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FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

Intra- and extra-articular differences in the collagen organization of the long head of the m. biceps brachii, clinical relevance? Observational pilot study

Promotor :
Prof. dr. Frank VANDENABEELE

Copromotor :
Prof. Dr. Carl DIERICKX

Jolien Cox , Michael Proesmans
Proefschrift ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

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Preface

Tendons are subject to many types of pathologies. Overuse is a common cause for tendinopathy and other tendon pathologies. Inflammation and degeneration are responsible for structural weakening of the tendons, which may eventually lead to tendon rupture (Sharma et al., 2006).

This is a descriptive morphological study, in which the (ultra)structural organization of the collagen in the long head of m. biceps brachii (LHB) is investigated. The LHB is a main stabilizer of the glenohumeral joint, allowing the humeral head to maintain position in the scapular labrum. Degeneration to the long head biceps tendon (LHBT) may lead to glenohumeral instability. This can lead to cranial translation and supraspinatus impingement.

This research is performed by light microscopy (LM) and transmission electron microscopy (TEM). Light microscopy is used to investigate the organization of collagen fibers and the amount of degeneration in different segments (origin +1/2/3 cm) of the LHBT. The TEM is used to examine differences of collagen fibers between the intra- and extra-articular part of the LHBT. Differences found between intra- and extra-articular parts can explain clinical features of LHBT pathology.

Tasks in this thesis were equally divided. Michael had a bigger part in writing and data acquisition. Jolien did most of the configuration, presentation, statistics and research. Communication was done in person, over the telephone and over the internet during Jolien's Erasmus program.

Research design is under supervision of , and approved by promotor Prof. Dr. F. Vandenabeele. Dr. C. Dierickx, an operating physician at the Jessa Hospital (Hasselt) specialized in the shoulder complex, acquired the specimens needed. Technical assistance concerning the TEM was provided by Mr. Marc Jans, head laboratory technician of the University of Hasselt.

Table of Contents

Intra- and extra-articular differences in the collagen organization of the long head of the m. biceps brachii, clinical relevance?

Observational pilot study

Intra- and extra-articular differences in the collagen organization of the long head of the m. biceps brachii, clinical relevance? Observational pilot study

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Abstract

Background: The long head of biceps tendon (LHBT) is remarkable due to its anatomical course through the intertubercular groove. This forms an intra- and extra-articular part. The intra-articular part is most prone to tearing, but further investigation is needed.

Method: 10 patients undergoing artroscopic shoulder surgery have the LHBT resected, biopsies are taken at regular intervals. These biopsies are examined by light microscopy (LM) and transmission electron microscopy (TEM), for fine and ultra-structural differences in collagen organization.

Results: LM showed most degeneration at 2cm distal from origin. During TEM the intra- (1cm distal from origin) and extra-articular (3cm distal from origin) sections were compared considering collagen density. The extra-articular section showed remarkably higher collagen density (215 fibers/μm^2), compared with the intra-articular section (161 fibers/μm^2). This was not statistically significant.

Conclusion: Light microscopy (LM) and transmission electron microscopy (TEM) seem to indicate an increased vulnerability to tearing in the intra-articular aspect of the long head biceps tendon (LHBT).

Goal of the research: Linking clinical signs of LHBT pathology to fine and ultra-structural LHBT morphology, by using light and transmission electron microscopy, particularly focused on collagen density.

Research question: Are there any intra- and extra-articular differences in the collagen organization of the long head of the m. biceps brachii, and what is their clinical relevance?

Keywords: Collagen organization, long head biceps tendon, LHBT, transmission electron microscopy, light microscopy, collagen density

Introduction

The m. biceps brachii has a long head, originating from the tuberculum supraglenoidale, and a short head, originating from the processus coracoideus. The two heads form a combined muscle belly near the insertion of the m. deltoideus, in the anterior midsection of the humerus. Distally, it attaches with a tendon to the tuberositas radii and secondly, by the aponeurosis m. bicipitis brachii or lacertus fibrosus which originates from the distal insertion of the m. biceps brachii and radiates medially into the fascia antebrachi.

The greater and lesser tubercles of the humerus form the sulcus intertubercularis. The transverse humeral ligament transverses the sulcus intertubercularis and forms a canal. The long head of the m. biceps brachii (LHB) runs through this canal, hence forming an intra- and extra-articular part. Both intra- and extra-articular parts can exhibit anatomical variations, and have different structural characteristics (Dierickx et al, 2009).

Joseph M. et al (2009) performed a histological and molecular analysis of the LHB. The intra-articular portion of the LHB showed disorganized collagen fibers and signs of tendinosis while the extra-articular section exhibits parallel collagen fiber orientation, as seen in healthy tendon (Joseph et al., 2009).

The LHB consists of an intra- and extra-articular segment, showing remarkable histological and molecular differences. A transition zone between the two segments is apparent when the tenotomized LHB is observed (9 out of 11 tendon samples, Joseph et al.,2009).

Figure 1. Tenotomized LHB with a with a clear distinction between degenerative and healthy tendon. Adapted from Joseph et al (2009).

The collagen fibers in the extra-articular parts are more parallel organized than the intra-articular portion, post-tenotomy (Joseph et al., 2009). This could be explained due to abnormal mechanical loading, proximal to the intertubercular groove.

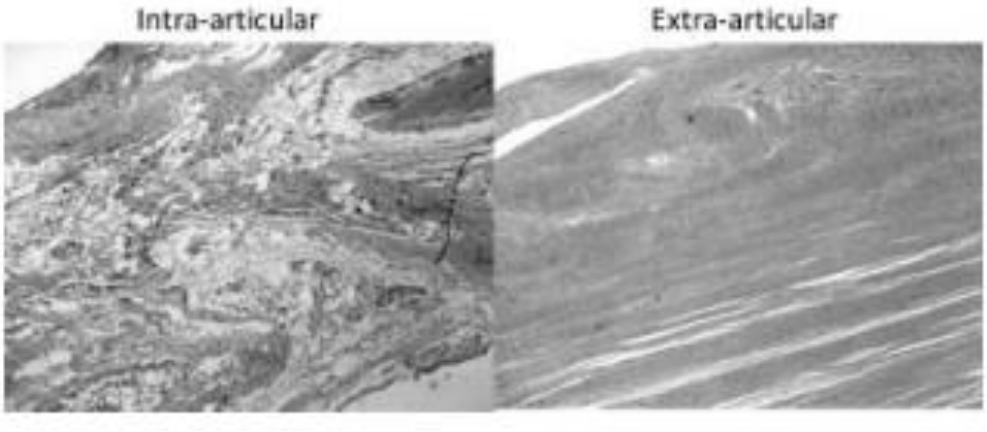

Figure 2. Differences in collagen orientation: disorganized collagen fibers in the intra-articular segment versus parallel collagen fiber orientation in the extra-articular segment. Adapted from Joseph et al (2009).

Bicipital tendinopathy can be a cause of pain and dysfunction. Diameter changes in LHBT (intra-articular segment versus intertubercular segment) are specific but not sensitive in diagnosing tendinopathy of the biceps tendon (Buck et al., 2009).

Alpantaki et al. showed that the LHBT is innervated by a network of sensory sympathetic fibers, important in the pathogenesis of shoulder pain (Alpantaki et al., 2005).

Additionally, the striation pattern of collagen is examined. Our co-promotor Dr. C. Dierickx found that pathologic tendons portrayed a ‘striation’ pattern in vivo during artroscopy, but not visible to the naked eye (see figure 3).

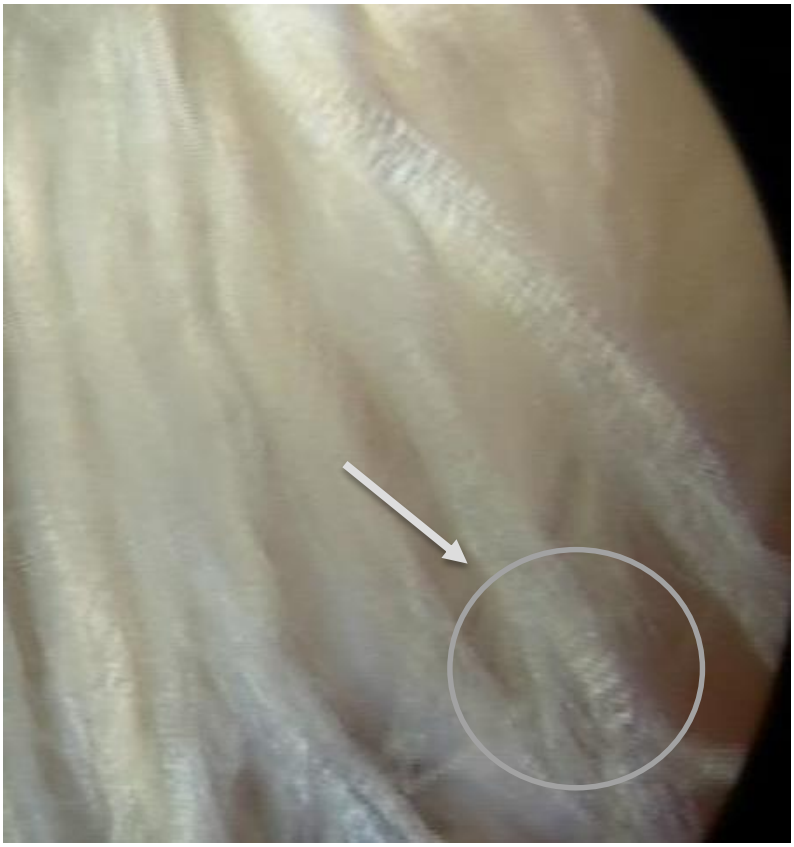

Figure 3: Image taken during arthroscopy of the shoulder joint. This frayed LHBT clearly has the ‘striped’ pattern, typically seen in pathologic tendon tissue (image courtesy of Dr. C. Dierickx).

