2013•2014
FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

Structural differences in collagen organization between the long head of the biceps and the supraspinatus tendon.

Promotor :
Prof. dr. Frank VANDENABEELE

Copromotor :
Prof. Dr. Carl DIERICKX

Kristof Schraepen , Niels Verheyen

Proefschrift ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

universiteit hasselt
KNOWLEDGE IN ACTION

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The authors are grateful to Mr. M. Jans to provide excellent technical support.

Finally an expression of appreciation to family and relatives for the support they have given throughout this process.

Kristof Schraepen, Grote Zouwstraat 29 – 3520 Zonhoven, 12-08-2014 K.S.

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PREFACE

The design of this study is founded in fundamental descriptive research.
The shoulder is characterized by a high mobility, obtained by a variety of muscles surrounding the shoulder joint. These muscle-tendon complexes generate stability. Loss of decent stability means that injuries are more likely to occur. For this reason it is important to understand the causes that affect this stability. Trauma to the shoulder complex leads to limitations in general daily life tasks. Eventually, resulting in a decrease in quality of life.

The focus of this research is situated on two tendons surrounding the shoulder joint, where injury is common and surgery is frequent: the tendon of the m. supraspinatus (SSP) and the long head of the m. biceps brachii (LHB).
The anatomical positions of the two investigated tendons are described. The proximal part of the LHB and the distal part of the SSP lie closely together. This makes it often difficult to differentiate between the tendons (Dierickx et al.).
Dr. Dierickx (2009) started a research on a broad population about the variety of muscles that enter the shoulder. During arthroscopy a striation pattern is found in the SSP and LHB tendons. Jespers and Vancopenholle did research on this unique striation pattern. The pilot study from Jespers and Vancoppenolle (2012) compared both tendons on an ultra-structural level. Conclusions deriving from this study stated that the crimp angle that shows a striation pattern is caused by the thickness of the collagen fibers.
The goal of this thesis is to confirm and extend their research. Differences in collagen organization between the LHB and the SSP will be investigated. During observations clinical and biomechanical correlations will be determined. Onset of the symptoms with the inactive period until invasive surgery is the main factor that will be considered while drawing conclusions.

Reference

Dierickx, C., Ceccarelli, E., Conti, M., Vanlommel, J., Castagna, A. (2009). Variations of the intra-articular portion of the long head of the biceps tendon: a classification of embryologically explained variations. Journal of Shoulder and Elbow Surgery, 18(4), 556-565.

Jespers, G., Vancoppenolle, G. (2012). Arthroscopic and fine structural differences in collagen organization between the long head of the biceps tendon and the supraspinatus tendon - A pilot study.

Specimens of the SSP and LHB will be investigated with the electron transmission microscope (TEM) at the University of Hasselt, Belgium. Biopsies will be recovered through arthroscopy of the shoulder (Dierickx C., orthopedic surgeon - Jessa Hospital, Campus Virga Jesse).

Tasks in completing this duo-thesis are equally divided. Collecting the data-acquisition is in cooperation with student and promoter. The data processing and writing tasks are divided in function of the students' possibilities.

The research design is created independently, led and approved by our promoter. An expert was required for recruiting the data. Guidance is provided for technical aspects of the TEM research.

Structural differences in collagen organization between the long head of the biceps and the supraspinatus tendon.

Clinical and biomechanical correlations

Structural differences in collagen organization between the long head of the biceps and the supraspinatus tendon.

Clinical and biomechanical correlations

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ABSTRACT:

Background Stability in the shoulder complex is vital. Decrease in stability and trauma causes limitations in daily life tasks. This paper aims to provide better knowledge in the structural organization of two tendons surrounding the shoulder: The long head of the biceps (LHB) tendon and the Supraspinatus (SSP) tendon.

Materials and Methods A total of 14 tendon samples are gained through arthroscopy (n=7). Biopsies are immersed in fixative to investigate by light and transmission electron microscopy (TEM). ImageJ is used to determine the amount of collagen fibers and collagen density. The Goutallier scale is used to grade the fatty degeneration of the involved muscles.

Results The LHB tendon shows larger collagen diameters when compared with the SSP. Differences between the diameters are more present in patients with a shorter inactivity period. In addition, the collagen diameter decreases as the time between onset of symptoms and surgery increases.

Discussion A smaller collagen diameter in patients with a longer inactivity period can be caused by degeneration and/or regeneration of collagen fibers. Hyperplasia and hypertrophy of fibroblasts in combination with the higher amount of fibers, suggests regeneration of collagen fibers. This is a mechanism that is activated to compensate the loss by degeneration and restore its load bearing capacity.

Conclusion The higher amount of small collagen fibers in patients with a longer inactivity period can be caused by regeneration of collagen fibers. In this way it maintains the same collagen density and restores its load bearing capacity. This is only a hypothesis and further research is necessary to confirm these results.

Keywords: Long Head Biceps, Supraspinatus, Tendon, Arthroscopy, Electron Microscopy

Introduction

Regular physical activity benefits general health, where injuries are a threat. Strains and sprains are most common. Biomechanically, an injury occurs when a muscle or tendon is under higher mechanical load compared to its capacity (Neumann, 2002). To lower the incidence of injuries, it is important to understand the biomechanical aspects of the shoulder complex.
The anatomical position and its structural composition are essential components in understanding the muscle's loading capacity of a tendon. This paper will focus on two tendons surrounding the shoulder complex: the long head of the m. Biceps brachii (LHB) and the supraspinatus tendon (SSP).

A tendon consists of collagen and elastin (macromolecular proteins) surrounded by a proteoglycan-water matrix including proteoglycans and glycoproteins (Culav, 1999).
Approximately 65-80% of a tendon consists of collagen fibrils. The triple matrix is the precursor of collagen, which determines the mechanical properties of collagen. It consists of 3 polypeptide chains, folded into a ropelike coil (Culav, 1999).
The fibrils in tendons are mostly collagen type I. Collagen Type III is the second most present (Järvinen, 2004). Fibrils unite in bundles of collagen fibers from 2 to 10μm (Junqueira en Carneiro, 2004).
The function of a tendon is to transfer forces from muscle to bone and resist tension. Therefore tendons need to be tensile and bear high forces in longitudinal, transversal and rotational movements. To bear these forces, the fibrils within one collagen fiber are oriented in all directions (Kannus, 2000). The strength of these tendons is determined by the size of collagen fibrils located in the tissue (Parry, 1978). This results in a complex ultrastructure of the tendon (Fig. 1).

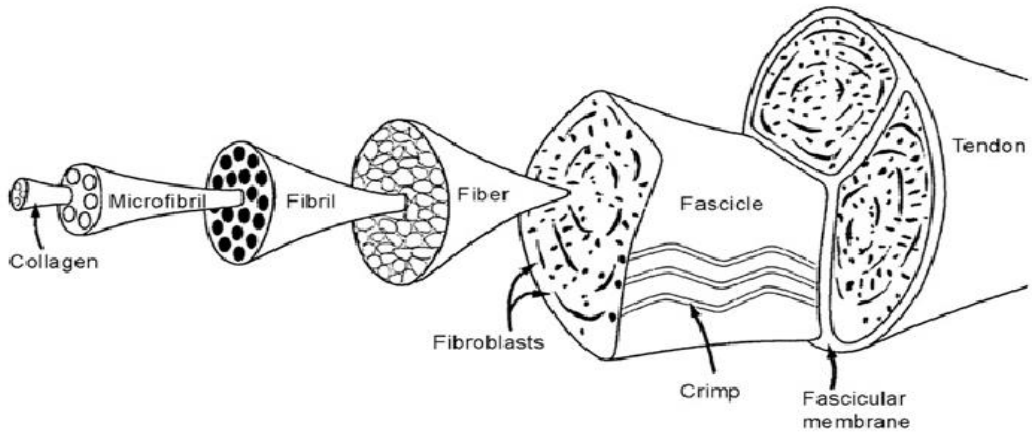


Figure 1 – Hierarchical organization of tendons (adapted from Killian, 2012).

The network of a tendon matrix shows a regular sinusoidal pattern called crimps. It acts as a shock absorber that allows small elongations in a longitudinal direction (1-3%), avoiding damage caused to the tissue (Järvinen, 2004).

m. supraspinatus

The origo of the SSP is located in the fossa supraspinata, where it partially merges with the superior joint capsule. It inserts at the upper plane of the tuberculum major. The m. SSP is innervated by the n. suprascapularis (C4-C6) (Platzer, 2008).

The m. infraspinatus fuses laterally with the m. supraspinatus and is difficult to separate from the SSP (Curtis, 2006).

The SSP is involved in shoulder abduction in conjunction with the m. deltoideus. By transferring the forces through the capsule it provides dynamic stability to the glenohumeral joint up to 120°.

Scapula-thoracic movement starts at 120° and has a range of motion until 180°. SSP also has a function in the static stability in the shoulder. It generates active forces that are directed almost parallel to the superior capsule force vector (Neumann, 2002).

Like all four rotator cuff muscles it stabilizes the caput humeri against the fossa glenoidale of the scapula. This helps in resisting forces against gravity in the upper limb.

m. biceps brachii

The anatomy of the LHB differs in a way that it passes through both intra- and extra-articular portions. The LHB originates from the tuberculum supraglenoidalis. Its insertion has multiple variations (Hill A.M., 2008). Dierickx et al describes that the SSP and the LHB embryonically have the same origin, resulting in 12 different variations of separation in the intra-articular LHB portion.

The LHB passes through the sulcus intertubercularis. After merging with the short head of the biceps, it inserts at the tuberositas radii. It radiates in the fascia antebrachii through an aponeurosis, the lacertus fibrosus (Platzer, 2008). It works as a sliding tendon within the sulcus intertubercularis and has a function in the caput humeri as a hypomochlion (Kolts, 1994).

The main movements of the LHB are abduction and internal rotation of the humerus. In cooperation with the biceps brachii caput breve, it provides an anteflexion in the humerus. The LHB also performs flexion at the elbow. It gives

supination in the forearm when the elbow is flexed. The LHB is innervated by the n. musculocutaneus (C5-6) (Platzer, 2008).
The LHB tendon runs close to the proximal aspect of the SSP when it enters the bicipital groove. The bicipital groove is covered with connective tissue, which extends over the greater tuberosity and blends with the supraspinatus. Therefore surgeons find it difficult to distinguish both tendons during arthroscopy when there is a full rupture (Boon, 2004).

This study is founded by the macroscopic observations during arthroscopy. While performing surgery a macroscopic striation pattern is observed by C. Dierickx in the LHB and SSP tendons.
By comparing these tendons ultra-microscopically in a pilot study, Jespers and Vancopenolle give an explanation for these patterns. Conclusions state that the unique striation derives from a crimp angle that is related to the thickness of the collagen fibers (Fig. 2).

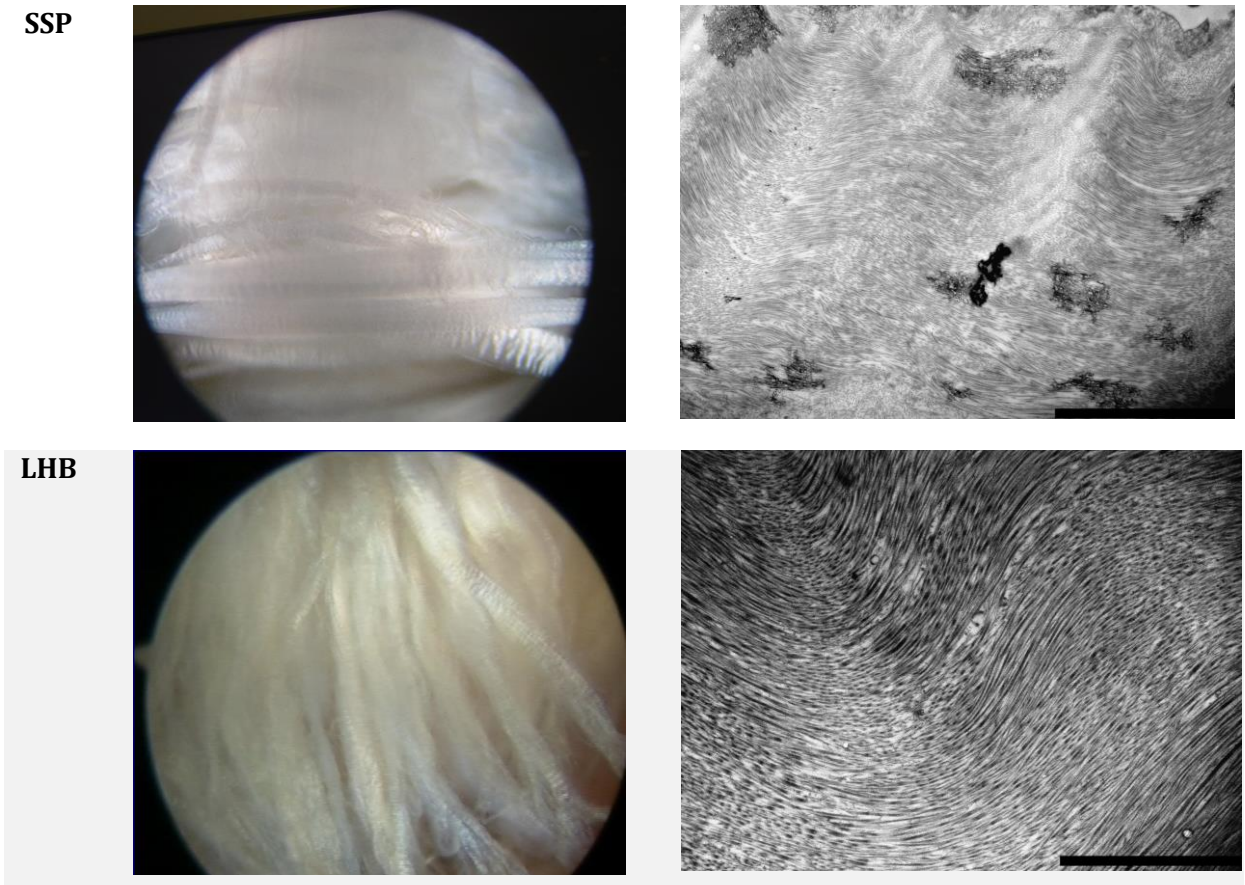


Figure 2 - Results adapted from Jespers and Vancopenolle showing striation patterns. LHB striation patterns are more frequently observable. SSP striation patterns have more zoom to find obvious patterns. TEM image – Bar = 5μm

The objective of this study is to determine structural collagen differences between the LHB and the SSP tendon. During observations clinical and biomechanical correlations will be determined. The results are linked with onset of symptoms with the duration of inactivity until surgery.

