitorum profundus and flexor digitorum superficialis tendons all lie inside the carpal tunnel. The rabbit's carpal bones are composed of three proximal bones, the radiale, intermedium, and ulnare, and a small accessory carpal bone on the lateral side of the wrist[28]. Most importantly, the rabbit is a common model to study nerve compression, so that there are extensive data available on the histopathology of the rabbit median nerve[29,30].

We have been intrigued by the superficial similarity in the pathological changes of progressive fibrosis and vascular changes induced by prolotherapy[26,31] and those seen in the SSCT of patients with CTS[14,32]. We hypothesized that prolotherapy could be adapted to induce a progressive change in the SSCT of an experimental animal that would, over time, lead to morphological changes in the median nerve, in essence reproducing the clinical course seen in patients with idiopathic CTS. If this hypothesis were supported, a new animal model would be available to study the cascade of events leading to CTS, including, possibly, therapies to abort the process before the neuropathy became established. We preferred to avoid compounds such as phenol, which have a direct toxic effect on nerve, and were intrigued by the possibility of using a physiological substance such as glucose, which has also been shown to have a prolotherapy effect when administered in hypertonic concentrations. The purpose of this study was, therefore, to evaluate, in a pilot study, the effect over time of a single injection of 10% dextrose solution in the SSCT of the rabbit carpal tunnel on the morphology of the SSCT and median nerve.

Methods

Fifteen adult New Zealand White rabbits, 14 male and one female, with a weight between 4- 4.5 kilograms, were used for this study. Our Institutional Animal Care and Use Committee approved this study.

The rabbits were anesthetized by an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (10mg/kg). Following the induction of satisfactory anesthesia, both forepaws were prepared and draped. One paw was randomly selected to receive an injection of 0.05 ml of 10% glucose solution, while the contralateral paw received a similar volume of saline solution as a control. The paw selected to receive the glucose solution was alternated between the right and left side among the animals. In each paw, the limb was exsanguinated with an elastic bandage, which was then used as a tourniquet. A small incision was made in the paw 1 cm proximal to the carpal tunnel. Localization of the carpal tunnel is facilitated in the rabbit, as the flexor retinaculum contains an easily palpated fibrocartilaginous disc (Fig. 1).

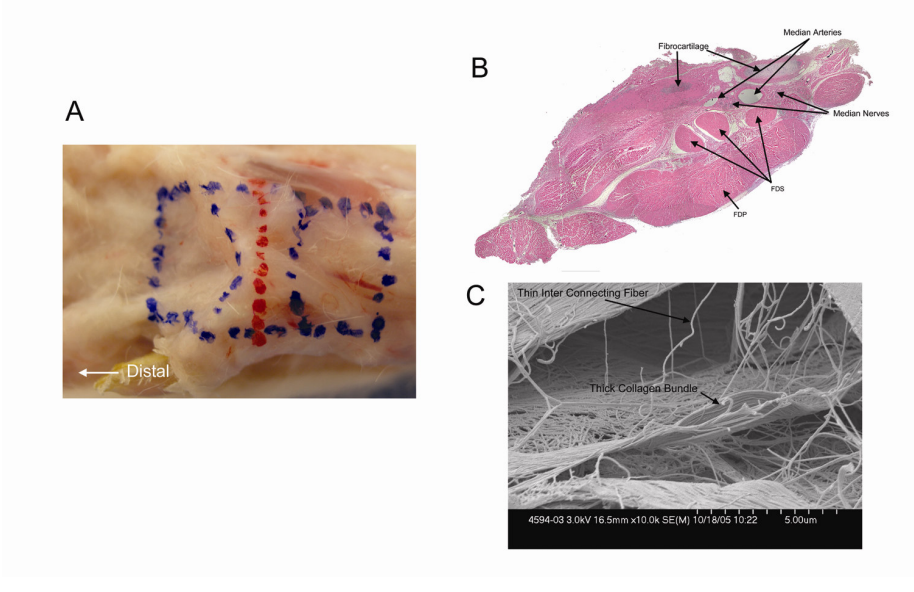

Figure 1: (a) Normal rabbit carpal tunnel outlined in blue in this dissected specimen. (b) Cross-section through area marked in red in Fig 1a. Note fibrocartilaginous disc within the flexor retinaculum, three separate flexor digitorum superficialis (FDS) tendons, and a single large flexor digitorum profundus tendon (FDP). Bundles of the median nerve, and the median arteries are noted. Hematoyxlin and eosin (HE) staining (x40). (c) SEM (x10,000) of rabbit carpal tunnel. Note thicker collagen bundles and thinner interconnecting fibers.

Dissection was carried out under 3.5 power loupe magnification. The flexor tendons were identified and the middle digit flexor was identified by moving that digit

passively. The injection was then made into the synovium around the middle digit flexor digitorum superficialis tendon, using a 30 gauge needle to minimize trauma. Care was taken to avoid any injection into the median nerve. The tourniquet was then removed. Hemostasis was achieved with local pressure, the wound closed with sutures of 5-0 Vicryl (Johnson and Johnson, New Jersey USA), and a sterile dressing was applied. Upon awakening, the rabbits were allowed full cage activity until the time of sacrifice. Three animals each were sacrificed at one, two, four, eight and twelve weeks after the injections. After sacrifice, the front paws were harvested and the total contents of the carpal tunnel were divided and prepared for light and scanning electron microscopy.

The contents of the carpal tunnel were marked with a marker pen, to orient the specimen proximal to distal and superficial to deep (Fig. 1). The biopsies for SEM were fixed in Trump's fixative (1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2[33]), and dehydrated through a graded series of ethanol solutions in a critical point dryer. Tissues were then rinsed for 30 minutes in 2 changes of 0.1 phosphate buffer, pH 7.2, and dehydrated in progressive concentrations of ethanol. The specimens were mounted on aluminum stubs and sputter coated with gold-palladium. Images were captured on a Hitachi S4700 cold field emission scanning electron microscope operating at 2KV (Hitachi S-4700, Hitachi High Technologies America, Inc., Pleasanton, CA, USA). Pictures were taken with the palmar side of the tissue up, and at different levels from proximal to distal throughout the harvested specimen. Specimens were evaluated qualitatively for collagen fiber organization and thickness.