LHBT biopsies were taken from patients undergoing rotator cuff surgery. These biopsies were examined under the light microscope (LM), and the Transmission Elektron Microscope (TEM). The goal is to find fine and ultra-structural differences in collagen organization between intra- and extra-articular portions of long head biceps tendon (LHBT).

Collagen is the most abundant protein in the human body. Collagens can be grouped into different categories such as fibrillar collagens (types 1 through 3), sheet-forming collagens and linking collagens. Fibrillar collagens have subunits that aggregate to form large fibrils visible through TEM. Collagen type 1 is the most abundant and widely distributed (Mescher et al., 2013 ;Stevens et al., 2007).

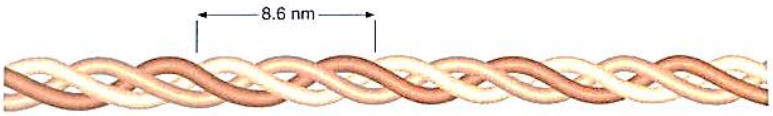

Figure 4: In collagen type 1, each procollagen molecule or subunit consists of two α1 and one α2-peptide chains, intertwined in a right-handed helix. The length of each molecule is 300nm, and its width is 1.5nm. Each complete turn of the helix spans 8.6nm (Steven et al., 2007). (Image taken from Junquiera's basic histology)

Most common types of collagen in tendon are types I and II. Type I is composed of three chains: two identical, termed α1 chains, and one α2 chain. Type I collagen is most present in skin, tendon, ligament, bone, cornea, etc. Type II is predominant in

Type	Molecule Composition	Structure	Optical Microscopy	Major Locations	Main Function
Fibril-Forming Collagens					
I	$[\alpha 1\ (I)]_2[\alpha 2\ (I)]$	300-nm molecule, 67-nm banded fibrils	Thick, highly picrosirius birefringent, fibers	Skin, tendon, bone, dentin	Resistance to tension
II	$[\alpha 1\ (II)]_3$	300-nm molecule, 67-nm banded fibrils	Loose aggregates of fibrils, birefringent	Cartilage, vitreous body	Resistance to pressure
III	$[\alpha 1\ (III)]_3$	67-nm banded fibrils	Thin, weakly birefringent, argyrophilic (silver-binding) fibers	Skin, muscle, blood vessels, frequently together with type I	Structural maintenance in expansible organs
V	$[\alpha 1\ (V)]_3$	390-nm molecule, N-terminal globular domain	Frequently forms fiber together with type I	Fetal tissues, skin, bone, placenta, most interstitial tissues	Participates in type I collagen function
XI	$[\alpha 1\ (XI)]\ [\alpha 2\ (XI)]\ [\alpha 3\ (XI)]$	300-nm molecule	Small fibers	Cartilage	Participates in type II collagen function
Sheet-Forming Collagens					
IV	$[\alpha 1\ (VIII)]_2\ [\alpha 1\ (IV)]_2$	2-dimensional cross-linked network	Detected by immunocytochemistry	All basal and external laminae	Support of epithelial cells; filtration
Linking/Anchoring Collagens					
VII	$[\alpha 1\ (VII)]_3$	450 nm, globular domain at each end	Detected by immunocytochemistry	Epithelial basement membranes	Anchors basal laminae to underlying reticular lamina
IX	$[\alpha 1\ (IX)]\ [\alpha 2\ (IX)]\ [\alpha 3\ (IX)]$	200-nm molecule	Detected by immunocytochemistry	Cartilage, vitreous body	Binds various proteoglycans; associated with type II collagen
XII	$[\alpha 1\ (XII)]_3$	Large N-terminal domain	Detected by immunocytochemistry	Placenta, skin, tendons	Interacts with type I collagen
XIV	$[\alpha 1\ (XIV)]_3$	Large N-terminal domain; cross-shaped molecule	Detected by immunocytochemistry	Placenta, bone	Binds type I collagen fibrils, with types V and XII, strengthening fiber formation

Figure 5: Table depicting general conditions of several different collagen fiber types. Tendon tissue primarily consists of types I and II. (Table taken from Junquiera's basic histology)

The clinical significance to pathology of the LHBT and the high variance of anatomic variations has been highly investigated in the past. Further study of the long head biceps tendon is needed to add to the scientific evidence that the intra-articular section and the extra-articular section show many differences.

The goal of the study is to find morphologic differences between intra- and extra-articular sections, mostly concerning density, diameter and organization of collagen fibers. Both LM and TEM were used.

The hypothesis is that the hypovascular zones are most prone to tissue degeneration and / or tears due to a lack of blood supply. A different collagen density was expected between intra- and extra-articular LHBT, possibly linked to LHBT degeneration.

Materials & Methods

Research design

During arthroscopy, a biopsy of about 1mm³ was taken. The surgical biopsies were immersed in 2% glutaraldehyde, before being stored in a cooling device. Later, the specimen were transported to the laboratory TEM, where a specialist postfixated the biopsies in 2% osmium tetroxide. They were imbedded in Araldite, before being cut into semithin (0.5µm) and ultrathin (0.06µm) sections. The semithin sections were investigated for vascular and neural structures under the lightmicroscope. The ultrathin sections were placed on a coppergrid, and examined through Transmission Electron Microscope.

Participants

Biopsies were taken from patients undergoing an arthroscopy (1-6-2013 to 1-6-2014), following rotator cuff tear, by operating physician Dierickx C., MD at the Jessa-hospital, Hasselt. All LHBT sections were taken during the months September 2013 to December 2013. All patients had a rotator cuff tear of the m. supraspinatus, and a complete tenotomy of the LHBT was performed in conjunction with cuff repair. Patient age ranged from 42 to 71 years old. Both male and female patients were included. 10 patients were included into this study, from which 40 LHBT sections were taken in total. Biopsies would be taken at 1cm intervals, starting from a margin just distally of the insertion. The first biopsy taken at 1 cm starting from the origin was a clear intra-articular section. The biopsy taken at 2 cm starting from the origin was a transitional zone between intra- and extra-articular LHBT. Biopsies taken past this point (3, 4, 5 cm respectively) were extra-articular LHBT sections. All patients had at least 3 biopsies taken. Some had 4 to 5 biopsies taken as LHBT length differed.

A complete summary of patient information can be found in the appendices.

Medical ethics

This study was a continuance of amendment study protocol 11.36/ortho 11.01 of the Ethical committee of the Jessa Hospital. Patients were given an informed consent, prior to the procedures of this research study. Biopsies were de-identified. Additional information concerning clinical signs, pathology and location (intra- / extra-articular) of the biopsy was obtained through the operating physician.

Intervention

The semithin sections were placed on glass, and examined by a light microscope (magnification 10x/40x), after which the ultrathin sections were prepared. The ultrathin sections were placed on a copperbed, and examined by a transmission electron microscope. For Transmission electron microscopy a Philips EM400 was used.

Outcomes

The outcome measure was the morphology of collagen fibers at various distances of the LHBT. Intra-articular sections (origin +1cm) were compared with extra-articular sections (origin +3cm).

Primary outcome measures

The primary outcome measure was the density of the collagen fibers in the intra- and extra-articular part of the long head of biceps tendon. The collagen density and number of collagen fibers were examined through Transmission Elektron Microscope. General condition and striation pattern were examined through Light Microscope.

Pictures of representative areas of collagen were taken at a magnification of 44000 (surface = 4.4µm²), using a TEM. Pictures of both intra- (1cm distal to origin) and extra-articular (3cm distal to origin) sections were taken. 4 pictures were taken from each section.

Collagen density was measured by counting the number of collagen fibers present in 1 picture at magnification 44000 (surface = 4.4µm²). From these 4 results, the average number of collagen fibers was calculated. Number of collagen fibers per µm² was calculated.

Data Analysis

The digital images taken under the transmission electron microscope are processed using AnalySIS.

SPSS was used for statistical research. A Q-Q-plot was made to determine the normality of the sample pool. A paired t-test was used to find statistically relevant changes in collagen density between intra- and extra-articular sections. A statistician was involved in the study to prevent statistical flaws.

Results

Light Microscopy

In 8 out of 10 specimens, tendon degeneration was seen at 2cm distal from the origin of LHBT. Signs of degeneration were lack of proper collagen organization, hypercellularity and signs of a present collagen striation pattern.

2 subjects had sings of degeneration in all 3 sections (origin +1cm, +2cm and +3cm). These subjects had a lesser collagen density and more cells in the tendon tissue, even in sites considered healthy in other subjects (origin +1cm / +3cm). These patients had a history of (recurring) LHBT luxation and anterior glenohumeral instability.

No pattern could be observed in the wavelength of collagen striation. The periodicity was not influenced by site nor pathology. Even within the same section, different periodicities of wavelength could be observed (fig 6).