Materials and Methods

Subjects

The research includes both genders aged between 43 and 73 years old (average age 60.5). The subjects had a shoulder arthroscopy, performed by C. Dierickx, Orthopedic surgeon, Jessa Hospital, Hasselt. The patients have no other health issues that could influence the state of their shoulder pathology.

The onset of symptoms can be divided in traumatic events (e.g. a fall) and due to an involvement of a chronic pathology (Frozen shoulder, chronic shoulder subluxation). Demographic features of all patients are collected in table 1.

Most patients underwent a standard procedure for a SSP suture: arthroscopic subacromial decompression in combination with a bursectomy and bicepsectomy if necessary. At first, removing of pathologic tissue followed by widening of the subacromial space. Secondary, the suture for the SSP tendon is placed.

By exception, one patient has a different procedure due to repetitive shoulder luxations with a chronic character. The placement of a reversed delta Xtend prosthesis was necessary.

This study is approved by the ethical committee (Appendices 1). Prior to participation all patients are asked to give their informed consent (Appendices 2).

Patient	Age (years)	Onset pathology	Surgery	Acute / Chronic
1	64	03 oct 13	18 dec 13	Acute
2	59	01 sept 13	11 dec 13	Acute
3	48	20 june 13	20 nov 13	Acute
4	71	April 2013	18 nov 13	Acute
5	43	May 2012	28 aug 13	Acute
6	73	August 2012	21 aug 13	Chronic
7	65	16 may 13	28 aug 13	Chronic

Table 1 – Demographic data from the included patients

Methods

- *Tendon biopsies and arthroscopy*

A total of 14 tendon samples are gained from patients (n=7) undergoing arthroscopic surgery (Jessa Hospital) due to a repair of the SSP or LHB tendon. Samples are harvested through identical arthroscopic procedures from pathologic tissue using a knee-arthroscopy type meniscus grasper (Fig. 3). SSP samples are taken as proximal to its insertion as possible (on the edge of the tissue rupture) and the LHB approximately 1 centimetre from its origin.

Figure 3 – Grapser used during arthroscopy to harvest the biopsies

Biopsies are taken from patients with shoulder pathology during an arthroscopy. Local anesthetic is provided by a scalenusblock. During arthroscopy the shoulder is rinsed with 0.9%NaCl with the use of hydrostatic pressure.

Tissue samples from the long head biceps and supraspinatus tendon are obtained. Sizes from the samples differ from 1-3 mm^3. All pathologic tissue is included.

Immediately after obtaining the biopsies, the specimens are immersed in a solution of 2% glutaraldehyde in 0,05 M cacodylate buffer (pH 7,3) for further investigation under the transmission electron microscope.

Surgeon Dierickx C. Jessa Hospital, Campus Virga Jesse, will perform all arthroscopies.

- *Transmission Electron Microscopy*

A transmission electron microscope (Philips EM400) is used to investigate the fragments on differences in collagen organization between the SSP and the LHB tendon.

While immersed in the fixative, fine dissection into 1-mm^3 blocks is performed in the laboratory under a stereomicroscope. The tissue is placed in fresh fixative and further fixed by immersion of the tissue for approximately 24 hours.

Selected samples are washed in cacodylate buffer, postfixed in 2% osmium tetroxide for 1 hour, stained with 2% uranyl acetate in 10% acetone for 20 minutes, dehydrated through graded concentration of acetone and embedded in Araldite.

Semi-thin sections (0.5 µm) are stained with a solution of thionin and methylene blue (0.1 % aqueous solution) and examined especially for collagen fibers organization (striation) and possible patterns.

Ultrathin (0.06 µm) slices of selected tendon tissue blocks are stained with uranyl acetate and lead citrate for examination under a Philips EM400 electron microscope. Mr. Jans M has done the processing of these steps.

- Image analysis

The processing program ImageJ is used to count collagen fibers and determine the covered surface area of collagen fibers.

The surface is counted from an image with an optical zoom of 22000, made with the TEM. The surface measured four square micrometers. The same surface area (22k) is used to determine the density of collagen fibers in this area.

- Goutallier grade

Goutallier grades the fatty degeneration of the rotator cuff muscles throughout CT or NMR scans (Table 6). All patients underwent a CT or NMR to evaluate every case. A severe grade (Three or higher) can indicate a higher grade of injury to the rotator cuff muscles. This scale is used to determine the grade of degeneration, to differentiate disuse of the compared muscles.

Results

All patients incorporated in the study are evaluated by onset of their pathology and time until surgery. This evaluation is our main interest and thus essential when drawing conclusions. Clinical and biomechanical correlations are evaluated throughout the results. The results are classified from macroscopic to microscopic findings.

Arthroscopy

Arthroscopic surgery in the shoulder shows the macroscopic striation pattern (Fig. 2) in the SSP and the LHB tendon. LHB shows more frequently striation patterns during arthroscopy. In comparison to the SSP, a higher magnification is necessary to have a clear view on the striation pattern. Because of that, the striation pattern in the SSP is less frequently observed than in the LHB.

Light microscopy

Microscopic findings are evaluated by light microscopy to investigate the striation patterns that are found during arthroscopy in both LHB and SSP tendons. The time between onset of symptoms and surgery is taken into account.

As seen in table 2, patients with a traumatic event and a shorter inactivity period (2-3 months) show a larger periodicity in the LHB striation pattern when compared to the SSP. Findings for patients with a pronounced inactivity period, due to more chronic pathologic involvement, are less obvious. Striation patterns coming from patients with a high inactivity period differ from these found in the traumatic patients. LHB striation patterns are smaller and therefore more similar to the SSP findings.

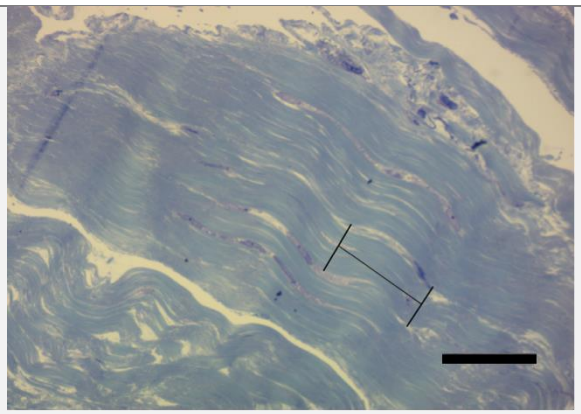
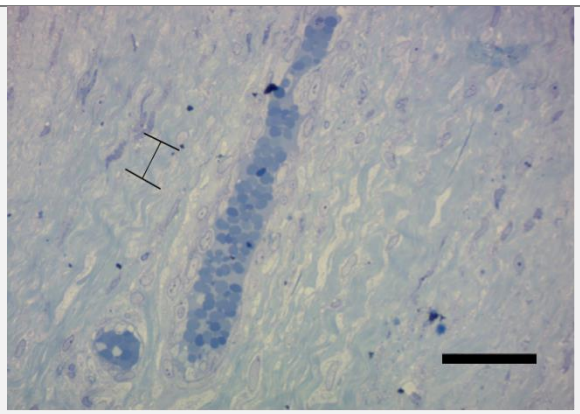
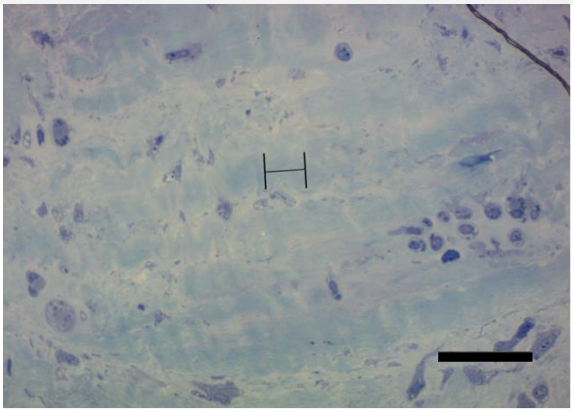
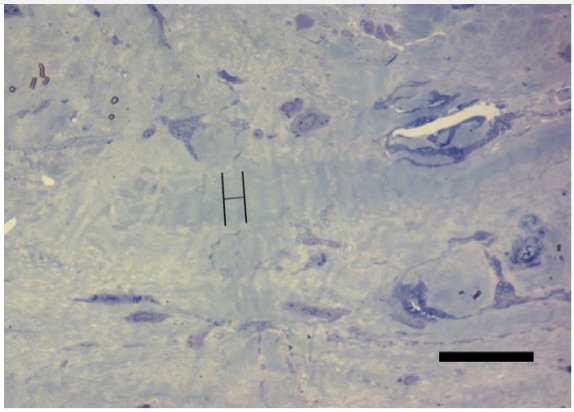
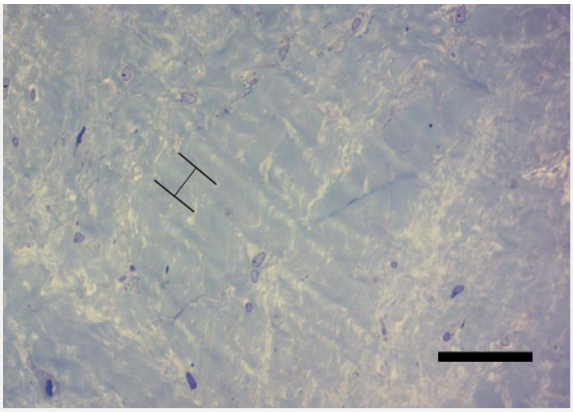
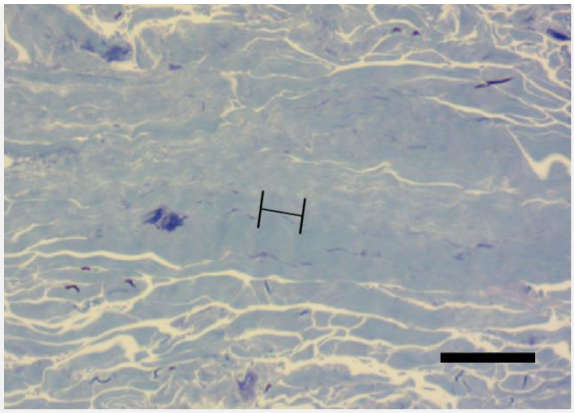
	LHB	SSP
ACUTE PHASE (2 MONTHS AFTER INJURY)		
ACUTE PHASE (15 MONTHS AFTER INJURY)		
CHRONIC PHASE (15 MONTHS AFTER DIAGNOSIS)		

Table 2 – Panel of representative light microscopic findings showing differences in striation patterns in the three groups of patients, classified according to the onset of symptoms and moment of surgery – Bar = 20µm

Next to the found striation patterns of collagen fibers, another remarkable observation is made through light microscopy. A hyper-cellularity in the tissue matrix is seen (Fig .4). This phenomenon is more present in patients with a longer inactivity period.