The tissue for histology was formalin fixed and paraffin embedded. Five μm sections were made and stained with standard Hematoxylin and Eosin or Luxol Fast Blue. Specimens were evaluated qualitatively for cellularity, neovascularization, fibrosis, and inflammation, as well as for evidence of median nerve demyelination.

Results

Postoperatively, all animals recovered without difficulty, and the wounds healed uneventfully. All rabbits then resumed normal behavior and skin wound healing proceeded uneventfully until the time of sacrifice, except for two of the three animals sacrificed at 12 weeks, who developed ulcerations on the dextrose injected paw in the week prior to sacrifice.

One of these two rabbits showed a 5×5 mm superficial ulceration just radial to the fibrocartilage disc and the other showed a 3×5mm size superficial ulceration also just radial to the fibrocartilage disc. There were no ulnar sided ulcerations. These small ulcerations did not connect to the carpal tunnel itself.

Compared to the normal SSCT, at one week after the dextrose injection the SSCT appeared to be somewhat hypercellular, but otherwise the collagen organization and vascularity appeared to be normal (Fig. 2).

There was no evidence of neutrophil invasion or any other histological evidence of inflammation. The findings in the saline injected paws at one week were nearly normal except for slightly increased cellularity. The results at two weeks were similar to the one week findings.

At four weeks after the dextrose injection, the cellularity appeared to increase further, and evidence of vascular proliferation was seen along with collagen remodeling (Fig. 3). The saline injected paws at four weeks appeared to be similar to the normal histology. Again there was no evidence of neutrophil invasion or any other histological evidence of inflammation.

By eight weeks after the dextrose injection, more angiogenesis and thicker collagen bundles were observed, without evidence of inflammation, while again the saline injected paws' histological appearance was unremarkable (Fig. 4).

Twelve weeks after the dextrose injection we observed vascular proliferation and thicker collagen bundles in the SSCT (Fig. 5). We also observed demyelination in all the median nerves after dextrose injection. (Fig. 5f) The saline injected paws were normal histologically.

Discussion

In this pilot study we wished to see if there was any evidence that local hypertonic dextrose injection could induce progressive morphological changes in the SSCT of an animal model, which might mimic the changes seen in carpal tunnel syndrome. We saw evidence of such changes, which appeared to progress throughout the twelve weeks of observation, culminating in histological evidence of median nerve demyelination in the animals observed for the longest time. We suspect that loss of sensibility associated with this demyelination may be responsible for the ulcerations which we observed in the dextrose injected paws of these animals.

While our findings in this small study are only qualitative, and the analysis was not blinded, we believe that the results are sufficiently suggestive of an effect from the dextrose injection to warrant a larger study with a more formal quantitative analysis, not only histologically and electrophysiologically, but also biologically, such as of cytokines, matrix macromolecules, proteases and protease inhibitors, and markers for apoptosis and cellular proliferation. These could then be compared with similar studies in tissue from humans with carpal tunnel syndrome, to establish whether this model possesses more than superficial similarity with human CTS.

The strength of this study is that we were able to document sequential structural changes in the SSCT and median nerve in this rabbit model. The limitations relate to the small sample size, lack of blinding, and the absence of any quantitative analyses or electrophysiological studies. However, we wished to avoid a study with more animals and more analyses before first knowing if the dextrose had any effect, and which tissues were most affected. The data we have collected here will allow us to design a far more comprehensive study of the effect of hypertonic dextrose on the rabbit carpal tunnel than would have been possible without this pilot data. We hope that others will also consider investigating and refining this new model, which we

believe may prove useful in the study of the causes and prevention of carpal tunnel syndrome.

Acknowledgements

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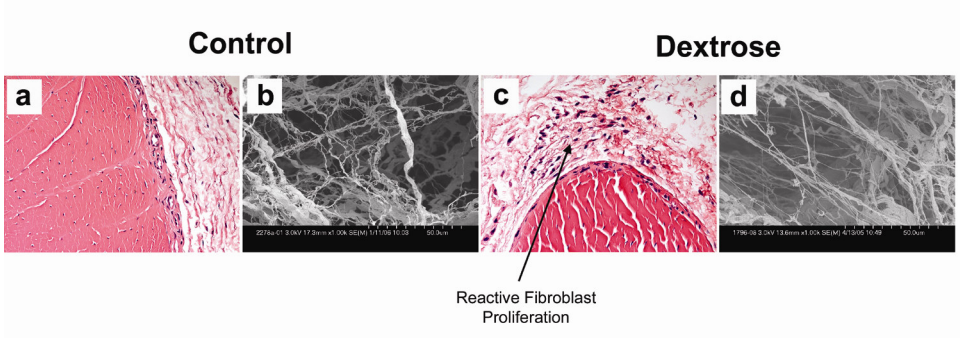

Figure 2: (a) 1 week saline injection HE (x400); (b) 1 week saline SEM (×1000); (c) 1 week dextrose HE (x400); (d) 1 week dextrose SEM (x1000). Early interstitial fibrosis with reactive fibroblast proliferation is identified in the specimen removed 1 week after dextrose injection. However, there is no associated inflammation. In contrast, the saline injected specimen shows no conspicuous changes compared to the normal control