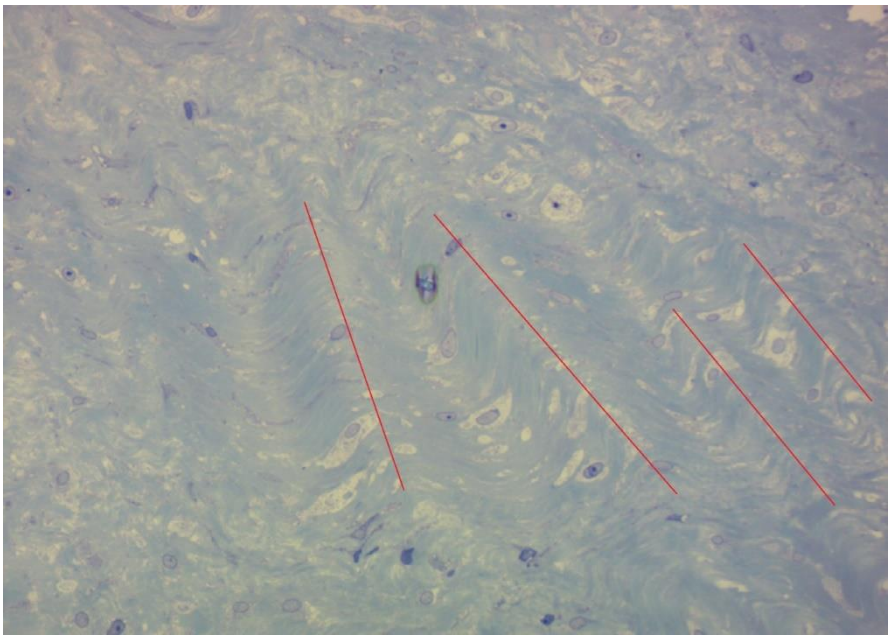

Figure 6: Striation of collagen has a divergent pattern in this section, leading to a high variance of wavelength, depending on point of measurement. (LHBT 1cm distal from insertion, as seen at 40x magn.)

Patients undergoing rotator cuff surgery seemed to have the least signs of degeneration in the LHBT. The most pathologic site was the section taken at 2 cm distal from LHBT origin.

Transmission Elektron Microscopy

10 patients were included in the study. During microscopy, a total of 80 pictures were investigated to compare the collagen density between intra- (origin +1cm) and extra-articular (origin +3cm) sections of LHBT. Average number of fibers measured on total surface (4.4µm²) was 707 intra-articular, and 944 extra-articular. This leads to an average of 161 fibers/µm² measured intra-articular, and 215 fibers/µm² extra-articular. Lowest measured was 36 fibers/µm², highest measured was 420 fibers/µm².

Normality was investigated using a Q-Q plot. The linearity of the points suggests that the data are normally distributed.

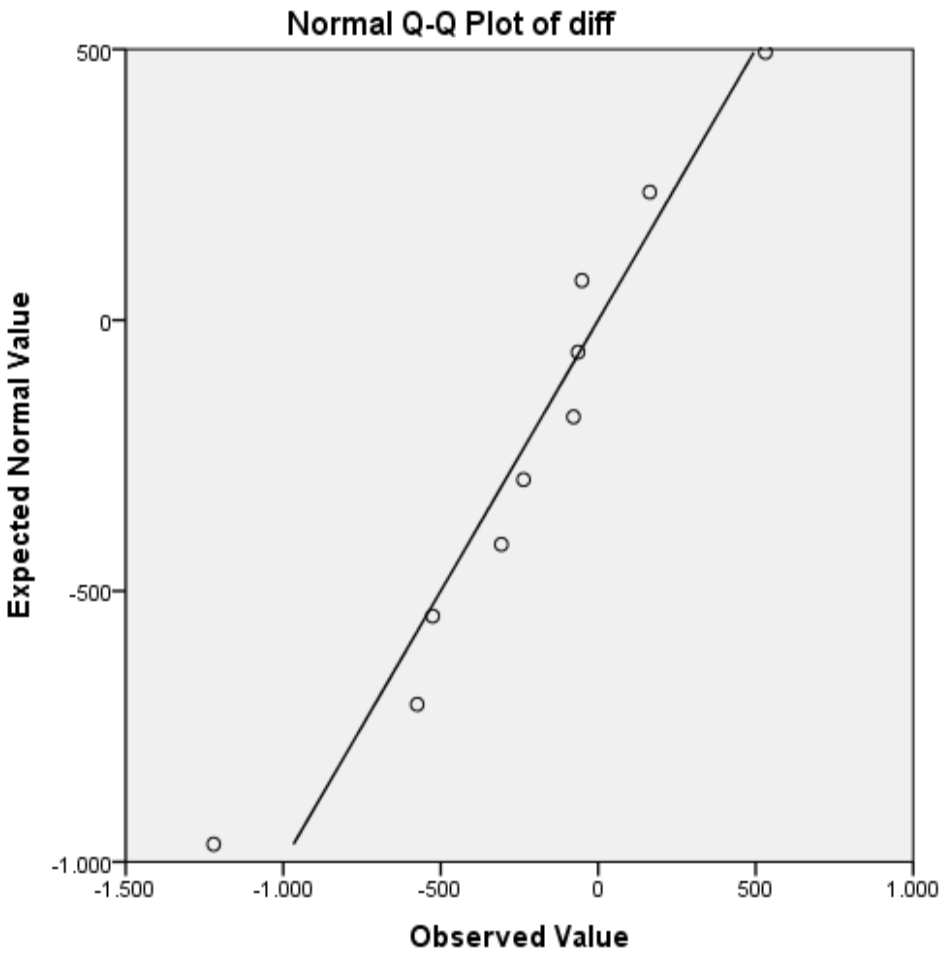

Figure 7: Q-Q plot to investigate the normal distribution of data. The test was performed using SPSS.

In order to examine differences between intra- and extra-articular LHBT, each patient had a minimum of 8 pictures taken: 4 intra- and 4 extra-articular. All intra-articular pictures were taken at 1 cm from LHBT origin. All extra-articular pictures were taken at 3 cm from LHBT origin. Representative areas of collagen were located at a magnification of 44000. Total fiber count was determined on 4.4µm².

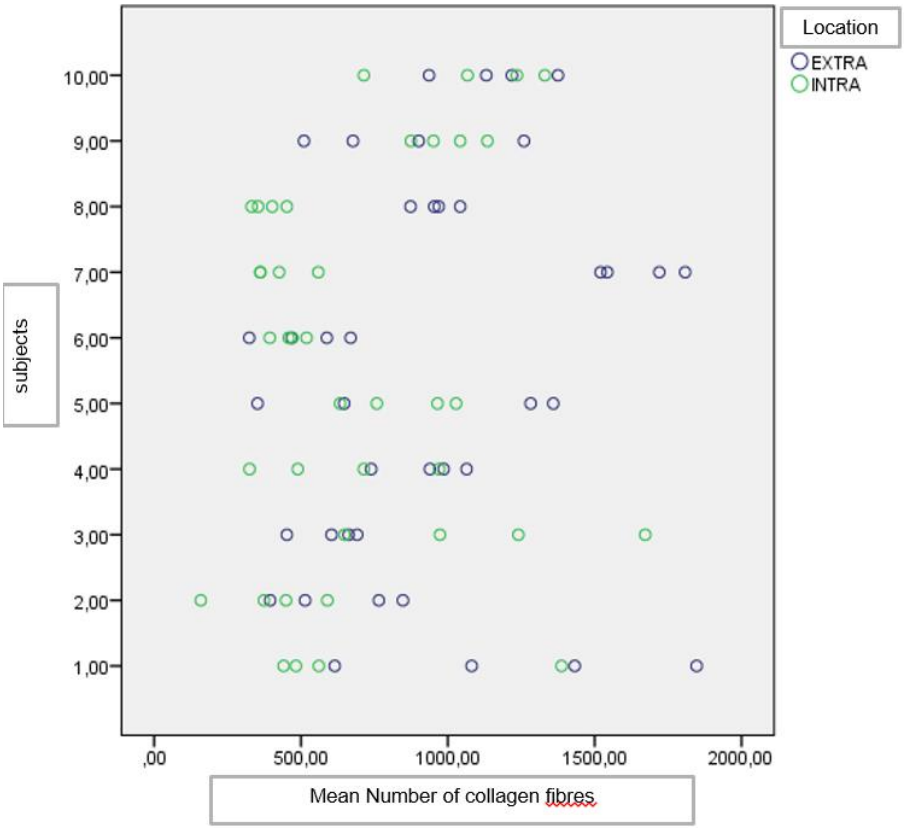

Figure 8: Scatter plot showing all measurements taken. Note how the extra-articular measurements lie more dominantly to the right hand side when compared to intra-articular measurements. Graph made using SPSS.

80% (8/10) of the subjects supported the hypothesis that the intra-articular section had a lower collagen density, when compared to the extra-articular section.