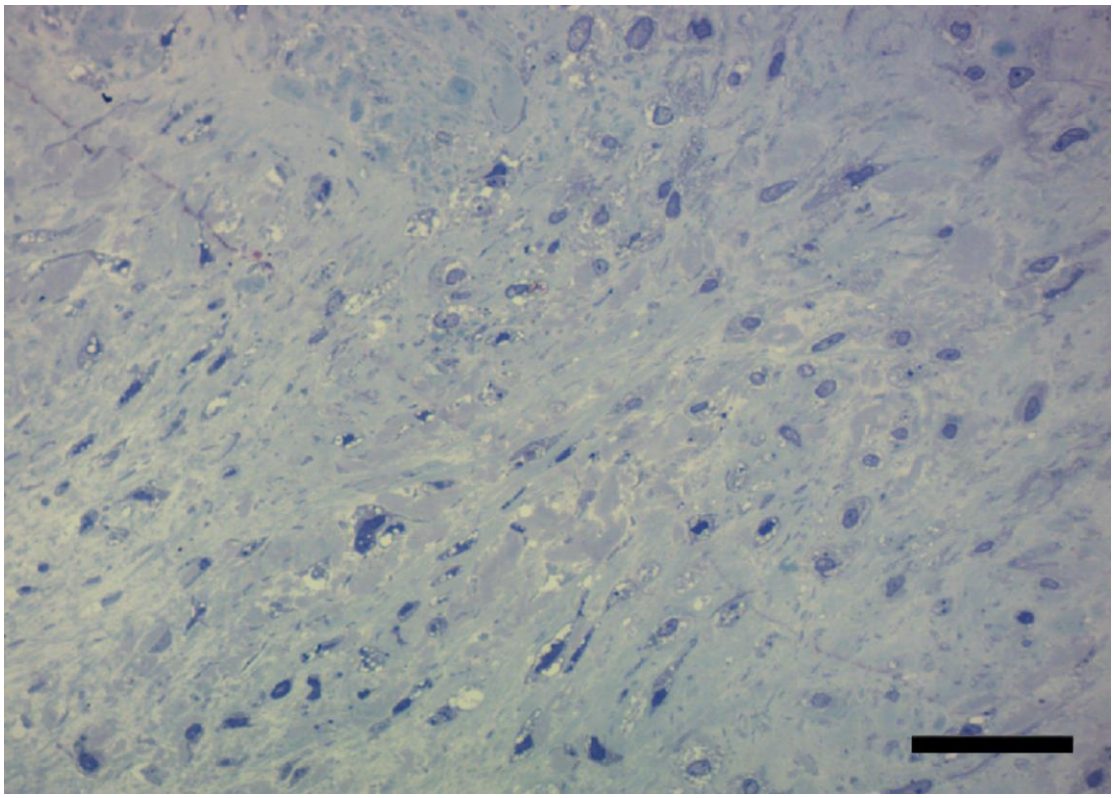


Figure 4 – Light microscopy: The typical hyper cellularity in patients with a longer inactivity period – Bar = 20μm

Transmission electron microscopy

Table 3 gives an inter-patient comparison in collagen fiber diameter between the SSP and LHB tendon. This is observed by the use of a transmission electron microscope. Results are evaluated in chronologic order by onset of the pathology and the duration of inactivity until invasive surgery is done.

Based on TEM, patients are classified into three groups:

1) Patients with a traumatic event and less inactivity (patients 1,2 and 3) show a larger diameter in the LHB when compared to the SSP.

2) Patients with a traumatic event and a longer inactivity period (patients 4 and 5) show more collagen fibers with a larger diameter but smaller in comparison to group 1 (shorter period of inactivity, 2-5 months).

3) Patients with a chronic pathology (patients 6 and 7) and a larger period of inactivity, show smaller differences in collagen diameter between LHB and SSP.

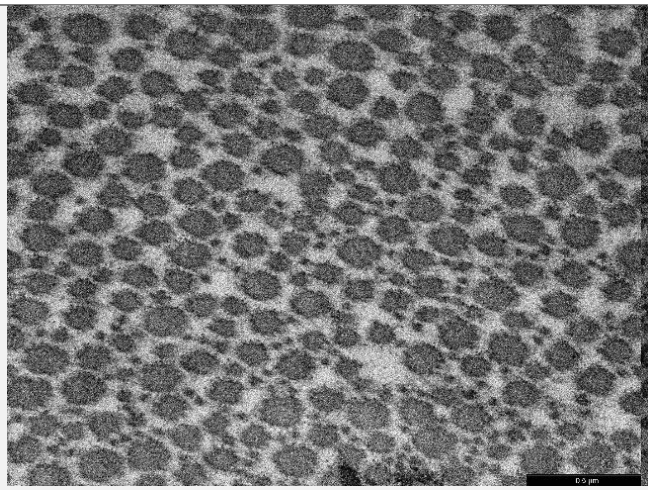
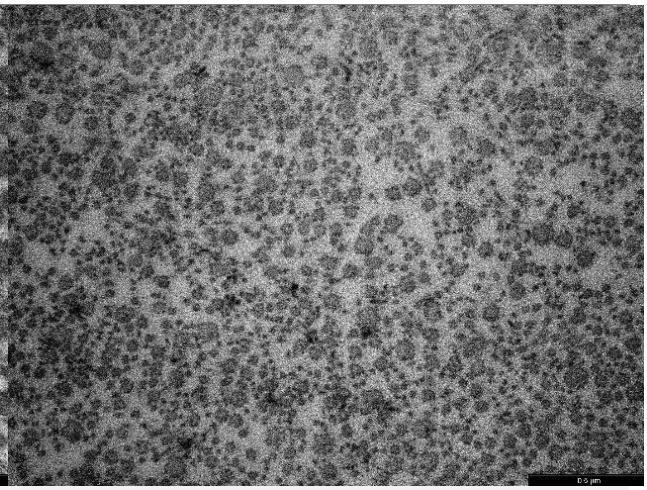
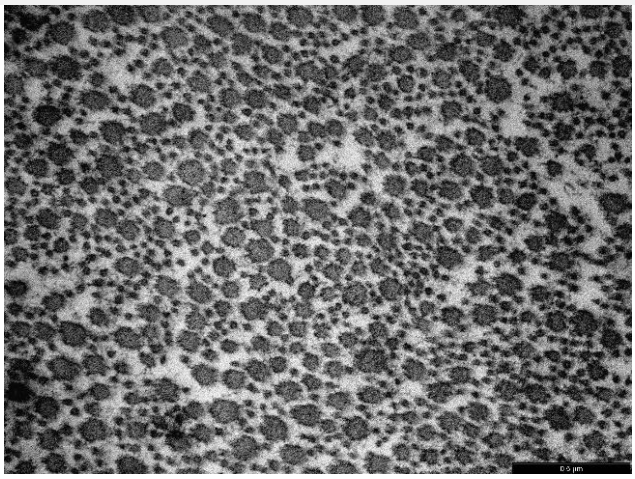
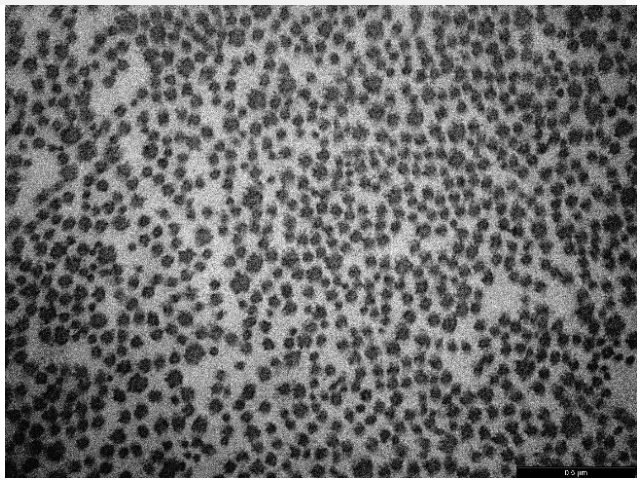
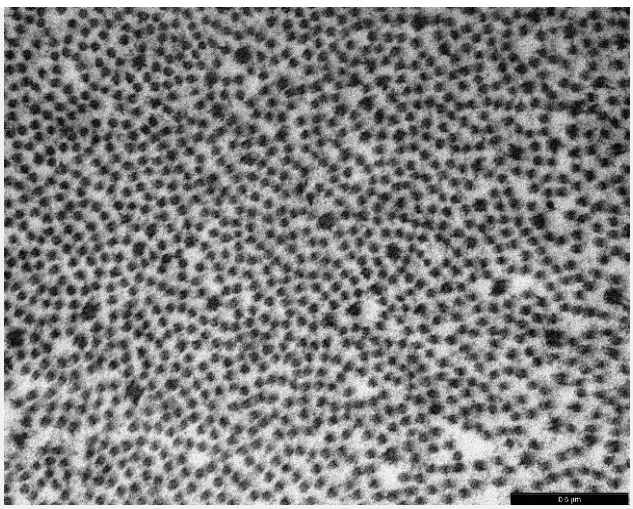
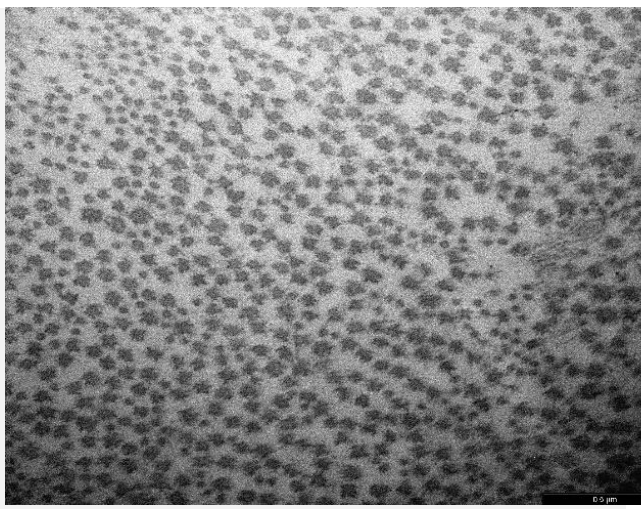
	LHB	SSP
ACUTE PHASE (2 MONTHS AFTER INJURY)		
ACUTE PHASE (15 MONTHS AFTER INJURY)		
CHRONIC PHASE (15 MONTHS AFTER DIAGNOSIS)		

Table 3 – TEM: differences in collagen fiber diameter and collagen density, classified into time between onset of symptoms and surgery – Bar = 0.5µm

Results deriving from the light microscopy showed a hyperplasia of cells in the tissue matrix. TEM results made it possible to show the activity of these individual cells.

Fig. 5 shows hypertrophy of a fibroblast in the tendon tissue matrix. This phenomenon indicates a higher activity of cells in the damaged tissue. Hyperplasia in combination with hypertrophy of fibroblast is more present in the patients with a longer period between pathology and surgery.

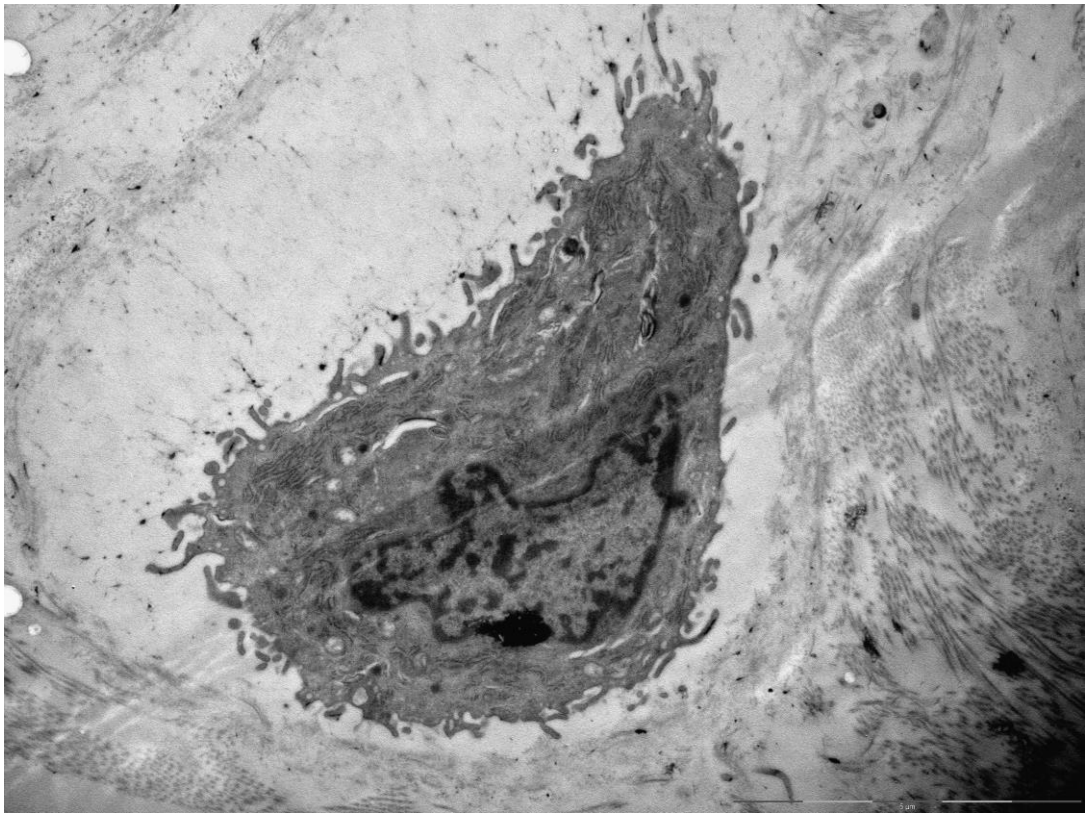


Figure 5 – TEM: Hypertrophy of a fibroblast indicates cellular activity in the tendon matrix. Showing abundant rough endoplasmatic reticulum – Bar = 5µm.

Table 4 shows results from counting collagen fibers on images taken with the TEM (x22000). Findings from the LHB in patients from group 1 show more fibers when compared to group 3. It is clear that the amount of fibers increases with a higher inactivity period.

Results deriving from the SSP show a smaller difference in the amount of fibers between group 1 and 3. SSP shows the opposite and gives a decrease in fibers when inactivity is longer. This trend is less obvious when compared to the LHB.

Table 5 shows the presence of collagen, relative to the whole tissue. Results give a small difference in density between the LHB and the SSP. SSP findings show a smaller covered surface area than the LHB, as shown in table 5. However, only a very small difference is found between group 1, 2 and 3 within both tendons.