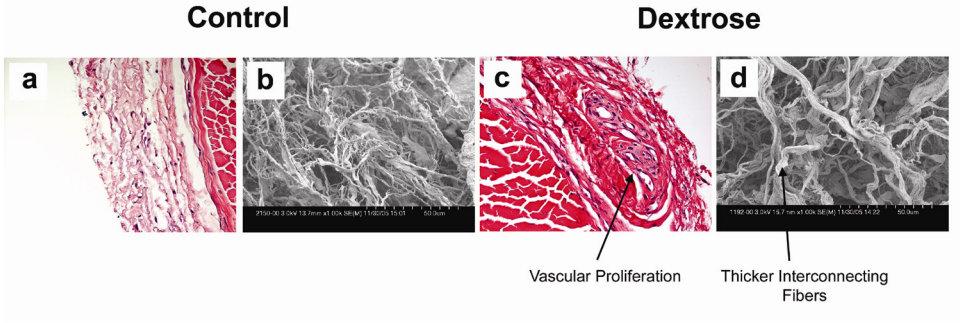

Figure 3: (a) 4 week saline HE (x400); (b) 4 week saline SEM (x1000); (c) 4 week dextrose HE (x400); (d) 4 week dextrose SEM (x1000). Interstitial, somewhat disorganized, collagenation with fibrosis is identified in association with vascular proliferation in the specimen removed 4 weeks after dextrose injection. On SEM, these findings correspond to thickened interconnecting fibers. In contrast, the saline injected specimen shows no conspicuous changes compared to the normal control.

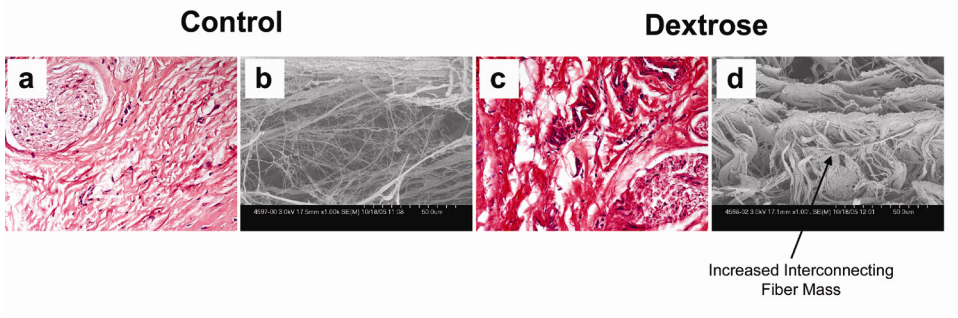

Figure 4: (a) 8 week saline HE (x400); (b) 8 week saline SEM (x1000); (c) 8 week saline HE (x400); (d) 8 week saline SEM(x1000). Dense collagenation and vascular proliferation become more prominent in the dextrose injected specimen compared to earlier specimens. In contrast, the saline injected specimen shows no conspicuous changes compared to the normal control. On SEM these findings correspond to thickened interconnecting fibers.

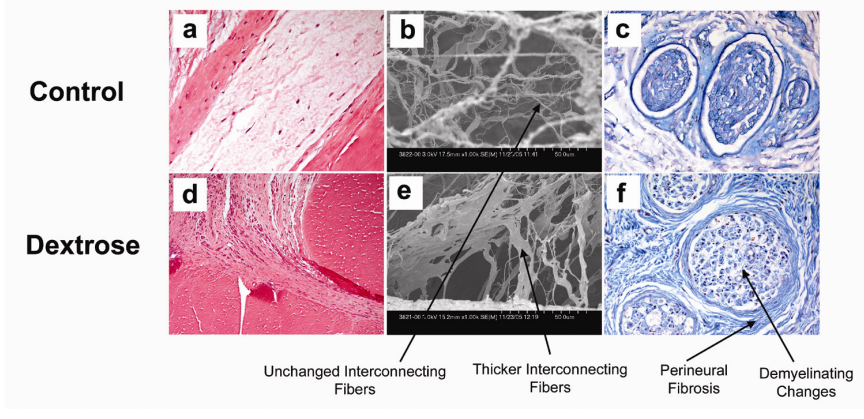

Figure 5: (a) 12 week saline HE (x400); (b) 12 week saline SEM (x1000); (c) 12 week saline Luxol fast blue staining (x400); (d) 12 week dextrose HE (x400); (e) 12 week dextrose SEM (x1000); (f) 12 week dextrose Luxol fast blue staining (x400). Demyelination is identified in association with interstitial organization fibrosis with collagenation and perineural fibrosis in the specimen removed 12 weeks after dextrose injection. In contrast, the saline injected specimen shows no conspicuous changes compared to the normal control.

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CHAPTER 6

General Discussion

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6.1
SSCT Morphology and Histology

The subsynovial connective tissue (SSCT) is an anatomic feature which is unique to the tendons in the carpal tunnel. The histopathology of the SSCT in patients with CTS is well documented[1-5]. Its role as a potential etiology of CTS has been discussed[2, 6].

We hypothesized that, if there was an insult to the subsynovial connective tissue in patients with carpal tunnel syndrome, there should be some histological and immunohistological evidence of it, and that these findings would not be present in unaffected individuals. Guimberteau[7] has postulated that an insult to the SSCT would be mechanical and Freeland[8] suggested an ischemia-reperfusion. Also Lluch[2] concluded that the edema and vascular fibroblastic proliferation in the synovium of the digital flexor tendons is a tissue reaction to the local ischemia, and therefore a consequence, rather than the cause, of carpal tunnel syndrome.

Our observation of SSCT obtained from patients with carpal tunnel syndrome show findings which are similar to those after injury to skin, tendon, and ligament and suggest that patients with idiopathic carpal tunnel syndrome may have sustained an injury to the subsynovial connective tissue which might lead to an increase in the volume of the contents in the carpal tunnel but also may alter its material properties, such as compliance and permeability to fluid flows[8-10] and vascularity[11, 12]. Scanning electron microscopy showed that the most severe changes in the SSCT were found close to the tendon, suggesting to us that these changes may be the result of a shearing injury[13].