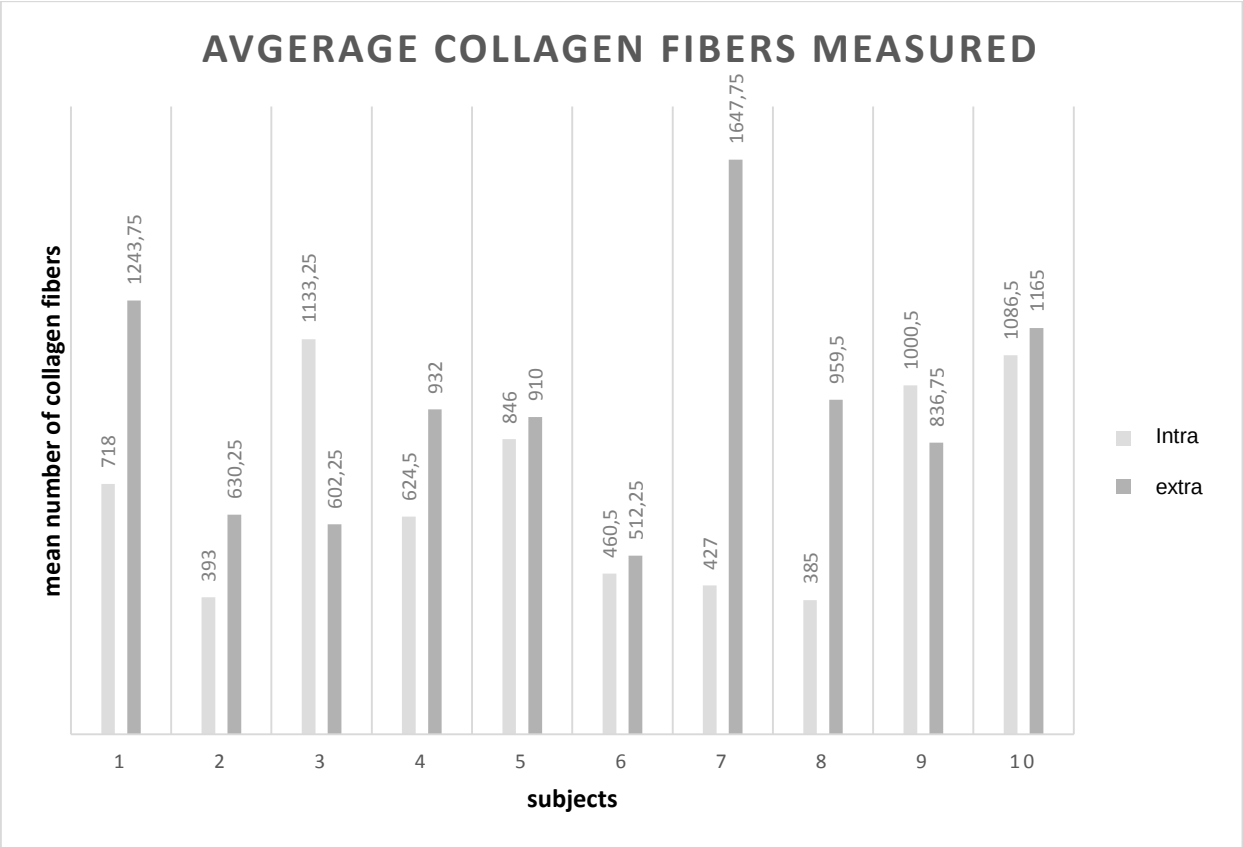

Figure 9: Average number of fibers measured (4.4µm²) for all patients. Grey lines represent the intra-articular measurements. Dark lines represent the extra-articular measurements. 8/10 support the hypothesis of a lower collagen density intra-articular as compared to extra-articular. 1 out of 2 exceptions had the most severe pathology, as luxation of LHBT might skew collagen density measurements.

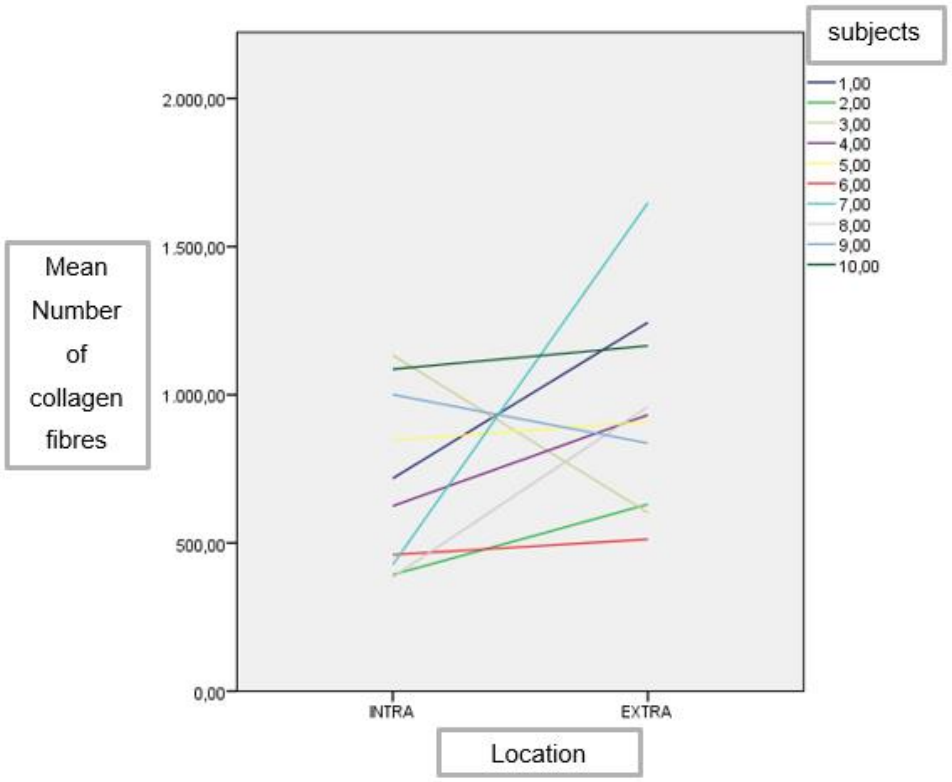

Figure 10: Comparison of mean values between intra- and extra-articular sections. Graph made using SPSS. Biggest differences in intra- and extra-articular measurements is in patients suffering from (chronic) luxation (patients 3 and 7)

A paired t-test was performed to look for statistically significant differences in the sample pool. With a significance of 0.148, the criteria of 0.05 was not met.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	INTRA - EXTRA	-236,52500	472,55903	149,43629	-574,57336	101,52336	-1,583	9	,148

Figure 11: Paired samples t-test performed on entire population. Significance level of 0.148 (>0.050) indicates no statistical significant difference between sample pools.

No statistically significant changes were found between the two samples (intra- and extra-articular).

The highest differences between intra- and extra-articular sections were patients suffering from (recurring) luxation of the glenohumeral joint (patients 3 and 7).

When a paired t-test was performed after both patients 3 and 7 were excluded, a significance level (2-tailed) of 0.051 was obtained with 8 patients included.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	INTRA - EXTRA	-209,43750	252,45791	89,25735	-420,49759	1,62259	-2,346	7	,051

Figure 12: Paired samples t-test performed with exclusion of both patients showing (chronic) luxation. The significance level obtained almost indicates a statistically significant change.

Discussion

During LM research, 8 out of 10 included subjects verified the supposition that most LHBT degeneration occurs at 2 cm distal from the insertion. This is in agreement with Cheng et al., 2010, who demonstrated that the LHBT is vascularized from 2 opposite sources. This leaves a hypovascular zone where tissue repair is impaired. Signs of degeneration were lack of proper collagen organization, hypercellularity and signs of a present collagen striation pattern.

Patients undergoing rotator cuff surgery seemed to have the least signs of degeneration in the LHBT during LM research. The most pathologic site was the section taken at 2 cm distal from LHBT origin due to a lack of collagen concentration and hypercellularity.

During TEM research, fibroblasts were found, as was expected during LM research. These fibroblasts are capable of synthesizing collagen, and are important in pathologic tendon. Tissue damage induces mitosis of fibroblasts, explaining the high number of fibroblasts in degenerated tendon.

No statistically significant changes were found between the two samples (intra- and extra-articular). This might be due to the low sample size (n=10), as 80% of included subjects did meet the hypothesis.

The highest differences between intra- and extra-articular sections were accounted by patients suffering from (recurring) luxation of the glenohumeral joint (patients 3 and 7). This luxation might damage the tendon at a location unrelated to natural shoulder biomechanics. As such, it might be beneficial to exclude severe shoulder pathology (such as luxation and humeral fractures) from this type of study.

When a paired t-test was performed after excluding both patients 3 and 7, a significance level (2-tailed) of 0.051 was obtained with 8 patients included. This might indicate a statistically significant change found in similar studies with higher sample size. Further research is needed to find a statistically significant change.

Because the highest differences of collagen density measurement were found in most pathologic LHBT tendon, it seems plausible that maladaptation occurs at the site

undergoing stress. Hypercellularity accounts for an increase in collagen density, as small fibers of low quality are released in the damaged tissue.

In 80% of included subjects, the collagen density seemed to be lower in the intra-articular section as compared to the extra-articular section (although this was not statistically significant). A possible reason for this assumption might be increased tissue damage due to an increased biomechanical loading of the LHBT during abduction of the glenohumeral joint. Additionally, lack of vascularization due to natural shoulder anatomy might play a role in maladaptation of collagenous structures. Tearing of the LHBT occurs most often in the intra-articular section, which has a notably lower collagen density in pathologic subjects. Furthermore, normal shoulder biomechanics maintain strain on the extra-articular part. This might lead to a parallel organized extra-articular LHBT while the intra-articular LHBT is disorganized (Joseph et al., 2009).

Collagen density ranged from 36 to 420 fibers/μm^2. It seems that neither an extremely low or extremely high collagen density account for healthy tendon. Healthy control specimen are needed to measure 'normal' collagen density in the LHBT.

All of our patients underwent surgery following rotator cuff pathology, mostly tears of the supraspinatus tendon, during which the long head of biceps tendon (LHBT) was resected. Although the intra-articular part of the LHBT was compared to the extra-articular part, no comparison was made between symptomatic and healthy tendon. It would have been beneficial to have healthy control samples, but this can't be done due to ethical restrictions.