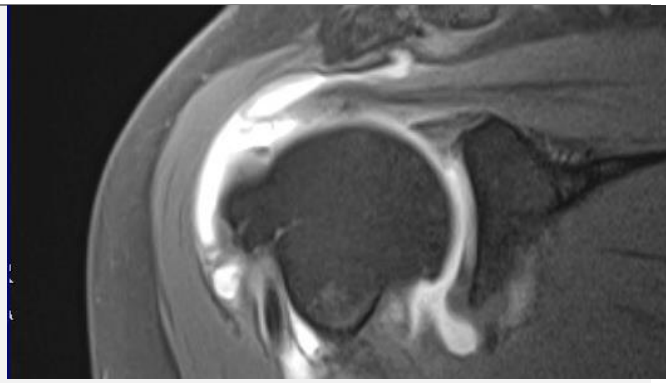
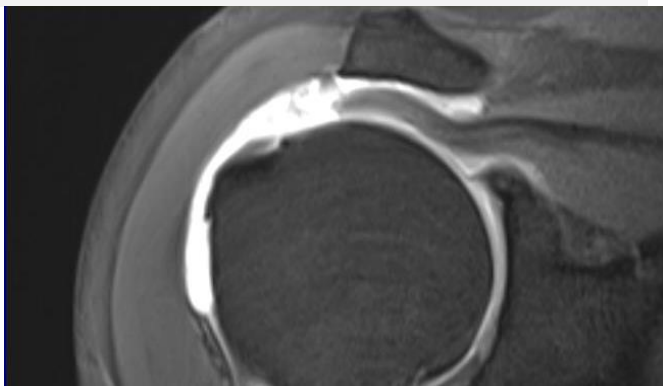
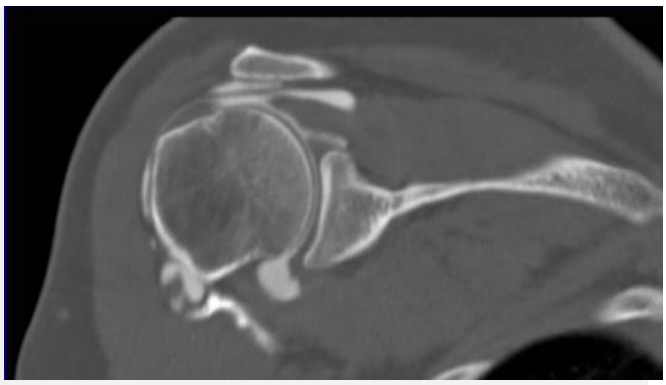
	LHB FIBERS (PER 4 µM²)	SSP FIBERS (PER 4 µM²)
ACUTE PHASE (2 MONTHS AFTER INJURY)	283 (158 – 377)	623 (598 – 649)
ACUTE PHASE (15 MONTHS AFTER INJURY)	550 (440 – 703)	563 (498 – 639)
CHRONIC PHASE (15 MONTHS AFTER DIAGNOSIS)	637 (428 – 846)	551 (506 – 597)

Table 4 – The amount of collagen fibers, classified into time between onset of symptoms and surgery

	LHB COLLAGEN DENSITY (%)	SSP COLLAGEN DENSITY (%)
ACUTE PHASE (2 MONTHS AFTER INJURY)	61,68 (61,52 – 61,84)	53,59 (53,41 – 53,76)
ACUTE PHASE (15 MONTHS AFTER INJURY)	60,23 (59,73 – 60,87)	54,88 (54,48 – 55,52)
CHRONIC PHASE (15 MONTHS AFTER DIAGNOSIS)	59,65 (59,59 – 59,70)	51,28 (50,36 – 52,19)

Table 5 – The collagen density, classified into time between onset of symptoms and surgery

In grading the scale of goutallier in patients before deciding if surgery is done, table 6 shows that the grade is higher in patients with a higher inactivity period (group 3) compared with the patients with less inactivity (group 1).

STAGES	PATIENT-GOUTALLIER GRADE	INACTIVITY PERIOD (MONTHS)	NMR/CT
ACUTE STAGE	Pt 1 = Grade 2	2m	
	Pt 2 = Grade 1	3m	
	Pt 3 = Grade 1	5m	

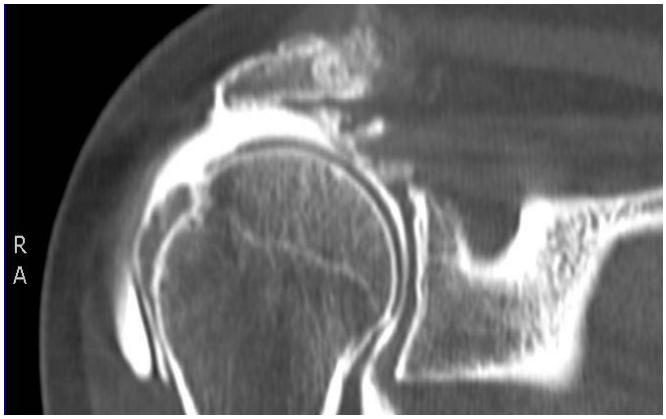
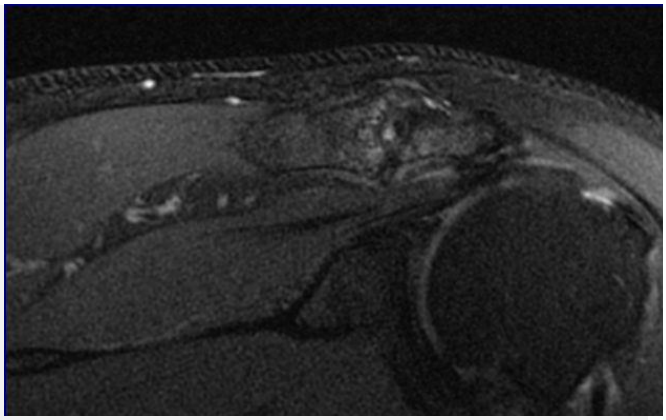
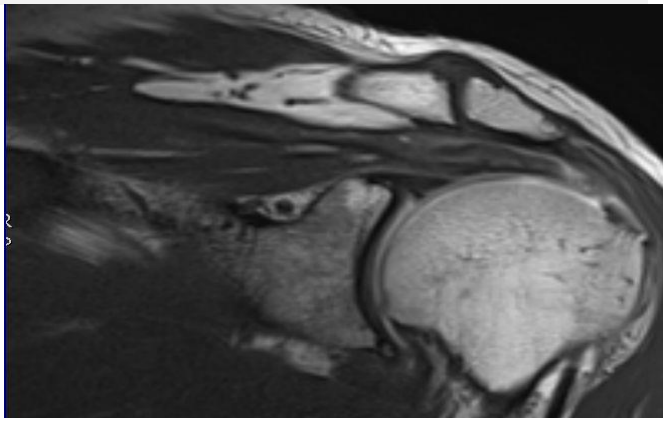
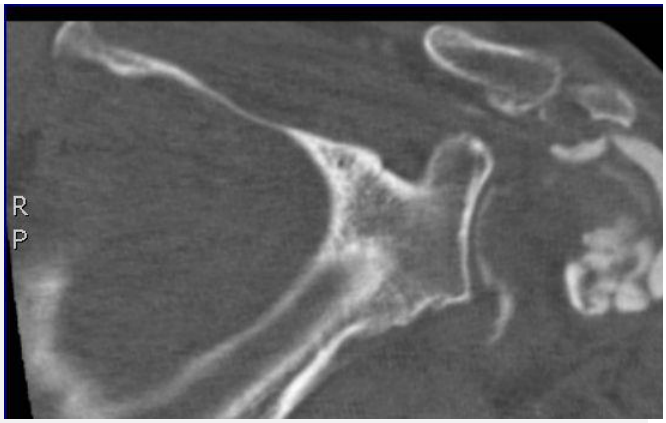
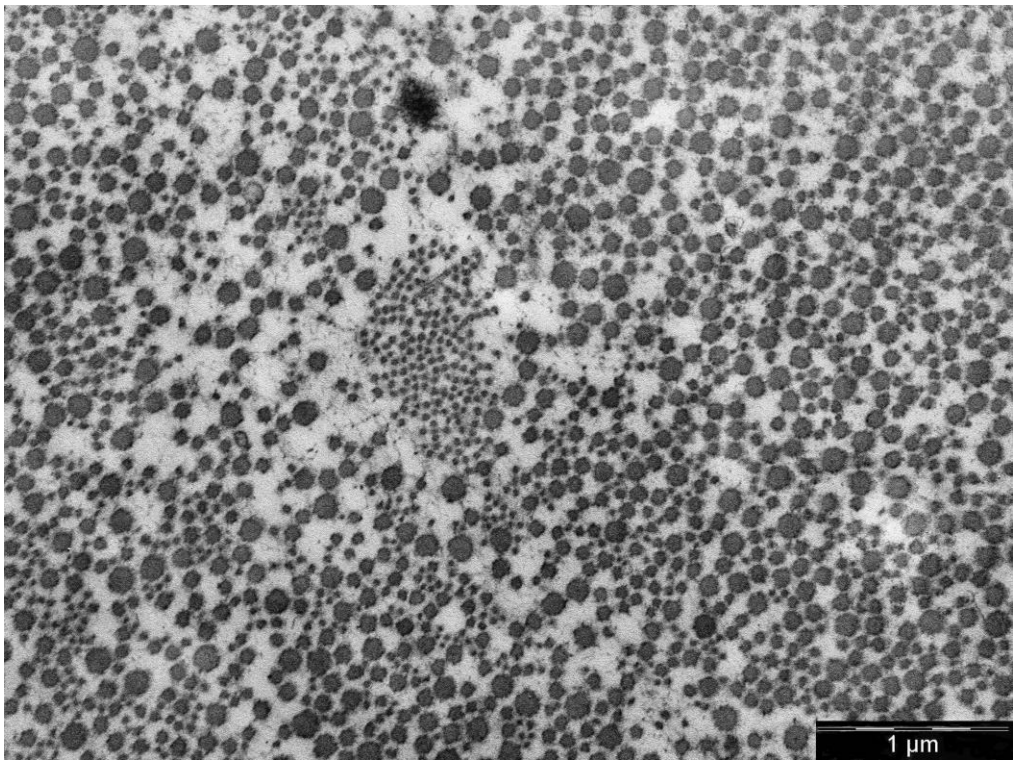
ACUTE STAGE	Pt 4 = Grade 4	7m	
	Pt 5 = Grade 3	15m	
CHRONIC STAGE	Pt 6 = Grade 2	12m	
	Pt 7 = Grade 4	15m	

Table 6 – Patients ranked in chronologic order where Goutallier grades are determined. CT and NMR show the fatty muscle degeneration in patients.

In patients with a high inactivity period (group 3), bundles of small collagen fibers are found, closely packed together between larger collagen fibers (Fig. 6a and 6b).



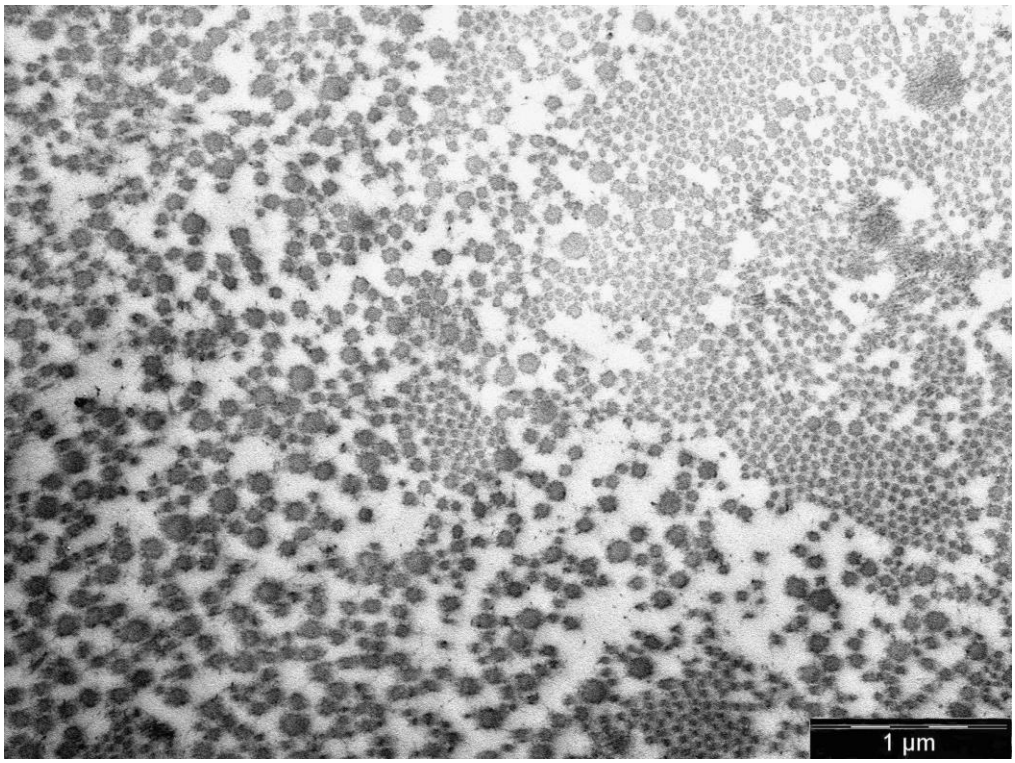


Figure 6a and 6b – TEM: Small collagen fibers that are packed closely together in between larger fibers. (Patients 6 and 7) – Bar = 1µm

Table 7 gives a summary from macroscopic to microscopic findings. This overview shows a red line through all stages of this research. Group 1 with a grade 1 goutallier shows a larger periodicity under light microscopy, resulting in larger collagen fibers that are seen in TEM research. In comparison, group 3 with a goutallier grade of 4 gives a smaller periodicity and smaller collagen fibers.