We believe that the pathofysiology of CTS can be explained thus: the normal sliding unit function is initially deranged as a result of injury or disease. In response, the synovium thickens in an attempt to repair the damage. This thicker synovium may be more resistant to injury but is also less compliant, and thus may restrict synovial gliding relative to the tendon, thus predisposing to the future cycle of injury, repair, and fibrosis. Ultimately, due either to the sheer mechanical effect of increased synovial bulk, or some combination of increased bulk and altered fluid flows within the synovium[9, 10], the pressure in the carpal canal increases. The increased carpal tunnel pressure (above 30mmHg) will affect the median nerve circulation[14] and thus initiating the symptoms of carpal tunnel syndrome.

6.2 Motion Characteristics of the SSCT

Based on Guimberteau's initial description[7] and more recent observations by scanning electron microscopy[13] and histology[12], the gliding mechanism of the flexor tendons in the carpal tunnel region appears to be a hybrid of the intrasynovial and extrasynovial mechanisms described by Gelberman[15].

The SSCT consists of fibrous bundles parallel to the tendon, which are connected to one another by smaller fibers (Figure 2).

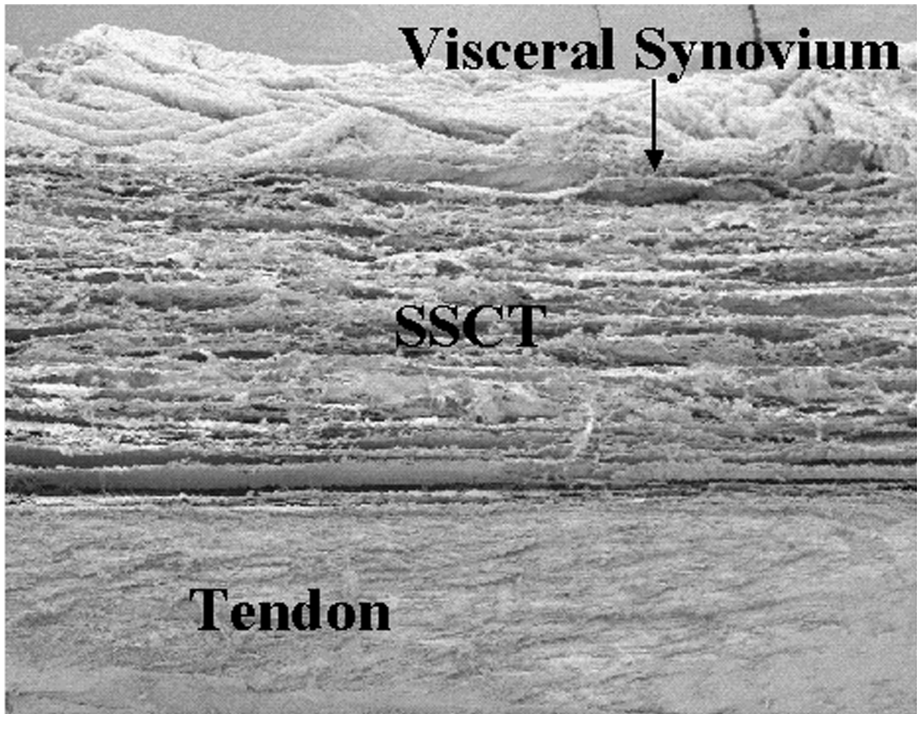

Figure 2. Scanning electron micrograph of normal subsynovial connective tissue (SSCT) (original magnification, x40)

In accordance with this concept, when the tendon moves, the fibrils connected to the tendons are stretched first, followed by the fibrils connected to the paratenon layers (Figure 3).

In this way, the lengthening propagates layer by layer until finally the visceral synovium (VS) moves. This is an extrasynovial mechanism. However, when the visceral synovium (VS) moves with the tendon, an intrasynovial type synovial sliding occurs between the visceral synovium and parietal synovium of the ulnar bursa of the carpal tunnel. Two factors theoretically will affect the relative motion between VS and flexor tendon. These are the mechanical properties of the SSCT and the moving resistance of the VS. Delayed VS motion indicates an increased elasticity of the SSCT; this would be the normal situation. If the SSCT becomes stiff, the VS motion will move simultaneously with the tendon. If there is no VS motion during flexor tendon motion, then this would imply that the SSCT is totally ruptured, and the tendon and VS are dissociated (Figure 4). While not observed in our study, we have

observed significant decreased movement of the VS in the patients and cadaver patients with tunnel syndrome.

We have shown that the SSCT does indeed serve as an intermediate layer between the tendon and VS functionally, so that there is normally a lag between the initiation of tendon motion and that of VS motion, and that this relationship is changed in patients with carpal tunnel syndrome, as well as in cadavers with a history of CTS, even though the clinical picture in the cadaver specimens was more varied than that in the patients.