Furthermore, due to the way biopsies were taken, it is impossible to say which exact location of the LHBT is damaged most during (normal) degeneration. All biopsies were taken at 1cm interval, starting from the insertion, and overall the section 2cm distally from origin showed most signs of degeneration. Because adjacent sections were taken at 1cm and 3cm, and because these sections showed markedly healthier tendon, it is safe to say that somewhere between this interval the tendon is most likely to degenerate and ultimately lead to clinical symptoms. This is in agreement with vascular assumptions where Cheng et al. (2010) have shown that the tendon is hypovascular around the bicipital groove (Cheng et al., 2010). This could also be linked to biomechanics, where the LHBT appears to be under the highest load of stress at the bicipital groove.

There was a lack of uniformity concerning the exact location of LHBT resection. Resection always started at its origin on the superior labrum. The first biopsy was taken one mm distal from the medial section. At the section itself the fiber would have been damaged by an electrocautery device (VAPR from Mitek, Depuy-Syntes). The other biopsies were taken with a simple ruler as a guideline, to take distal biopsies towards the extra-articular portion of LHBT. The biopsies were taken from the most rounded side of the LHBT which seemed the healthiest. Because there was no exact guideline (apart from distance) at what side of LHBT the biopsies were taken, it is unclear whether these were taken from the superior, inferior, anterior or posterior compartment.

One of the questions regarding this topic is the existence of a certain wavy pattern of collagen, found in pathologic tendon sections. Little is known about the formation of this 'wavy' collagen striation pattern. This pattern is visible during shoulder artroscopy, but only in pathologic tendon tissue. This wavy collagen pattern, or striation, is most likely created by crimp-formation in tendon-like tissue. The tensile stress-strain behavior tendons starts with a toe region. This is believed to result from the straightening of crimped collagen fibrils (Hansen et al. 2002). The measurement of crimp by high-field MRI could serve as an in vivo index of physiological strains in collagenous strains (Mountain et al. 2011). Crimp is caused by contraction of tendon fibroblasts. This contraction is a sufficient mechanical impulse to create a planar wavy pattern (Herchenchan et al. 2012). A higher number of fibroblasts might increase this wavy pattern. Flattening of tendon crimps may occur at the start of tendon stretching (Franchi et al. 2007), so ruptured tendons might show increased tendon crimps (or striation pattern).

While Alpantaki et al. (2005) found that the LHBT was innervated, little is known about the specifics of nerve endings found in the tendon. Furthermore, little is known about the progression: while the tendon may be innervated, there will be areas which have a lower / higher concentration of free nerve endings. It is likely this may be appear similar to the vascularization of the tendon. Superficially, we suspect a dense nervous network, but deeper into the tissue, and especially posteriorly (where the tendon lies against bone directly), the amount of nervous tissue will be lowered. Whether it's a lower concentration or a complete absence of nervous tissue in the deeper structure

of the LHBT, is unclear. Additionally it would be interesting to find any differences in medial and lateral compartments.

Collagen types I and II are most abundant in tendon tissue. A distinction between these two types could not be made based on diameter differences. It might be of clinical significance whether or not the type distribution alters between intra- and extra-articular sections of the same tendon.

Conclusion

Both light (LM) and transmission electron microscopy (TEM) seem to indicate an increased vulnerability to pathology in the intra-articular part of the long head biceps tendon (LHBT).

During LM hypercellularity is visible in the intra-articular section (origin +2cm). This might indicate an increased inflammatory response to injury. Additionally, extra-articular LHBT showed more extensively parallel oriented collagen fibers when compared to intra-articular parts..

By TEM, the collagen density was measured and compared between intra- and extra-articular sections. 1 cm distal from origin was considered intra-articular, 3 cm distal from origin was considered extra-articular. The extra-articular section showed a higher collagen density (215 fibers/µm² extra-articular, 161 fibers/µm² intra-articular). This might indicate that the intra-articular section is more prone to pathology, due to there being less tensional resistance. Although this difference was not significant, 80% of patients supported the hypothesis that the intra-articular section had a lower collagen density.

Furthermore, when excluding the heavy pathologic patients (chronic luxation) from the sample pool, a significance level (2-tailed) of 0.051 was found during a paired t-test. This might be insignificant due to low sample size.

Acknowledgements

These acknowledgements express the author's appreciation to those participating and aiding during this study.

First and foremost the authors would like to thank promotors Prof. Dr. F. Vandenabeele and co-promotor Dr. C. Dierickx. Without their guidance it would have been impossible to create this descriptive study. We would like to thank Prof. Dr. F. Vandenabeele for his supervision and evaluation during this process, and Dr. C. Dierickx for his advice, brainstorm sessions and collection of the biopsies.

Secondly, the authors would like to thank Mr. M. Jans for his exceptional technical support.

The authors would also like to thank staff of both UHasselt and Jessa-Hospital, and patients included in this study.

Finally, thanks go out to friends and family, for support and encouragement during this process.

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Jolien Cox, Tongersesteenweg 116, 3723 Kortessem J.C.

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Addendum

Addemdum 1: Descriptive exploration total sample size

Descriptives

LOCATIE				Statistic	Std. Error
VEZELS	1	Mean		707,4250	56,72291
		95% Confidence Interval for Mean	Lower Bound	592,6921	
			Upper Bound	822,1579	
		5% Trimmed Mean		687,6111	
		Median		575,5000	
		Variance		128699,533	
		Std. Deviation		358,74717	
		Minimum		159,00	
		Maximum		1672,00	
		Range		1513,00	
		Interquartile Range		542,50	
		Skewness		,807	,374
		Kurtosis		-,140	,733
	2	Mean		943,9500	64,63612
		95% Confidence Interval for Mean	Lower Bound	813,2111	
			Upper Bound	1074,6889	
		5% Trimmed Mean		928,5278	
		Median		918,5000	
		Variance		167113,126	
		Std. Deviation		408,79472	
		Minimum		324,00	
		Maximum		1847,00	
		Range		1523,00	
		Interquartile Range		625,75	
		Skewness		,548	,374
		Kurtosis		-,472	,733

Figure 16: Descriptive exploration of the sample pool. Intra-articular mean of 707 fibers measured (161 fibers/µm²). Extra-articular mean of 944 fibers measured (215 fibers/µm²). Range is comparable, while both minimum and maximum measurements are higher in the extra-articular sample pool.

Addendum 2: Informed Consent

Cadaveric study of anatomic variants of the long head of the biceps (LHB).

Informed consent formulier:

Confidentieel

Voor onze thesis doen wij, masterstudenten in de kinesitherapie en revalidatiewetenschappen, onderzoek naar de verschillen in structuur van de de m. Biceps Brachii pees. De betrekkende pees bevindt zich in de schouder. Aangezien er verschillende cellulaire en structurele varianten van deze pees bestaan, zouden wij graag kleine stukjes weefsel willen gebruiken om aan de hand van microscopie erachter te komen wat de gevolgen hiervan zijn.

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.
Deelnemen aan dit onderzoek heeft geen invloed op de behandeling.

Via ons onderzoek hopen wij een beter inzicht te krijgen in de cellulaire structuur van de betreffende pees.
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, ... geeft hierbij zijn/haar toestemming voor het nemen van enkele fragmenten peesweefsel tijdens de chirurgische ingreep die ik onderga op ..../..../..... en bevestig hierbij volledig vrijwillig deel te nemen aan deze studie. Ik verklaar hierbij dat ik de mogelijkheid heb gehad om vragen te stellen, dat alle vragen voldoende beantwoord werden en dat ik voldoende informatie heb ontvangen.

Datum:

Handtekening deelnemer:

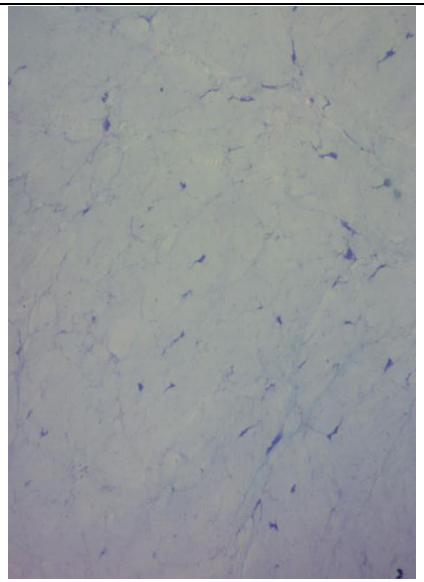
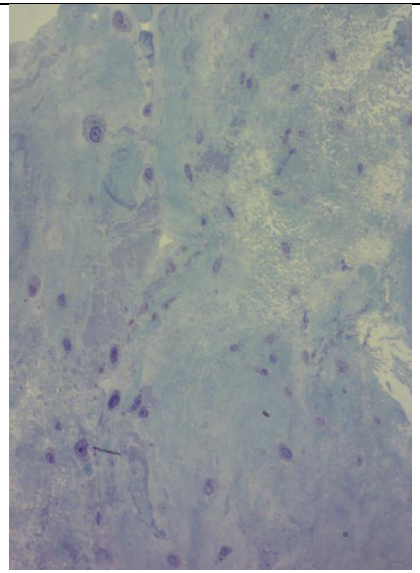
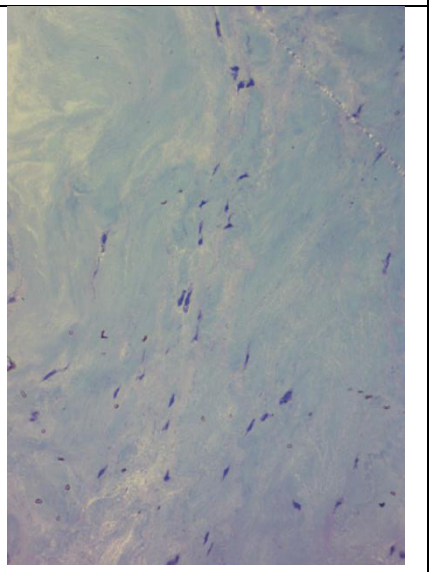
Naam en handtekening onderzoekers:

Student : Michael Proesmans (michael.proesmans@student.phl.be)

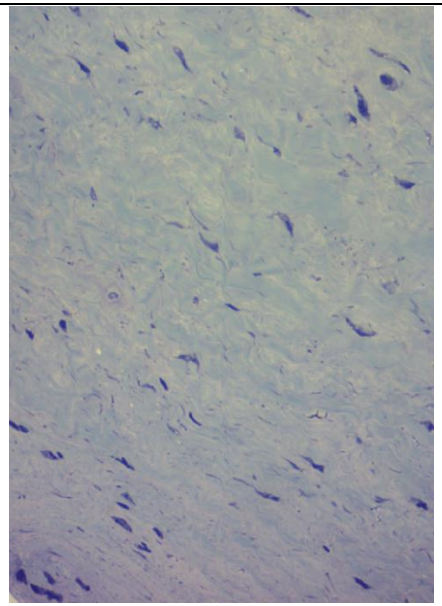
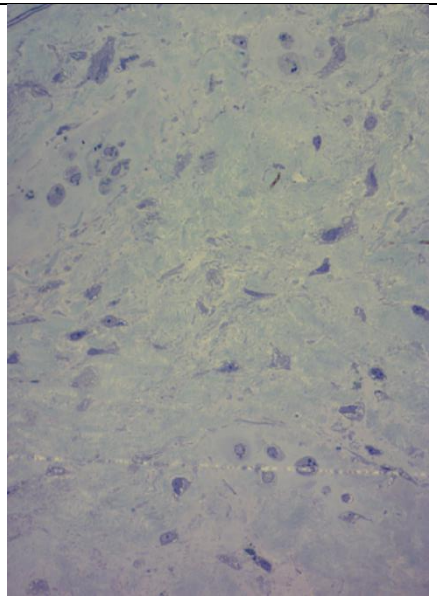
Jolien Cox (jolien.cox@student.phl.be)

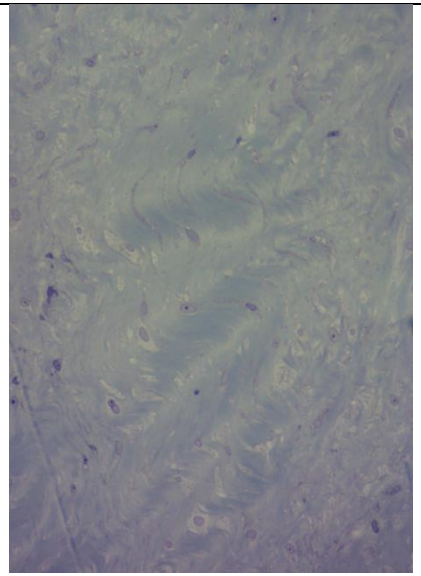
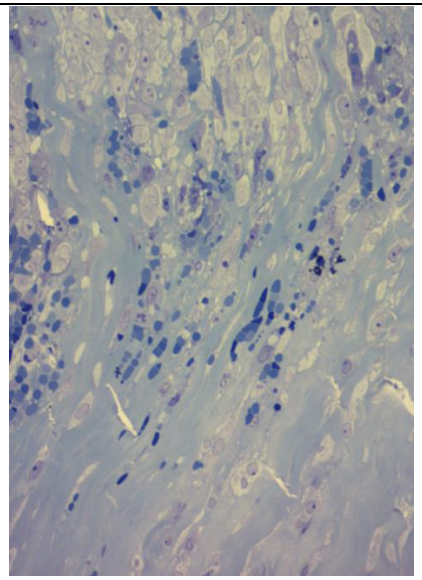
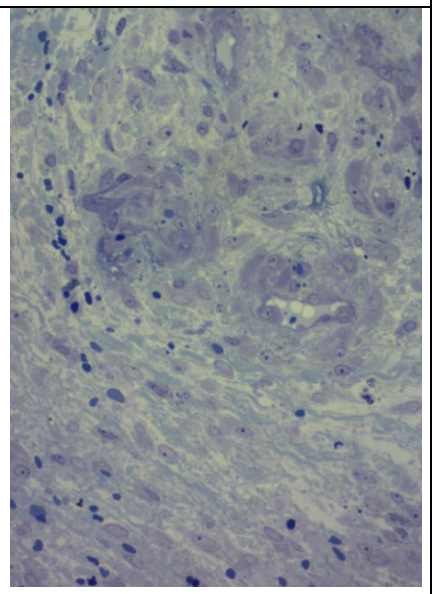
Promotor Vandenabeele F, MD, PhD:

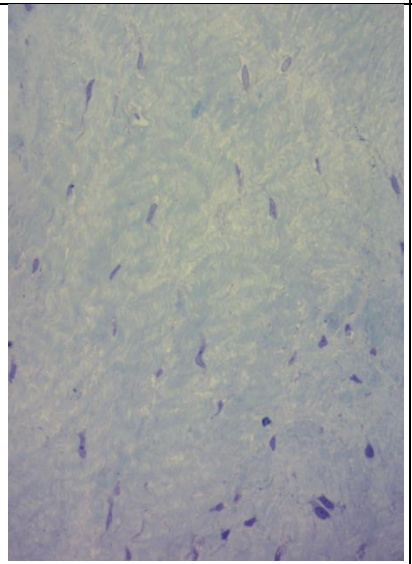
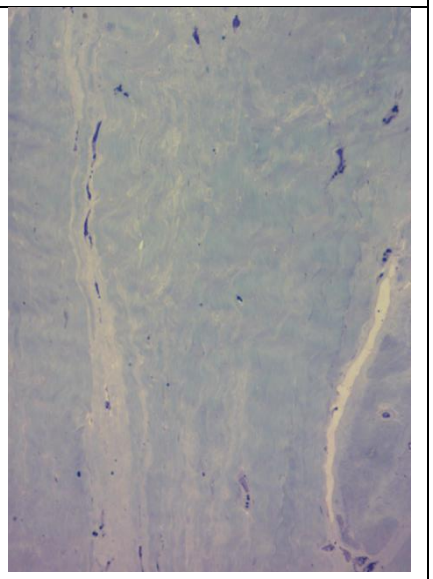
Addendum 3: Summary patient information

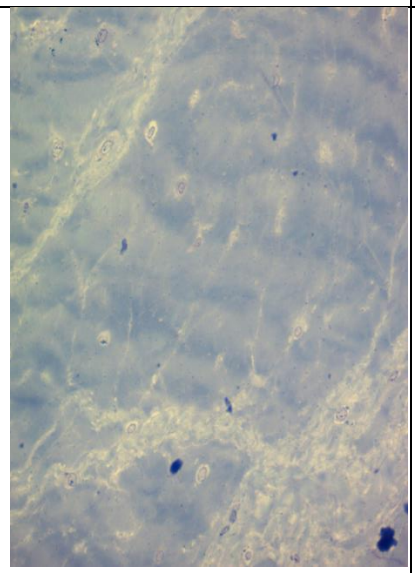
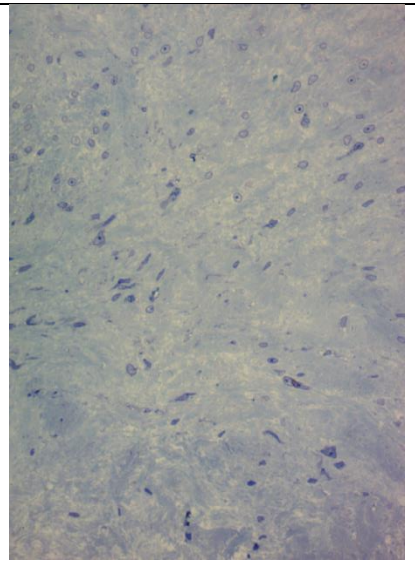
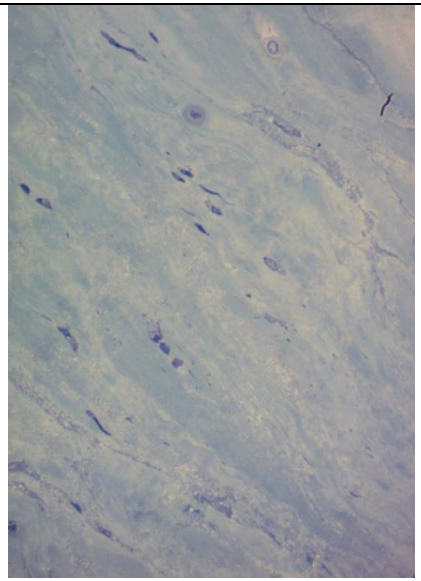
<table>
<tr><td colspan="2">Patient 1</td><td colspan="3">Biopsy Light microscopy</td></tr>
<tr><td>info</td><td>pathology</td><td>+1cm</td><td>+2cm</td><td>+3cm</td></tr>
<tr><td>BM
07/04/1971
male
Laborer</td><td>• Left traumatic (fall incident) rotator cuff tear en AC arthrosis
• Arthroscopic subacromial decompression, AC resection, biceps tenotomy, bursectomy and arthroscopic SSP repair</td><td rowspan="2">  </td><td rowspan="2">  </td><td rowspan="2">  </td></tr>
<tr><td colspan="2">Collagen count TEM