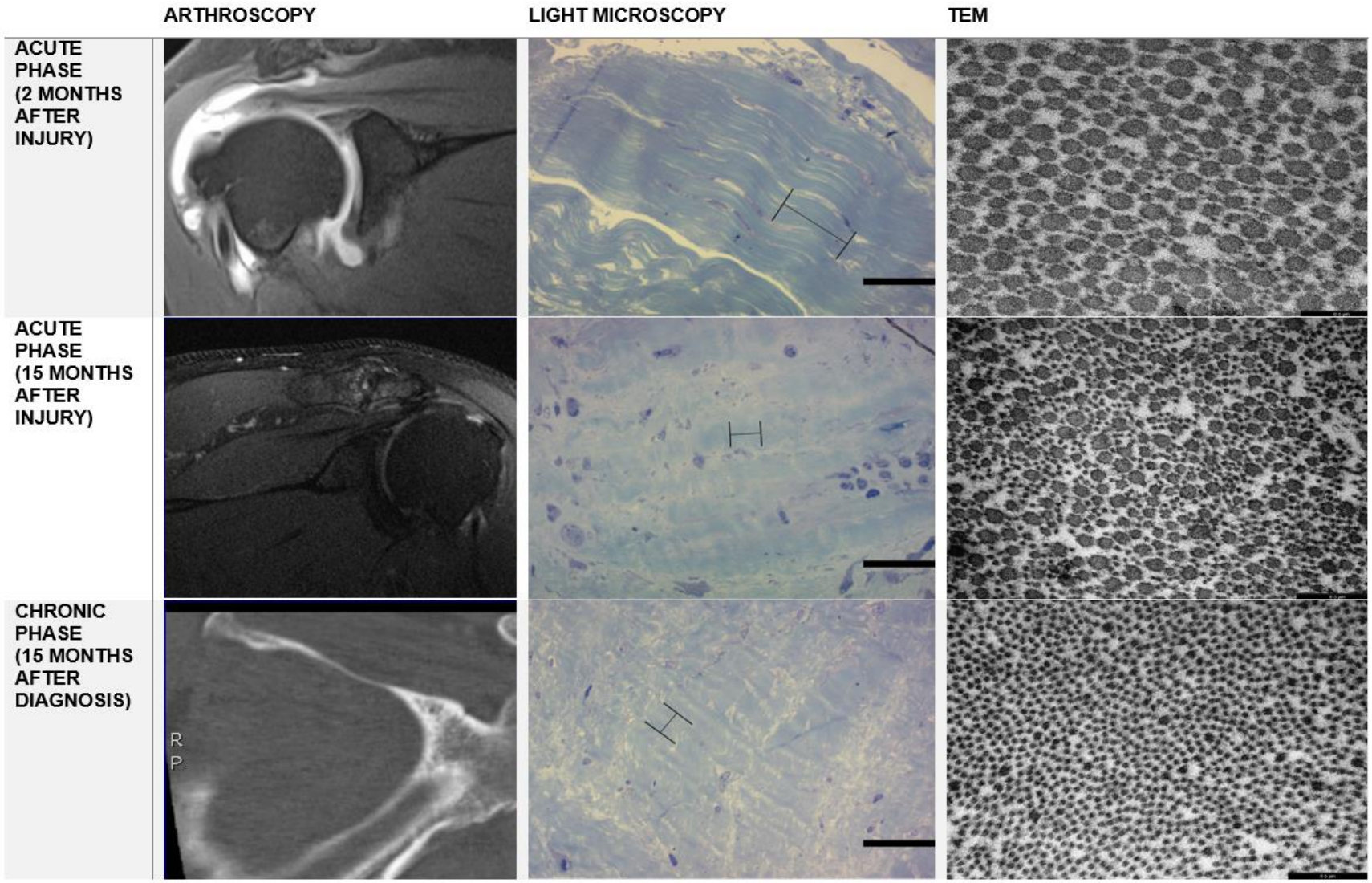


Table 7 – Summary: macroscopic to microscopic findings in chronologic order with the stages of inactivity.

Discussion

LHB and SSP tendon observations during light microscopy reveal a striation pattern. Organization patterns between the tendons are similar, besides a greater periodicity in the LHB in comparison to the SSP. Järvinen (2004) stated that thicker fibers exposed to higher mechanical loads demonstrate a larger crimp angle (LHB), while tendons that carry out lighter strains have smaller collagen fibers with a smaller crimp angle (SSP).
Conclusions from the study Jespers and Vancoppenolle (2012) stated that the crimp angle that shows a striation pattern is caused by the thickness of the collagen fibers. These findings are confirmed by the observations through the light microscopy (table 2).
Findings in the transmission electron microscope about striation patterns are not clear due to the use of pathological tissue. As a result of this pathologic tissue the fibers in these tendons are non-aligned. With a large magnification it is nearly impossible to find clear results.

Chronic pathological tissue with a larger inactivity period (group 3) (Appendices 3) shows a smaller collagen diameter of the LHB tendon when compared to traumatic pathologic tissue (group 1 and 2). Hijioka (1993) states that the amount of tendons that degenerate and show tears increase in the fifth to sixth decade of life. A higher age is in accordance with larger ruptures. However, there is no increase of incidence. Biopsies taken from these patients with an average age of 60.5 validate this theory.
Degeneration occurs by thinning and becoming more fragile of collagen fibers. In addition, large vacuoles occur with dense fine granular materials between the collagen fibers (Hashimoto, 2003). Riley et al. (1994) stated that there is a possibility of cell intrusion, creating a new matrix with collagen type III. There is no clear evidence that these results are a secondary response to degeneration or a primary cause of weakening the tendon that will eventually lead to rupture.

Findings in figures 4 and 5 indicate hyperplasia and hypertrophy of fibroblasts in the damaged tissue.
A possible hypothesis to explain these results is that collagen regeneration is possible after degeneration due to a higher activity of fibroblasts within the tissue matrix. In this way, larger damaged collagen fibers will be replaced by thinner collagen fibers.

Although the thick fibers are replaced by thinner fibers, density remains approximately the same in both acute and chronic stages. This means that the amount of new thinner fibers needs to be higher than the previous thicker fibers

to have the same density. In this way the tendon contains its strength and the capacity to bear heavy loads.
Density taking from pathologic tissue is lower than healthy tissue. According to Culav (1999), approximately 65-80% of a tendon consists of collagen fibrils. Findings reveal a range of 59,65% to 61,68% for the LHB and 51,28% to 53,59% for the SSP. An explanation can be found in the degeneration characteristics of tendons.

The presence of local bundles of small fibers packed together could attribute to this hypothesis. Further more, these local accumulations can be seen as a ‘locus minoris’. This is only present in patients with a longer inactivity period (group 3). In these patients fibroblasts can intrude the tissue matrix to start regeneration and restrict the degeneration process.
A hypothesis deriving from these results is that mechanical loads that interfere in a tendon with a ‘locus minoris’ can cause ruptures due to a different load bearing capacity of the tendon. There is always one fiber bundle that is weaker and the primary area where the rupture occurs. Killian et al. stated that mechanical properties in tendons derive from type I collagen fibers. Organization of these fibers arrange along the axis of the tendon. Collagen fibers are tightly packed for transmission of loads. This creates a high stiffness in the direction of the fiber orientation.
This is a hypothesis, based on biomechanics and the mechanical properties of a tendon.

Evidence to strengthen the hypothesis that a ‘locus minorus’ is a result of degeneration is hard to find. Hereby the hypothesis where a ‘locus minoris’ is the cause of degeneration cannot be excluded and should be taken into account. Results show only bundles of smaller collagen fibers in patients with an inactivity period of 1 year and more. Future investigations are necessary to strengthen these results.

The observations in this paper show a link to fatty degeneration as based on CT and NMR findings. This theory is confirmed by a higher Goutallier grade in patients with longer inactivity periods (Table 6). Tendon bundles become thinner until rupture or severe instability occurs. Goutallier et al. stated that a higher grade does not interfere with a higher prevalence in rupture of the SSP tendon. If a higher goutallier grade is present, than the rupture that occurs (trauma) will be larger. A strength loss of the muscle is present due to degeneration.

Further studies are necessary to confirm the observations in this pilot study. A larger sample size is required to validate collagen organization between the LHB and SSP tendon.
Because of the large variation in collagen diameter, it is hard to draw conclusions from the absolute amount of collagen fibers present in the tissue. Although the difference in diameters between these patients is certainly present, there is not any form of statistical analysis applied; therefore the findings still remain hypothetical.
Additionally, it would be useful to characterize the types of collagen using immunohistochemistry.

Tissue samples were harvested from patients undergoing a shoulder arthroscopy or surgery after a traumatic event or chronic pathology. It would be very useful to compare these findings with normal healthy tendon tissue, but this is ethically not possible.

From a physiotherapists' point of view, the question is how useful it would be for a tendon to be fully restored when it has a fatty degenerated muscle that is not able to bear normal biomechanical forces. The effects of degeneration and/or a prolonged inactivity are visible in the tendons, but how severe are the consequences when linked to its muscle. Maybe this is one of the reasons why, according to Hijoka (1993), the incidence of ruptures have no correlation with the severity of degeneration.

Conclusion

The pilot study from Jespers and Vancoppenolle showed a smaller collagen diameter in the SSP compared with the LHB. This study includes the time between the onset of symptoms and surgery, which can be interpreted as acute or chronic. Results show a smaller collagen diameter in patients with a longer inactivity period, although the amount of collagen fibers increases. This can indicate a regeneration of fibers at the place where the original fibers are damaged. The hyperplasia and hypertrophy of fibroblasts confirm this hypothesis as they suggest a higher cell activity. Due to the higher amount of fibers, density remains the same so that the tendon does not lose its bearing capacity.

Acknowledgements

The authors gratefully acknowledge the help of Dr. C. Dierickx, Department of Orthopeadics, Jessa Hospital, Hasselt (Belgium) in obtaining the tendon biopsies. We wish to express appreciation to Mr. Marc Jans, University of Hasselt, Belgium, for his excellent technical support during this research.

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Appendices – Appendix 1

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JESSA ZIEKENHUIS

Ethische Toetsingscommissie

ADVIESFORMULIER

- ☐ studieprotocol
- ■ amendement protocol
- ☐ medical need program

VOORZITTER
dr. Koen Magerman

SECRETARIAAT
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ONS KENMERK
11.36/ortho11.01

Hasselt, 7 mei 2013

Titel protocol:	**Structural differences between the long head of the biceps tendon and the supraspinatus tendon. A pilot study.**
Amendement:	1
Inhoud amendement:	Wijziging hoofdonderzoeker + onderzoekers
Belgisch registratien°:	B243201213192
Hoofdonderzoeker:	Dr. F. Vandenabeele
Onderzoekers:	Dr. C. Dierickx Gertjan Jespers en Geertjan Vancoppenolle worden vervangen door Michael Proesmans, Jolien Cox, Niels Verheyen en Kristof Schraepen

Geachte collega,

De Ethische Toetsingscommissie van het Jessa Ziekenhuis heeft het hierboven vermeld amendement bestudeerd en heeft geen bezwaren tegen de wijziging in het onderzoeksteam.

De Ethische Toetsingscommissie is georganiseerd en handelt volgens de richtlijnen van GCP/ICH.

Met vriendelijke groeten,

Dr. Koen Magerman
Voorzitter Ethische Toetsingscommissie
Jessa Ziekenhuis

10 mei 2013

Studie: 'Arthroscopic and fine structural differences in collagen organization between the long head of the biceps tendon and the supraspinatus tendon.'

Informed consent formulier:

Confidentieel

Voor onze thesis doen wij, masterstudenten in de kinesitherapie en revalidatiewetenschappen, onderzoek naar de verschillen tussen de structuur van de m. Supraspinatus pees en de m. Biceps Brachii pees. De betreffende pezen bevinden zich in de schouder.

Om dit onderzoek te kunnen uitvoeren, maken wij gebruik van peesbiopten (stukjes peesweefsel) die genomen worden tijdens een chirurgische ingreep uitgevoerd door dr. Dierickx. Deze weefselfragmenten (maximaal 1 à 2 mm^3) worden nadien onder de elektronen microscoop onderzocht (dit om te kijken welke verschillen in structuur we zien bij de verschillende pezen).

Voor het nemen van biopten worden er geen extra chirurgische ingrepen uitgevoerd. Het nemen van biopten heeft geen consequenties voor de spier-peesfunctie en zal geen extra pijn veroorzaken. Zoals bij elke ingreep zijn er risico's verbonden aan het nemen van biopten (al is dit gereduceerd omdat er voor een deel "defect" weefsel afgenomen wordt en slechts 1 mm^3 gezonde pees) deze zijn: ontsteking wonde, lokale bloeding en blauwe plek. Het nemen van peesbiopten kan ongemakken veroorzaken, maar hebben meestal geen zware gevolgen. Voor het reduceren van deze ongemakken raadt men ijsapplicaties aan. Indien nodig zijn deze beschikbaar in het ziekenhuis.
Deelnemen aan dit onderzoek heeft geen invloed op de behandeling.

Via ons onderzoek hopen wij een beter inzicht te krijgen in de differentiatie tussen deze pezen.
De deelname aan de studie is geheel vrijwillig en u kan zich op elk moment uit het onderzoek terugtrekken indien gewenst. Hier zijn geen consequenties aan verbonden. Het is dan ook mogelijk om deze beslissing te nemen in overleg met familie/bekenden.
Uw gegevens worden vertrouwelijk behandeld. In geval van eventuele publicaties, worden geen gegevens openbaar gemaakt (wet op privacy 8 december 1992).
Voor dit onderzoek is geen vergoeding voorzien voor de deelnemers.