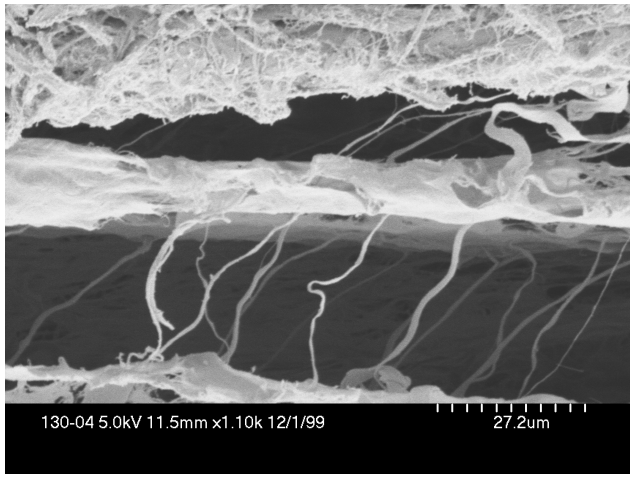

Fig 3A

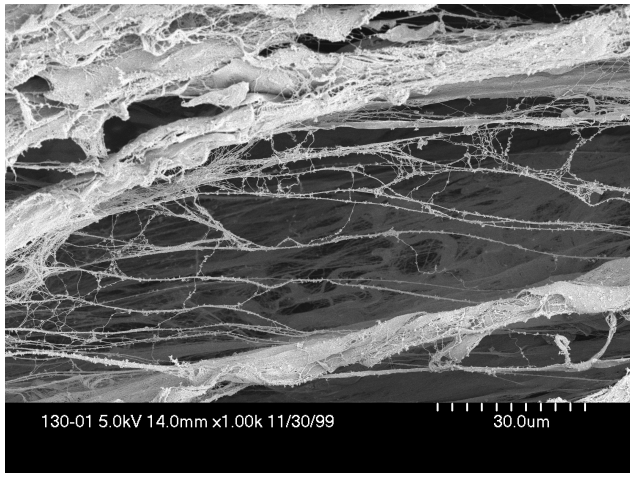

Fig 3B

Figure 3. 3A. Loose vertical fibers join adjacent layers in the SSCT (SEM, original magnification x1.10K). **3B.** The loose vertical fibers between adjacent layers are stretched during flexor tendon movements (SEM, original magnification x1.00K).

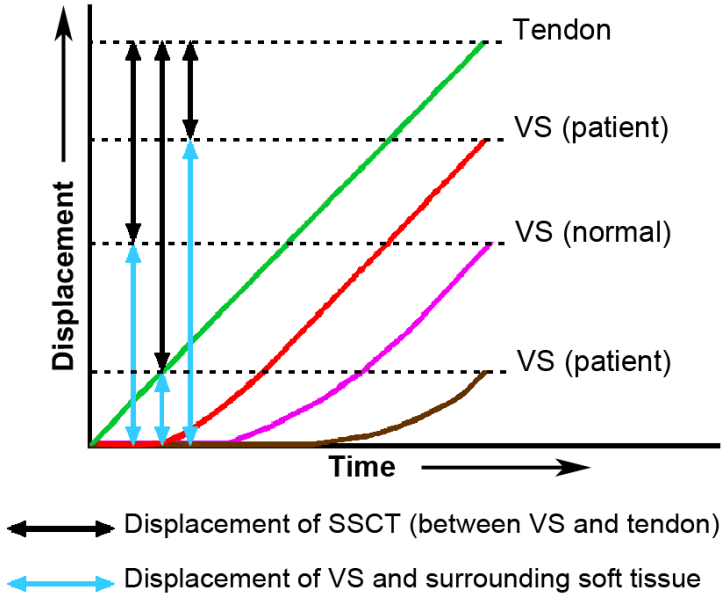

Figure 4. Hypothesis: the motion of the FDS III tendon and visceral synovium in the non-carpal tunnel syndrome situation (VS normal) and in the patients with carpal tunnel syndrome.

The moving resistance of the VS includes the surface gliding resistance between VS and PS and the mechanical resistance of moving the synovial bursa. The VS will start to move when the force applied to the VS (the force transferred from tendon through the SSCT layer by layer) is greater than the VS moving resistance. As the stiffness of the synovium increases, a greater force will be needed to move the VS, which may lead to earlier muscle fatigue or, if the force is great enough, further damage to the SSCT, establishing a vicious cycle. In our study, there was a trend for greater VS motion in CTS patients than in controls, which may indicate greater adherence of the SSCT to the tendons in carpal tunnel syndrome patients. Such adherence may limit or increase the tendon force needed for independent, differential tendon motion; could result in tethering[16-21] of the adjacent median nerve; and could also predispose the SSCT to shearing injury with differential tendon movement.

Our findings suggest that patients with carpal tunnel syndrome have changes in the functioning of the SSCT which could well explain the known pathophysiology of this disorder. If fibrosis of the SSCT does indeed result in adherence between the SSCT and the underlying tendons, then the normal delayed recruitment of SSCT motion will not occur, and this in turn may predispose to shear injury of the SSCT, further adherence, further shear, until either the tendon becomes totally fixed to the SSCT or pulls completely free. These are the two scenarios observed in our CTS patients. The resulting SSCT fibrosis could also be expected to disrupt fluid flows[8, 22, 23]

and raise hydrostatic pressure in the carpal canal[24-29], consistent again with observations of the fine microvasculature of the SSCT in CTS patients[11, 12].

Our observations that isolated motion of the FDS have a greater effect on SSCT motion than does group tendon action suggest that such motions may particularly predispose to SSCT shear injury, and indeed there is other recent evidence to support this concept[30].

Similar changes are also identified post mortem in the CTS patient, suggesting to us that once the SSCT has been damaged and altered to form fibrosis, these changes are irreversible. The changes in the SSCT could potentially aggravate or even cause median nerve pathology. If the etiology of CTS represents irreversible changes or damage to the SSCT, one would prefer a method that can detect changes before they are causing carpal tunnel syndrome and we would want a therapy that could prevent further damage to the SSCT.

We believe that by focusing on the characteristics of the subsynovial connective tissue (SSCT) there might be an additional parameter to focus on for the diagnosis of carpal tunnel syndrome.

High-resolution ultrasound is a very precise method to display the anatomy of the tendon and SSCT within the carpal tunnel and it is possible to detect their different velocities with Doppler. Non-invasive and real time assessment of the thickness and velocity of the tenosynovium in carpal tunnel syndrome by high-resolution sonography might potentially be a new diagnostic tool for disorders affecting the SSCT, especially carpal tunnel syndrome. Our ultrasound study focused for the first time on the SSCT, rather than on the nerve or carpal canal morphology when, and we distinguished motion of the middle finger tendon from the SSCT.