<table><tr><th>patient</th><th>intra 13/15</th><th>extra 13/17</th></tr><tr><td>1</td><td>561</td><td>615</td></tr><tr><td></td><td>483</td><td>1432</td></tr><tr><td></td><td>1387</td><td>1847</td></tr><tr><td></td><td>441</td><td>1081</td></tr><tr><td>mean</td><td>718</td><td>1243,75</td></tr></table></td></tr>
<tr><td colspan="2"></td><td>Star-shaped cells
Firm en good organized collagen</td><td>Chondrometaplasia (rounded cells, less structured</td><td>Star-shaped cells
Firm collagen</td></tr>
</table>

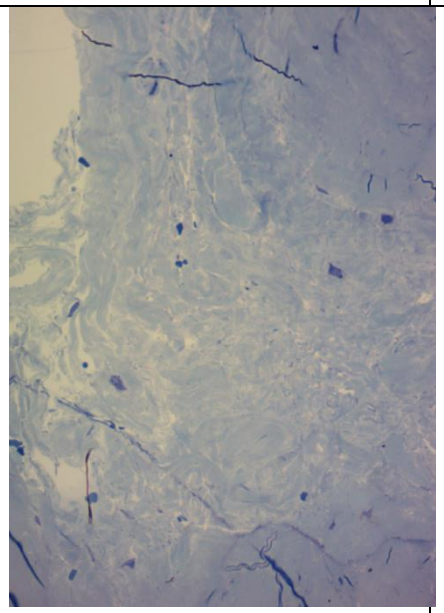
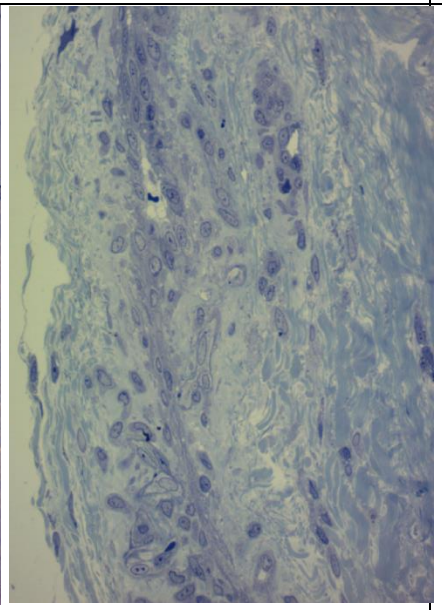
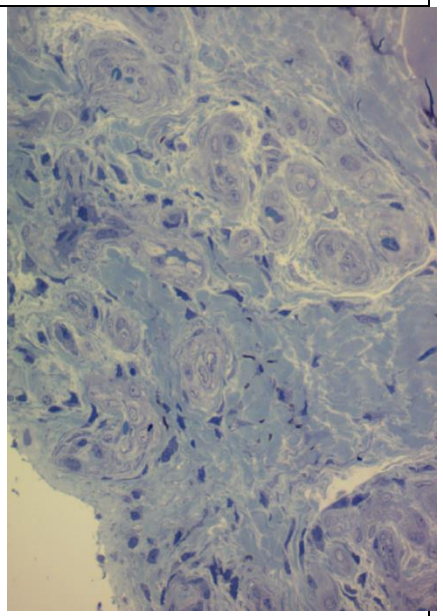
<table>
<tr><td colspan="2">Patient 2</td><td colspan="3">Biopsy Light microscopy</td></tr>
<tr><td>info</td><td>pathology</td><td>+1cm</td><td>+2cm</td><td>+3cm</td></tr>
<tr><td>VG
04/04/1942
Male
In retirement</td><td>Wide left rotator cuff tear, frozen shoulder and AC arthrosis
Arthroscopy left shoulder with arthrolysis, subacromial decompression, AC resection, bursectomy, biceps tenotomy and arthroscopic SSP repair</td><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="2">Collagen count TEM
<table><tr><th>patient</th><th>intra 13/19</th><th>extra 13/21</th></tr><tr><td>2</td><td>374</td><td>765</td></tr><tr><td></td><td>590</td><td>395</td></tr><tr><td></td><td>159</td><td>514</td></tr><tr><td></td><td>449</td><td>847</td></tr><tr><td>mean</td><td>393</td><td>630,25</td></tr></table></td><td>Rich in cells + , star-shaped cells, firm</td><td>Rich in cells ++, groups of cartilage cells</td><td>Firm</td></tr>
</table>

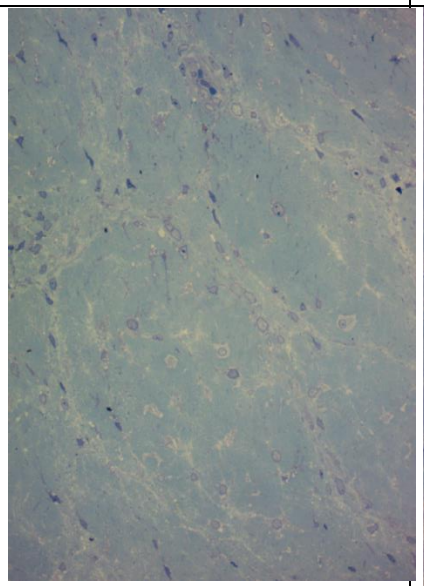
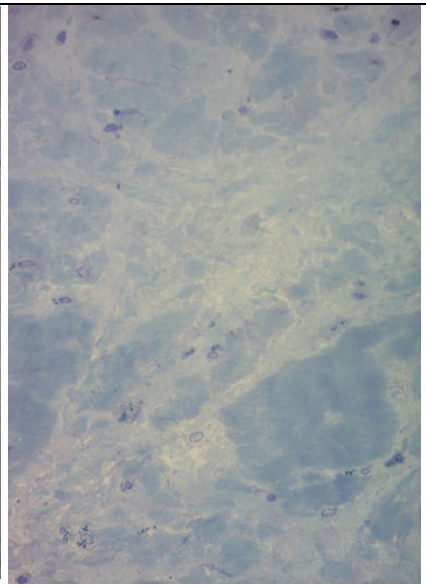
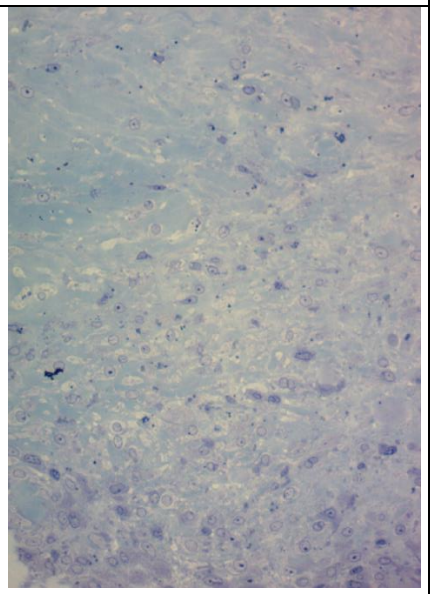
<table>
<tr><td colspan="2">Patient 3</td><td colspan="3">Biopsy Light microscopy</td></tr>
<tr><td>info</td><td>pathology</td><td>+1cm</td><td>+2cm</td><td>+3cm</td></tr>
<tr><td>HJ 23/07/1949
Male
In retirement</td><td>• Left rotator cuff tear artropathy and chronic luxation of the shoulder
• Total shoulderprothesis: type reversed delta Xten
• Traumatic recurring shoulderluxation Left</td><td rowspan="2">  </td><td rowspan="2">  </td><td rowspan="2">  </td></tr>
<tr><td colspan="2">Collagen count TEM
<table><tr><th>patient</th><th>intra 13/10</th><th>extra 13/12</th></tr><tr><td>3</td><td>973</td><td>662</td></tr><tr><td></td><td>1240</td><td>452</td></tr><tr><td></td><td>1672</td><td>691</td></tr><tr><td></td><td>648</td><td>604</td></tr><tr><td>mean</td><td>1133,25</td><td>602,25</td></tr></table></td></tr>
<tr><td colspan="2"></td><td>Cells around the paratenon, rich in cells , less structured</td><td>Rich in cells +++, vascularization+++</td><td>Fibrin, rich in cells +++, degenerative chondrometaplasia, vascularization</td></tr>
</table>