Alle deelnemers worden verzekerd in overeenstemming met de Belgische wet van 7 mei 2004 inzake experimenten op de menselijke persoon zodat eventuele lichamelijke, materiële en/of immateriële schade vergoed kan worden.

Ondergetekende, ... (voornaam, naam, leeftijd) geeft hierbij zijn/haar toestemming voor het nemen van enkele fragmenten peesweefsel tijdens de chirurgische ingreep die hij/zij ondergaat op ..../..../..... en bevestigt hierbij volledig vrijwillig deel te nemen aan deze studie. Hierbij wordt ook verklaard dat er de mogelijkheid is geweest om vragen te stellen en dat alle vragen voldoende toegelicht werden.

Datum:

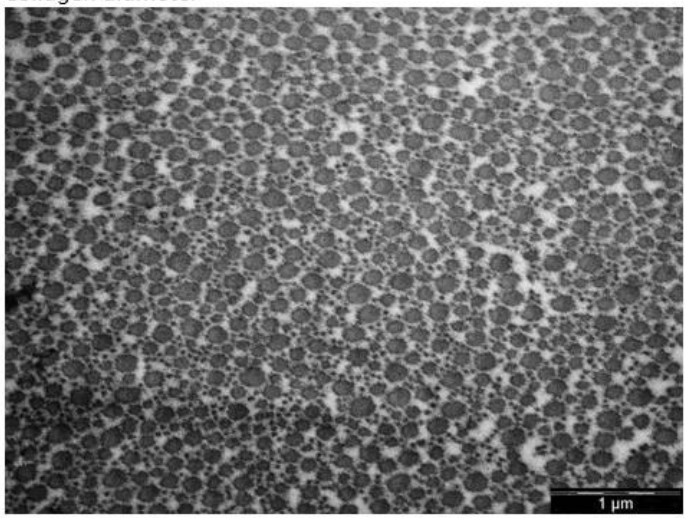
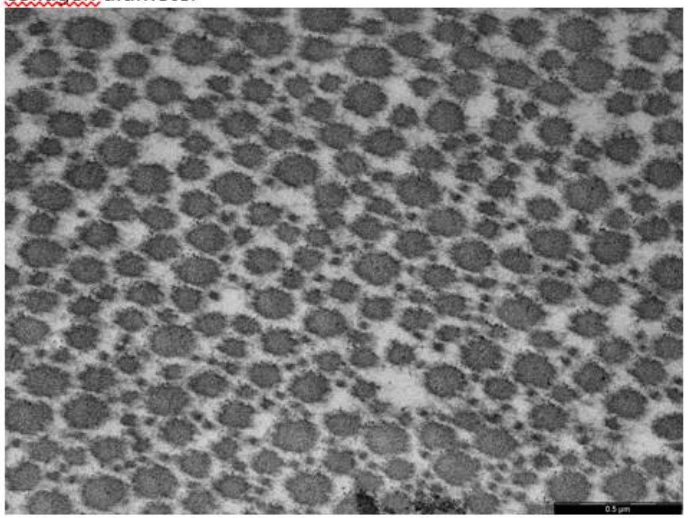
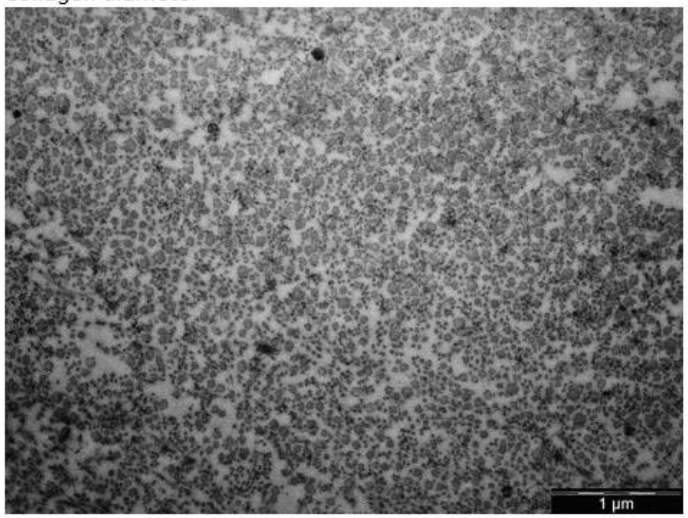
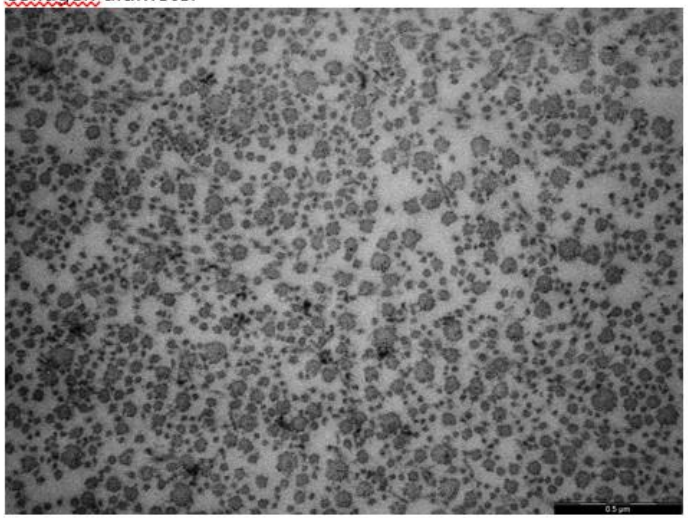
Handtekening deelnemer:

Naam en handtekening onderzoekers:

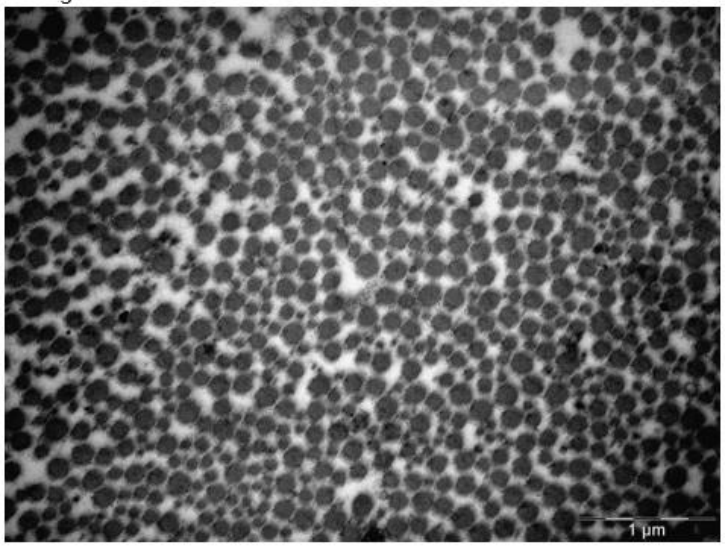
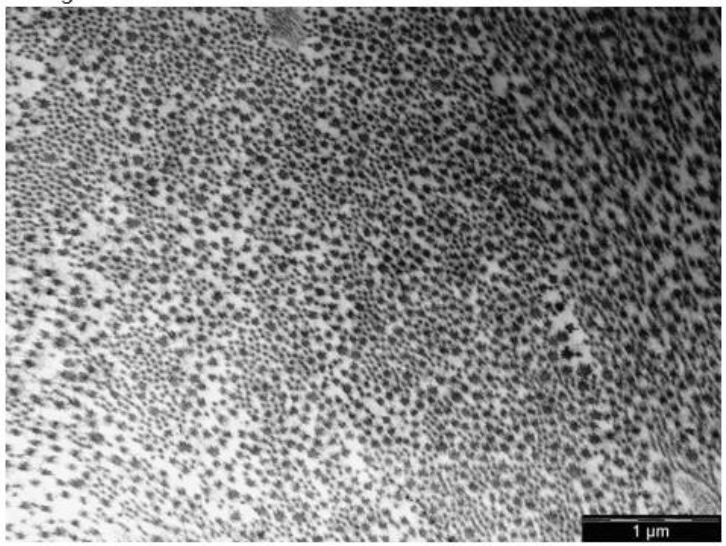
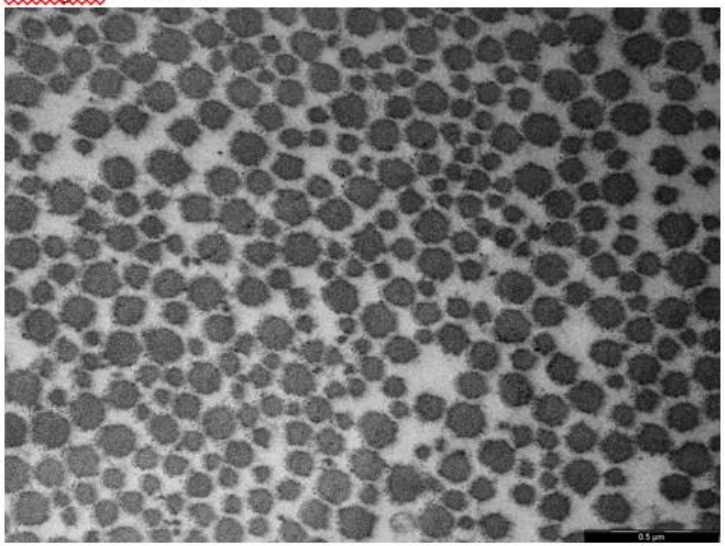
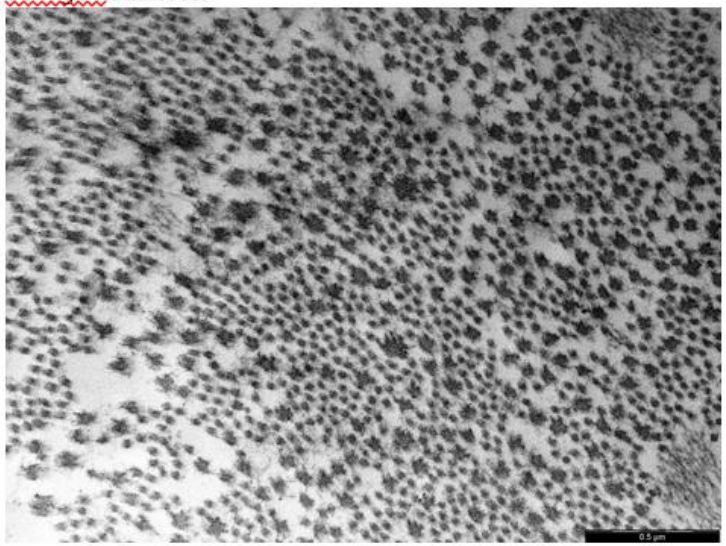
Student Kristof Schraepen - Bereikbaarheid: kristof.schreapen@student.phl.be
Niels Verheyen - Bereikbaarheid: niels.verheyen@student.phl.be

Promotor Vandenabeele F, MD, PhD
Co-Promotor Diericks C, MD (Orthopedisch Chirurg)