6.3 Gliding Characteristics of the SSCT

CTS is a compression neuropathy of the median nerve. The most commonly noted pathological feature of carpal tunnel syndrome is fibrosis of the SSCT[1, 2, 4, 5, 12]. The most commonly noted epidemiological association with carpal tunnel syndrome is high force, high repetition activity[31-35]. The connection, if any, between these two observations is unknown, although some studies have shown that the SSCT of patients with carpal tunnel syndrome is less permeable than normal SSCT[2, 23], and might therefore predispose to pressure elevation.

Flexor tendon gliding characteristics have been well studied in the synovially lined spaces in the digits[36, 37]. However, the gliding characteristics in the mixed environment within the carpal tunnel have not been reported. In our study we present both a method to measure gliding resistance within the carpal tunnel, and reference values from cadaver hands with no recorded antemortem diagnosis of carpal tunnel syndrome.

The anatomic structure of the carpal tunnel has been well studied[38]. Flexor tendons occupy roughly 90% of the carpal tunnel cross sectional area. The flexor tendons in the carpal tunnel are surrounded by a multi-layered matrix of collagen with abundant vasculature[7] commonly termed the subsynovial connective tissue (SSCT).

The SSCT is histologically similar to paratenon[39, 40]. Uniquely, the carpal tunnel also contains two synovially lined bursae, so that tendon movement is associated with both synovial and paratenon-related (extrasynovial) sources of friction.

During hand and wrist motion, the flexor tendons glide against the more or less fixed neighboring bone and ligament, and the median nerve[41]. In addition to motion at the bursal surface, tendon motion results in sequential sliding of the layers of the SSCT and stretching of the connective tissue between SSCT layers. The resulting gliding resistance includes bursal friction, the surface friction of the SSCT layers, and any stretch of the collagen fibers which connect the SSCT layers[13].

Wrist position affected the flexor tendon gliding resistance in our study. Both the mean and peak gliding resistance were highest with the wrist in 60 degrees of flexion, while positioning the wrist in 0 degrees and 30 degree extension had the least gliding resistance. This is consistent with clinical studies which have shown that carpal tunnel pressure is increased with wrist flexion[24, 28, 42]; our data provides further evidence that the wrist flexion position is adverse for tendon mechanics within the carpal tunnel.

We noted that gliding resistance was higher while moving the middle finger FDS tendon alone. This is logical, as the SSCT is shared by all three FDS tendons that we tested. Moving any single tendon would lead to stretching of the neighboring SSCT and thus would increase gliding resistance. This observation also suggests that differential finger motion could create a risk of SSCT shear injury.

It is not obvious why single digit motion with wrist flexion should result in higher gliding resistance than single digit motion with an equivalent degree of wrist flexion, as in each case the arc of contact is the same. We believe that our data suggest a viscoelastic component to the PGR, which would be consistent with previous descriptions of the SSCT [7, 12]. Specifically, the lower PGR in wrist extension may indicate that the SSCT is stretched somewhat in an extension direction in that starting position; then when the tendon is moved proximally in our model, the SSCT first relaxes from its extended position and is then slightly stretched into a flexed position, yielding a modest PGR. In contrast, with the wrist flexed the SSCT starting position may be already slightly stretched in a flexion direction, to which is then added the additional proximal tendon motion, resulting in a much higher PGR.

In conclusion, our study assessed for the first time the flexion tendon gliding resistance within the carpal tunnel. We demonstrated that the middle finger FDS gliding resistance was elevated when the wrist was in 60 degrees of flexion. Differential finger motion increased the peak gliding resistance, especially with the wrist in flexion. Based on this data, future studies can be designed to study in more detail the viscoelastic properties of the SSCT, and the role that SSCT mechanics and injury may play in the etiology of carpal tunnel syndrome.

6.4 Search for an Animal Model to Investigate the Etiology of Carpal Tunnel Syndrome in Vivo:

Carpal tunnel syndrome (CTS) occurs frequently and has been studied by many

investigators. Many animal models have been used for CTS research. In these models, CTS is induced by tightening the flexor retinaculum[2], nerve banding with a silastic tube[43-45], inserting an inflatable device[26] or fluid into the tunnel[46-48] or placing a tourniquet around the limb[49, 50]. Although progressive demyelination of the median nerve has been observed in those experiments, such models only replicate those cases of CTS caused by acute space occupying lesions within the carpal tunnel, such as hematoma or abscess, or acute alterations in carpal canal anatomy or perfusion, as may occur after wrist fracture or dislocation. These animal studies may be more appropriately characterized as compression neuropathy models, rather than models designed to test hypotheses related to the etiology of CTS.
Because most studies have focused on the chronic fase in the development of carpal tunnel syndrome, animal models will help us hopefully better understand the acute phase and the etiology of CTS. We have started our search for an animal model to investigate the etiology of CTS by first identifying a suitable animal in which we can focus on the SSCT. And secondly, to study in this animal possible identical changes as seen in human patients with CTS. Hopefully in the future we will be able to test the etiology of CTS, by making a model in which we can induce CTS by pre-stretching the SSCT and subsequently assess the biological process in a more acute phase of the disease.
Our prolotherapy model may be useful for future studies to investigate the effect of changes in the SSCT on median nerve function, which may in turn have relevance to the pathogenesis of carpal tunnel syndrome.

6.5 Carpal Tunnel Syndrome, current Therapy and the Future

The mainstay of diagnosis remains a clinical assessment of the patients' symptoms and history, with nocturnal paraesthesiae in the median nerve distribution being the most characteristic symptom. The clinical diagnosis of CTS can be confirmed by abnormal finding at the electrophysiological tests[51, 52]. Electrophysiological tests have been found to be highly sensitive and specific [53].

Although the underlying disease mechanism for CTS is increased carpal tunnel pressure[24-29, 54], numerous causes may lead to this final pathway. These causes may be classified as idiopathic, intrinsic, or extrinsic[55]. In patients with associated diseases such as diabetes the nerve neuropathy appears to represent a combination of metabolic and vascular factors. Alterations in the fluid balance seen during pregnancy, hemodialysis and hypothyroidism increase the volume of the contents of the carpal tunnel. Any condition that increases the volume of the contents of the carpal tunnel tends to compress the median nerve. Benign tumors, such as lipomata, hemangiomata, and ganglia may encroach upon the carpal tunnel[5]. Despite the long list of causes leading to increased pressure on the nerve, most of the patients are idiopathic CTS patients.