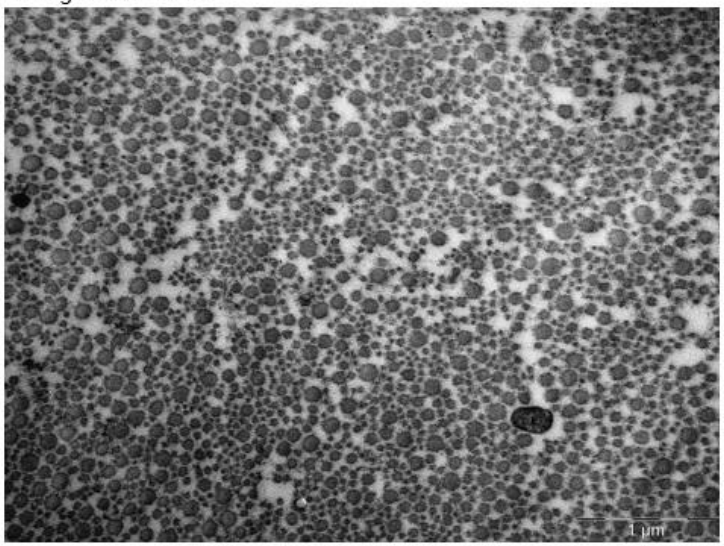
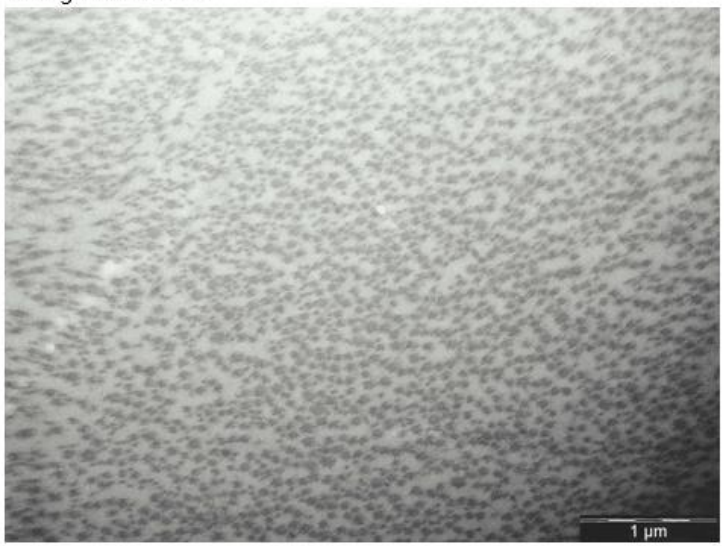
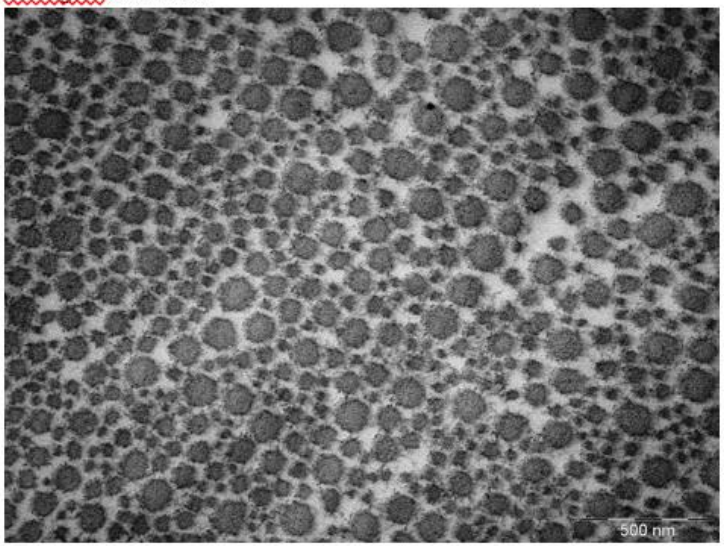
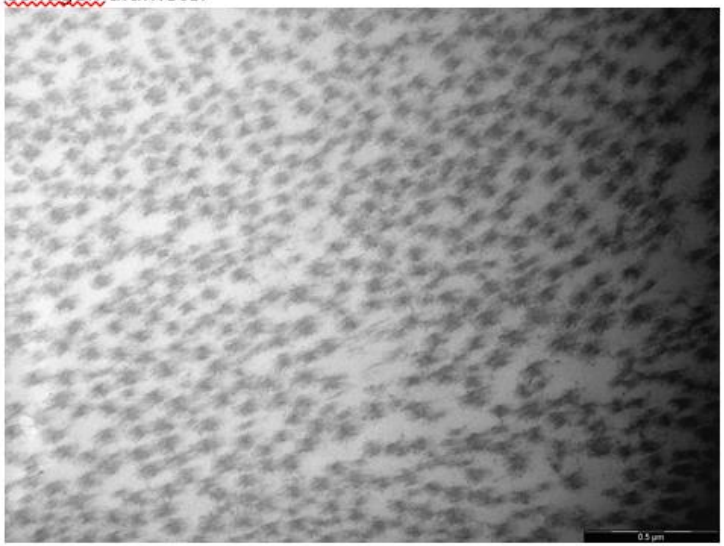
PATIENT 1 - FEMALE – 1949

DATA	LHB	SSP
PATHOLOGY: – ROTATOR CUFF TEAR RIGHT (TRAUMA) PERFORMED SURGERY: – SUBACROMIAL DECOMPRESSION – BURSECTOMIE – BICEPSTENOTOMY – SSP SUTURE TIME BETWEEN ONSET AND SURGERY: 2M	*Collagen diameter*  *Collagen diameter* 	*Collagen diameter*  *Collagen diameter* 

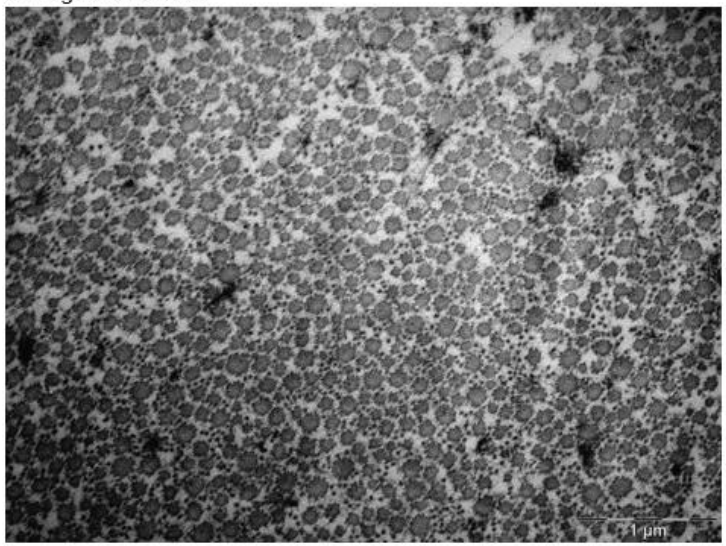
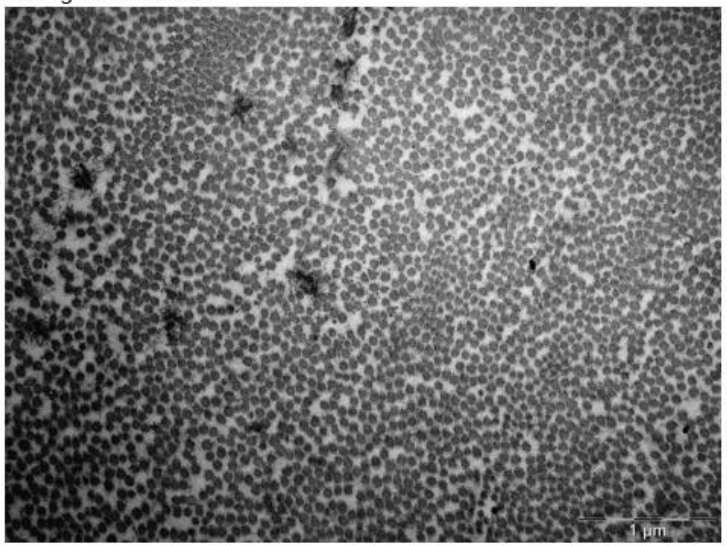
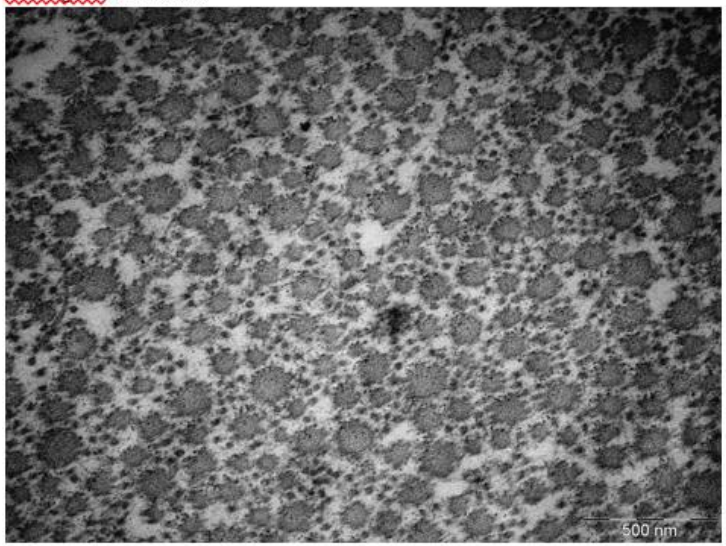
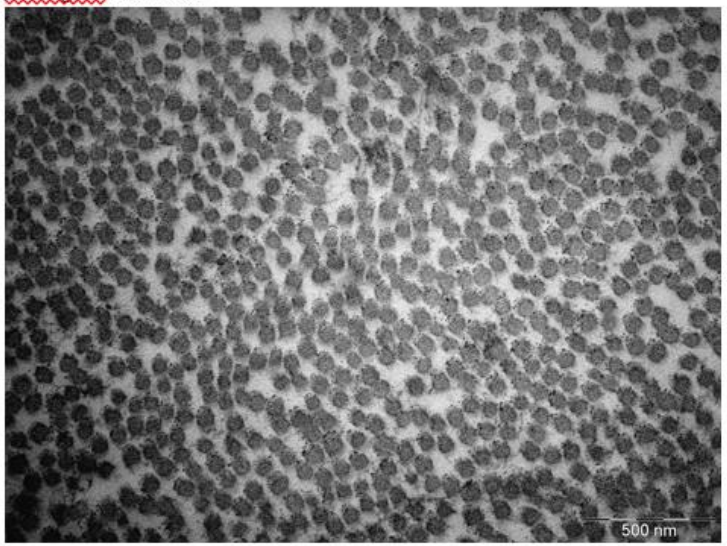
PATIENT 2 – MALE – 1955

DATA	LHB	SSP
PATHOLOGY: – ROTATOR CUFF TEAR RIGHT (TRAUMA) – INFLAMMATION LHB PERFORMED SURGERY: – SUBACROMIAL DECOMPRESSION – BURSECTOMIE – BICEPSTENOTOMY – SSP SUTURE TIME BETWEEN ONSET AND SURGERY: 3M	*Collagen diameter* 	*Collagen diameter* 
	Collagen diameter 	*Collagen diameter* 

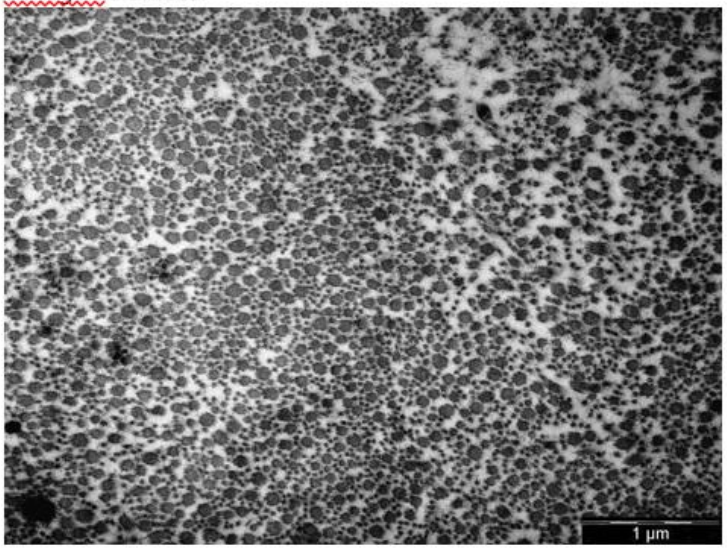
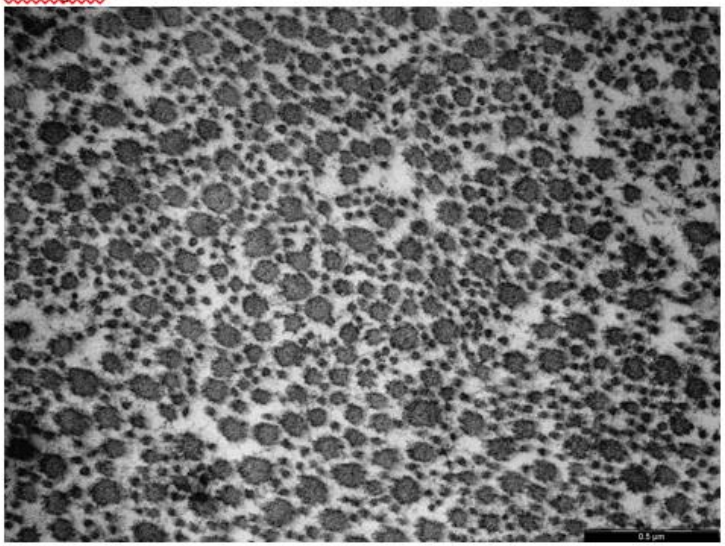
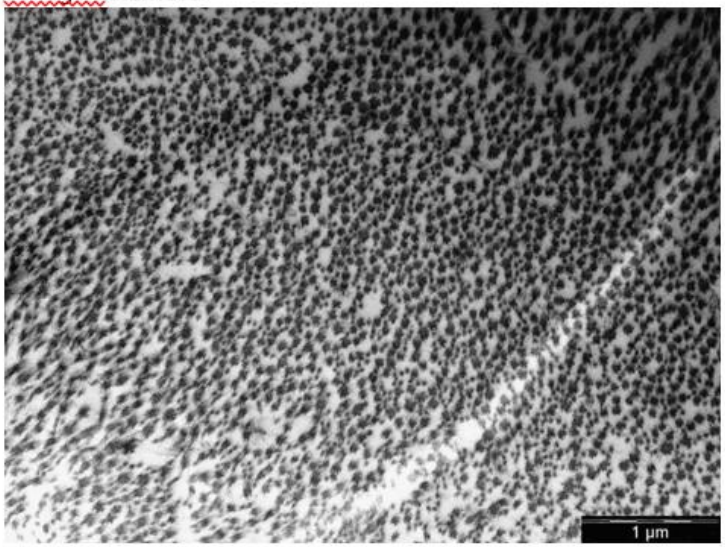
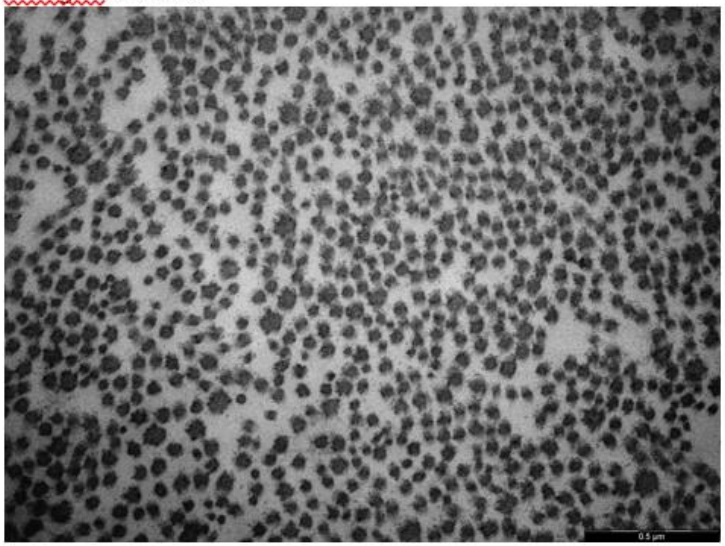
PATIENT 3 – FEMALE - 1965