6.5.1 Conservative Therapy

There is no commonly accepted treatment program for mild or moderate CTS at this time, but typically the initial approach to carpal tunnel syndrome, when reversible etiologies have been excluded, involves no intervention, analgesics, wrist splinting, and steroid injection.

Splinting to prevent wrist flexion. Initially this is on a continual basis, but later only at night[5]. When after 6 weeks there has not been any improvement of the symptoms this is not an effective treatment option.

Since the initial reports on cortisone causing dramatic improvements in patients with rheumatoid arthritis, corticosteroids have become established as the most potent anti-inflammatory agents in the pharmacotherapy of various chronic inflammatory diseases[56, 57]. Corticosteroids reduce local inflammation reaction and formation of edema by their effect on vasoconstriction and microvascular permeability [58]. Local corticosteroid injections in the carpal tunnel provides great clinical improvement in patients with carpal tunnel syndrome but with limited long-term effectiveness[59-63].

Though in many cases conservative treatment is started, there is only limited evidence of the efficacy of any of these conservative treatment options[63] and especially the long-term effectiveness[62, 64].

6.5.2 Carpal Tunnel Release

In severe cases of carpal tunnel syndrome or if conservative therapy fails, surgical release of the carpal tunnel is the preferred method of treatment[61, 65].

Until recently, an open carpal tunnel release has been the standard procedure[65, 66]. An incision at the base of the palm is made through the skin, subcutaneous fat, and palmar aponeurosis down to the transverse carpal ligament, which is incised at the medial edge of the carpal tunnel.

Though the carpal tunnel release procedure is usually curative, some patients experience postoperative complications, such as scar sensitivity, pilar pain (incidence 6%-36%)[67, 68], recurrent symptoms[69], and grip weakness, troublesome painful neuromas of the palmar cutaneous nerve, regardless of whether the release was done through an open, mini-open, or endoscopic approach[70].

The endoscopic method is a relative new procedure and has been developed with some reduction in the size of scar and in the recovery time[71] and scar tenderness, although this is not a dramatic improvement[65]. These possible benefits have to be balanced against a probable increased risk of nerve damage[72-75] through restriction of view, and increased cost of the procedure in terms of additional disposable items. The place of endoscopic carpal tunnel release is not yet established.

6.5.3 Tenosynovectomy

Flexor tendon synovectomy is not part of classic carpal tunnel decompression. Many investigators have examined the flexor tenosynovium with respect to its role in increasing the volume of contents within the carpal tunnel. A non-specific chronic

tenosynovitis had long been thought to contribute to CTS. However, several histological studies have demonstrated that inflammatory changes of the tenosynovium are extreme rare and non-inflammatory fibrosis [1-5, 67, 76] and edema[2, 77, 78] are usually noted instead. Our findings also support these findings of non-inflammatory fibrosis.
Most studies have observed neither an added benefit nor an increased rate of morbidity in association with the performance of a flexor tenosynovectomy[76] or a epineurotomy[79] at the time of a carpal tunnel release. Ketchum[67] proposed a flexor tenosynovectomy when the transeverse ligament is not divided in workers who use the palm of the hand in heavy manual work because of decrease in pillar pain.

6.5.4 Future Directions

There has recently been increased attention paid to the structure and potential function of the SSCT[7]. The histopathology of this tissue in patients with CTS is well documented[1-5]. Its role as a potential etiology of CTS has been discussed[2].

If our hypothesis about ultrastructural changes of the synovium in carpal tunnel syndrome patients due to shearing injury is confirmed, then new treatment options for carpal tunnel syndrome may appear. Empiric experience to date suggest that tenosynovectomy does not improve the outcome of carpal tunnel release[76], but if the changes which we observe in the SSCT could be detected earlier in the course of carpal tunnel syndrome, then perhaps an intervention might be considered which could avoid the need for later surgery.

We have initiated a new line of research, which in contradiction to most studies, which have focused on the chronic phase in the development of carpal tunnel syndrome, will investigate the acute phase of the disease.

Hopefully in the future we will be able to test the etiology of CTS, by making a model in which we can induce CTS by pre-stretching the SSCT. The pre-stretching of the SSCT can be done by either surgically shortening one of the tendons or by injecting a solution in to the muscle, that stimulates cellular proliferation and neovascularization, which in turn will cause a muscle to contract by fibrosis. By surgically shortening the tendon there could be an extra problem, given the wound healing of the tendon in the area of interest, which could affect reliability of the setup.

This line of research could investigate the etiology of carpal tunnel syndrome in a step by step approach and subsequently assess the biological process in a more acute phase of the disease.
It would be interesting to investigate the viscoelastic properties of the SSCT, as edema is commonly seen in the histologic samples of synovium of patients with idiopathic and non-idiopathic carpal tunnel syndrome[2, 6, 8, 77, 78]. Edema might be a potential factor also in the stretching of the SSCT.
When this animal model is established the step by step approach of the acute phase will hopefully give more answers.

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CHAPTER 7

Summary

CHAPTER 7

7.1 Summary

Carpal tunnel syndrome (CTS) is the most common compression neuropathy, yet the cause of the compression is in most cases idiopathic. The most common clinical finding associated with the nerve compression is non-inflammatory fibrosis of the subsynovial connective tissue (SSCT) which surrounds the flexor tendons in the carpal canal. The normal SSCT has a multi-layer gliding mechanism, reminiscent of a series of sleeves around the tendon, with each successive sleeve being connected to its neighbors with fine collagenous fibers. We have conducted a series of experiments which have outlined the pathology, kinematics and material properties of the SSCT in normal individuals and in individuals with CTS, and have identified specific features within the SSCT of individuals with CTS that suggest that injury to the SSCT may play a role in the etiology of CTS. Specifically, we have identified evidence of a shearing injury of the SSCT, with fibrosis, and obliteration of the gliding, sleeve-like mechanism, being greatest close to the tendon, and with a tendency of greater degrees of fibrosis being observed in more severe cases of CTS. This fibrosis may be associated with tethering of the SSCT to the underlying tendon, which may increase the work of finger movement in patients with CTS, and impair differential finger movement. Based on these observations, we propose a vicious cycle of SSCT injury and repair as a working hypothesis of the etiology of CTS, and have developed a rabbit model to test this hypothesis in vivo.

7.2 Dutch Summary / Nederlandse Samenvatting

Carpaal tunnel syndroom (CTS) is de meest voorkomende compressie neuropathie, waarvan de oorzaak in de meeste gevallen idiopatisch is. De meest voorkomende pathologische bevinding bij carpaal tunnel syndroom is non-inflammatoire fibrose van het subsynoviale bindweefsel (SSCT) dat zich om de pezen en de nervus medianus in de carpale tunnel bevindt. De SSCT is in de normale situatie een geleidingsstructuur bestaande uit multipele lagen, waarbij elke laag aan de naastliggende vastzit met behulp van fijne collageen vezels. We hebben een serie experimenten uitgevoerd om de pathologie, kinematiek en eigenschappen van de SSCT in normale individuen en in patiënten met CTS in kaart te brengen. Daarbij hebben we specifieke kenmerken van de SSCT in patiënten met CTS aan het licht gebracht, die aanwijzingen geven dat een trauma van de SSCT een rol speelt bij de etiologie van CTS. Bovendien hebben we bewijs gevonden dat er een trauma van de SSCT plaatsvindt door de werking van schuifkrachten, waarbij fibrose en obliteratie van de gelaagde geleidingsstructuur met name in de lagen die het dichtst bij de buigpezen liggen ontstaat en er een toename is van de fibrose in patienten met ernstig CTS. Door de fibrose van de SSCT raakt deze SSCT verstrengeld met de onderliggende buigpees, wat in patienten met CTS een toename met zich mee kan

brengen van de arbeid die een vinger moet verrichten bij een beweging en waarbij ook de ‘differentiële vinger beweging' aangedaan kan zijn. Op basis van deze observaties stellen wij ons een vicieuze cirkel voor van trauma en wondgenezing van de SSCT als de etiologie van carpaal tunnel syndroom en hebben wij een dierexperimenteel model ontwikkeld om deze hypothese in vivo te testen.

CHAPTER 8

Acknowledgement

CHAPTER 8

Acknowledgement

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I also want to thank Dr Kai-Nan An for giving me the opportunity to work in the biomechanics lab with so many excellent doctors, engineers, technicians and students. His teaching and thoughts have made a great impression on me.
Dr. Zhao was a supervisor for all my projects and has helped me out in many ways and it was always a great pleasure to work together. Thanks! You are a great friend and taught me a lot.
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CHAPTER 9

Curriculum vitae and Publications

CHAPTER 9

Curriculum vitae

The author of this thesis was born on December 5th, 1975 in Almelo, The Netherlands. She was raised in Enschede, where she also finished the Atheneum B at the Jacobus College.
At the age of 18 she started medical school at the Erasmus University Rotterdam. In 1998 she started her first research project with Prof.dr. S.E.R. Hovius and Dr. A.B. Mink van der Molen at the Department of Plastic, Reconstructive and Hand Surgery of the Erasmus Medical Centre in Rotterdam.
After medical school she started her research in the Orthopedic Biomechanics Laboratory of the Mayo Clinic, Rochester, MN, USA, for one year with Prof. P.C. Amadio and Dr. K.N. An.
In 2002 she came back to The Netherlands and became a resident in general surgery at the Meander Medical Centre in Amersfoort under the supervision of Prof.dr. T.J. van Vroonhoven and Dr. G.H.M. Verberne.
In the beginning of 2004 she went back to the Biomechanics Lab in Rochester to continue her research now focusing on carpal tunnel syndrome. In November that year she got her first research award, the Patrick Kelly Award for basic research, for her first publication as a first author: *A Histological and Immunohistochemical Study of the Subsynovial Connective Tissue in Idiopathic Carpal Tunnel Syndrome*
In 2005 she worked full-time as a Ph.D. student on this thesis.
Since March 2006 she worked as a resident at the Department of Plastic, Reconstructive and Hand Surgery under the supervision of Prof.dr. S.E.R. Hovius, who has supported her throughout this thesis.

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CHAPTER 10

Inspiration for Change

CHAPTER 10
Inspiration for Change

Dies slowly

Pablo Neruda

Dies slowly he who becomes a slave of habit, repeating every day the same pathways,
who does not change brand, does not risk to wear a new color and does not talk to someone he does not know.

Dies slowly he who avoids a passion, who prefers black to white
and the dots on the "i" to a whirlpool of emotions, just those ones that recover the gleam from the eyes, smiles from the yawns, hearts from the stumbling and feelings.

Dies slowly he who does not overthrow the table when is unhappy at work,
who does not risk the certain for the uncertain to go toward that dream that is keeping him awake.
Who does not allow, at least one time in life, to flee from sensate advises.

Dies slowly he who does not travel, does not read, does not listen to music, who does not find grace in himself.

Dies slowly he who destroys his self-esteem, who does not let anybody help.

Dies slowly he who passes his days complaining of his bad luck or the incessant rain.

Dies slowly he who abandons a project before starting it, who does not ask questions on subjects that he does not know or who does not answer when being asked about something he knows.

Let's avoid death in soft quotes, remembering always that to be alive demands an effort much bigger than the simple fact of breathing.
Only a burning patience will lead to the attainment of a splendid happiness

* * *