DATA	LHB	SSP
PATHOLOGY: – ROTATOR CUFF TEAR RIGHT PERFORMED SURGERY: – SUBACROMIAL DECOMPRESSION – BURSECTOMIE – BICPESTENOTOMY – SSP SUTURE TIME BETWEEN ONSET AND SURGERY: 5M	*Collagen diameter* 	*Collagen diameter* 
	Collagen diameter 	*Collagen diameter* 

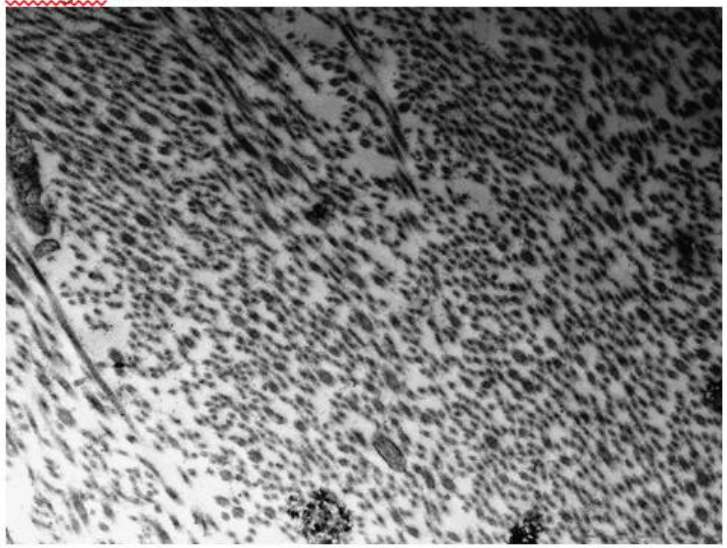
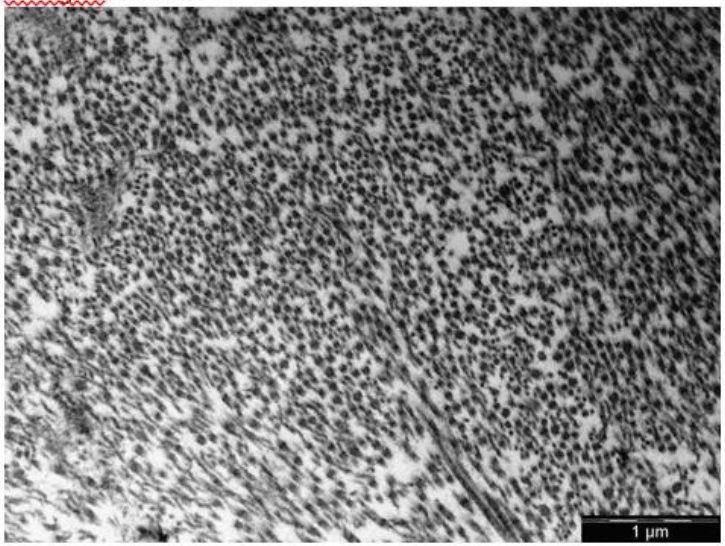
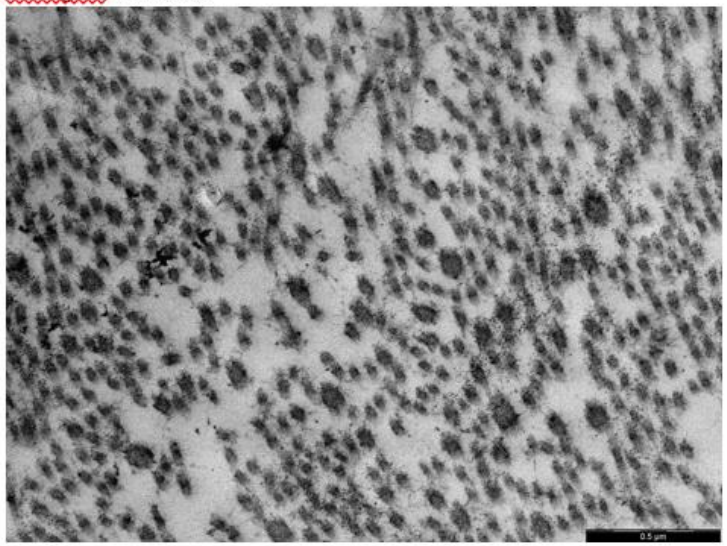
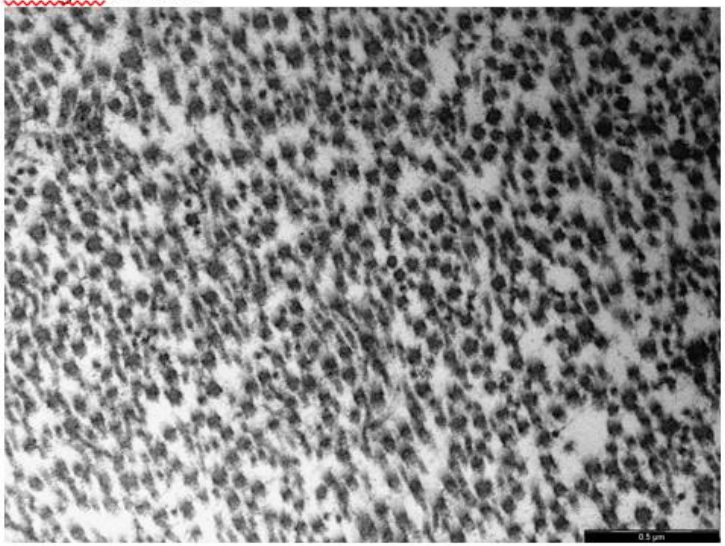
PATIENT 4 – FEMALE – 1943

DATA	LHB	SSP
PATHOLOGY: – ROTATOR CUFF TEAR RIGHT – RECURRENT INSTABILITY WITH SUBLUXATIONS IN A CRANIAL DIRECTION PERFORMED SURGERY: – SUBACROMIAL DECOMPRESSION – BURSECTOMIE – BICEPSTENOTOMY – SSP SUTURE TIME BETWEEN ONSET AND SURGERY: 7M	*Collagen diameter* 	*Collagen diameter* 
	Collagen diameter 	*Collagen diameter* 

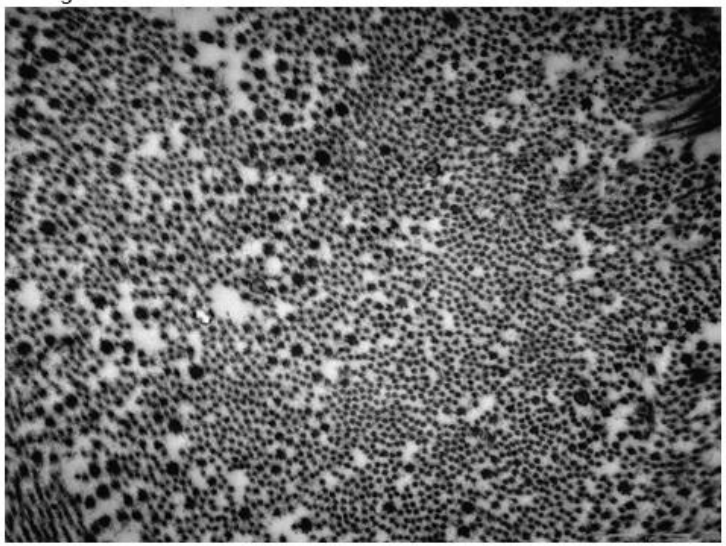
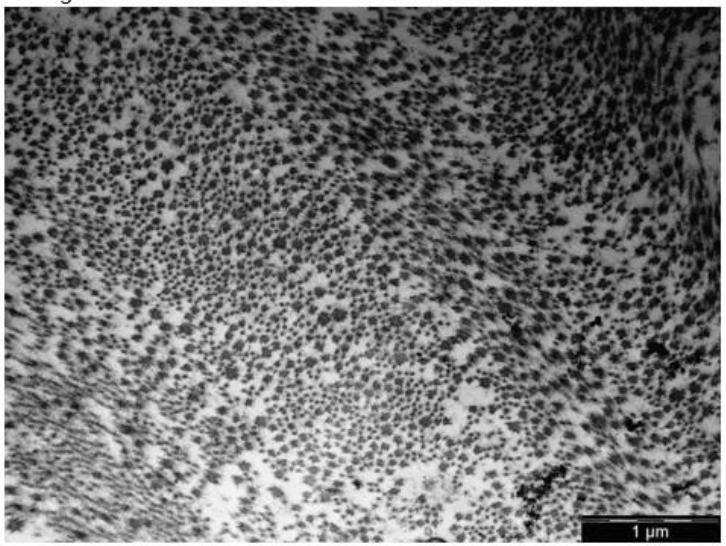
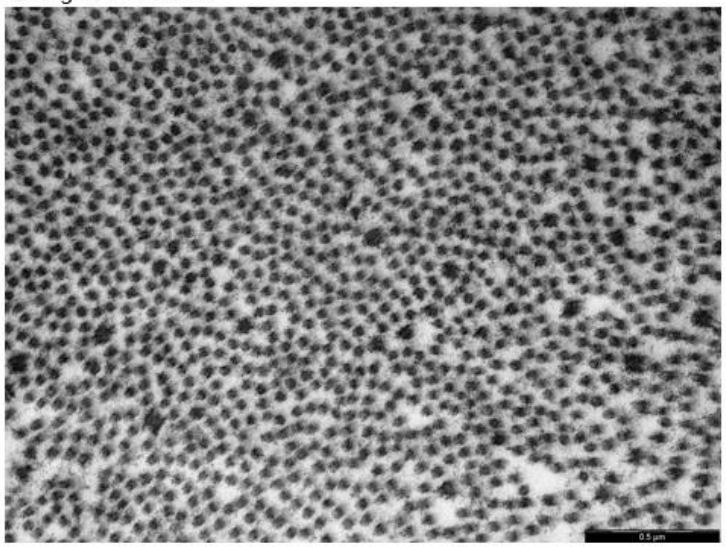
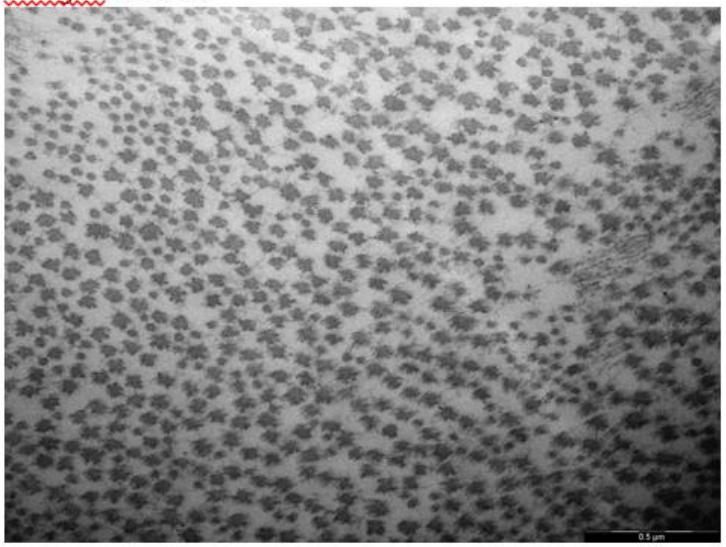
PATIENT 5 – MALE – 1971

DATA	LHB	SSP
PATHOLOGY: – ROTATOR CUFF TEAR LEFT (TRAUMA) - AC ARTHRITIS – PARTIAL TEAR LHB LEFT PERFORMED SURGERY: – ARTHROSOPIC SUBACROMIAL DECOMPRESSION – BICEPSTENOTOMY – BURSECTOMY. – ARTHROSCOPIC SSP SUTURE TIME BETWEEN ONSET AND SURGERY: 1Y3M	*Collagen diameter*  *Collagen diameter* 	*Collagen diameter*  *Collagen diameter* 

PATIENT 6 – MALE – 1942

DATA	LHB	SSP
PATHOLOGY – ROTATOR CUFF TEAR LEFT – FROZEN SHOULDER – AC OSTEOARTHRITIS PERFORMED SURGERY – ARTHROLYSIS – SUBACROMIAL DECOMPRESSION – BURSECTOMIE – BICEPSTENOTOMY – SSP SUTURE TIME BETWEEN ONSET AND SURGERY: 1Y	*Collagen diameter* 	*Collagen diameter* 
	Collagen diameter 	*Collagen diameter* 

PATIENT 7 – MALE – 1949

DATA	LHB	SSP
PATHOLOGY: – ROTATOR CUFF TEAR LEFT WITH CHRONIC SHOULDER LUXATION - AFUNCTIONAL FOR 3 MONTHS PERFORMED SURGERY: – TOTAL SHOULDER PROSTHESIS: REVERSED DELTA XTEND – BICEPSTENOTOMY TIME BETWEEN ONSET AND SURGERY: 1Y3M	*Collagen diameter* 	*Collagen diameter* 
	Collagen diameter 	*Collagen diameter* 

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