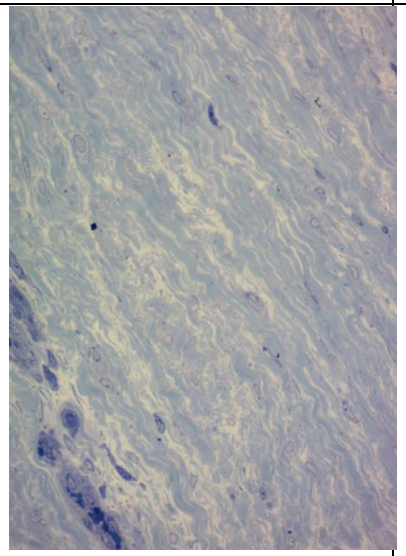
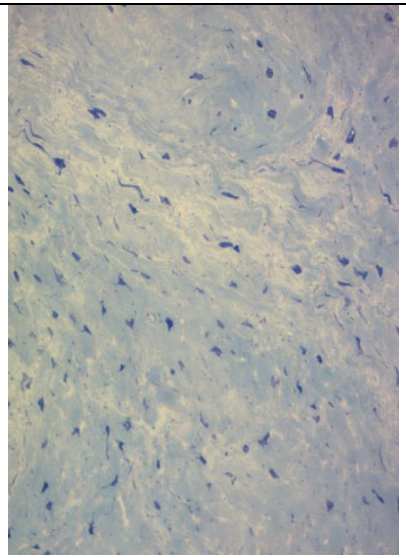
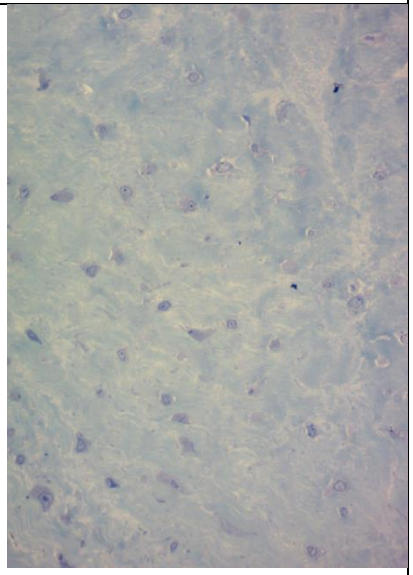
<table>
<tr><td colspan="2">Patient 4</td><td colspan="3">Biopsy Light microscopy</td></tr>
<tr><td>info</td><td>pathology</td><td>+1cm</td><td>+2cm</td><td>+3cm</td></tr>
<tr><td>OA 21/03/1954
Male
Vendor</td><td>Left rotator cuff tear and AC arthrosis.
Arthroscopy of left shoulder with arthroscopic subacromial decompression, AC resection, bicepstenotomy, bursectomy and arthroscopic SSP repair</td><td rowspan="2">  </td><td rowspan="2">  </td><td rowspan="2">  </td></tr>
<tr><td colspan="2">Collagen count TEM
<table><tr><th>patient</th><th>intra 13/2</th><th>extra 13/4/5</th></tr><tr><td>4</td><td>714</td><td>739</td></tr><tr><td></td><td>489</td><td>986</td></tr><tr><td></td><td>970</td><td>1064</td></tr><tr><td></td><td>325</td><td>939</td></tr><tr><td>mean</td><td>624,5</td><td>932</td></tr></table></td></tr>
<tr><td colspan="2"></td><td>Waved healthy structures</td><td>Rich in cells +, chondrometaplasia, organisation ok</td><td>Healthy and well organized</td></tr>
</table>

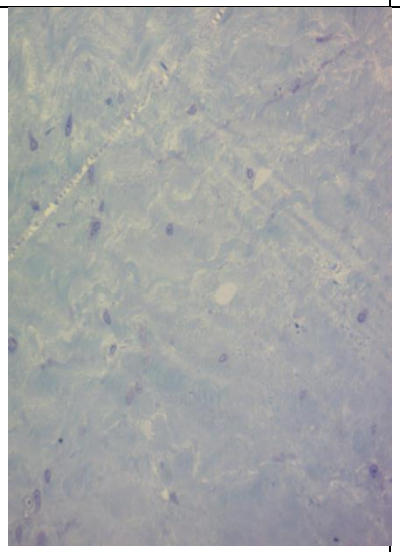
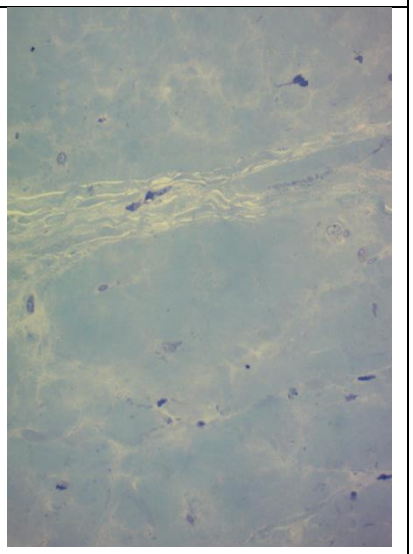
<table>
<tr><td colspan="2">Patient 5</td><td colspan="3">Biopsy Light microscopy</td></tr>
<tr><td>info</td><td>pathology</td><td>+1cm</td><td>+2cm</td><td>+3cm</td></tr>
<tr><td>FS 08/12/1949
female
In retirement</td><td>• Traumatic right rotator cuff tear and AC arthrosis
• Arthroscopy of the right shoulder with arthroscopic subacromial decompression, AC resection, bicepstenotomy, bursectomy and arthroscopic SSP repair</td><td rowspan="2">  </td><td rowspan="2">  </td><td rowspan="2">  </td></tr>
<tr><td colspan="2">Collagen count TEM

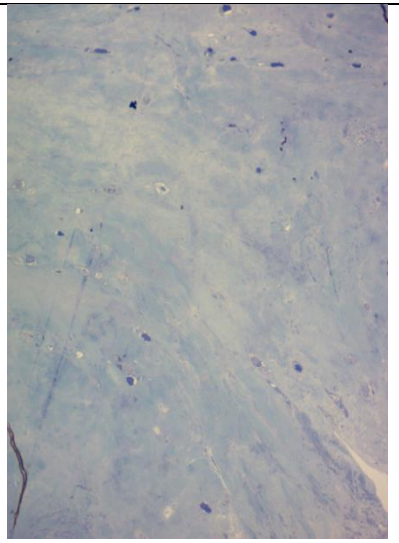
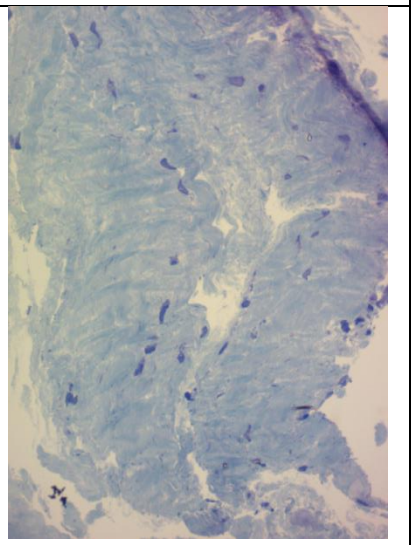
patient 5
intra 14/1: 758, 965, 633, 1028
extra 14/3: 352, 1282, 1359, 647
mean: 846 / 910</td></tr>
<tr><td colspan="2"></td><td>Firm healthy collagen</td><td>Rich in cells ++ less structured</td><td>Star-shaped cells, well structured</td></tr>
</table>

Patient 6		Biopsy Light microscopy		
info	pathology	+1cm	+2cm	+3cm
BJ 20/06/1955 Male Teacher	• Right rotator cuff tear after a fall. • Arthroscopy of the right shoulder with arthroscopic subacromial decompression, bicepstenotomy, bursectomy and arthroscopic SSP repair			
Collagen count TEM patient 6: intra 14/6: 394, 471, 458, 519; extra 14/8: 324, 669, 468, 588 mean: 460,5; 512,25		Less structured	Rich in cells +++, chondrometaplasia	Rich in cells+++, chondrometaplasia

Patient 7		Biopsy Light microscopy		
info	pathology	+1cm	+2cm	+3cm
VA 04/07/1943 female In retirement	• Massive rotator cuff tear ot right shoulder, recurring instability of the humerus head with cranial (sub) luxation • Arthroscopy of the right shoulder with arthroscopic synovectomy, bursectomy, biceps tenotomy and arthroscopic resection SSP			
Collagen count TEM patient 7: intra 14/12: 426, 361, 362, 559, mean 427; extra 14/14: 1808, 1520, 1543, 1720, mean 1647,75		Star-shaped cells, firm	Less structured, chondrometaplasia, rich in cells +	Rich in cells, star-shaped cells

<table>
<tr><td colspan="2">Patient 8</td><td colspan="3">Biopsy Light microscopy</td></tr>
<tr><td>info</td><td>pathology</td><td>+1cm</td><td>+2cm</td><td>+3cm</td></tr>
<tr><td>DM 28/10/1965
female
cleaninglady</td><td>• Right rotator cuff tear and AC arthrosis after a traumatic fall.
• Arthroscopy of the right shoulder with arthroscopic subacromial decompression, AC resection, bicepstenotomy, bursectomy and arthroscopic SSP repair</td><td rowspan="2">  </td><td rowspan="2">  </td><td rowspan="2">  </td></tr>
<tr><td colspan="2">Collagen count TEM
<table><tr><th>patient</th><th>intra 14/17</th><th>extra 14/19</th></tr><tr><td>8</td><td>402</td><td>969</td></tr><tr><td></td><td>354</td><td>873</td></tr><tr><td></td><td>452</td><td>954</td></tr><tr><td></td><td>332</td><td>1042</td></tr><tr><td>mean mean</td><td>385</td><td>959,5</td></tr></table></td></tr>
<tr><td colspan="2"></td><td>Firm collagen star shaped cells</td><td>Rich in cells, less organized</td><td>Rich in cells, less organized</td></tr>
</table>

Patient 9		Biopsy Light microscopy		
info	pathology	+1cm	+2cm	+3cm
RT 21/05/1961 Male Truck driver	• Right rotator cuff tear and AC arthrosis • Arthroscopy of the right shoulder with arthroscopic subacromial decompression, AC resection, bicepstenotomie, bursectomie and arthroscopic SSP repair			
Collagen count TEM patient 9: intra 15/1: 951, 874, 1042, 1135; extra 15/3: 677, 510, 901, 1259 mean: 1000,5; 836,75		Firm healthy collagen	Rich in cells, less organized	Healthy and well organized

Patient 10		Biopsy Light microscopy		
info	pathology	+1cm	+2cm	+3cm
VE 22/01/1947 Male In retirement	• Broad right rotator cuff tear and AC arthrosis • Arthroscopy of the right shoulder with arthroscopic subacromial decompression, AC resection, bicepstenotomy, CA –depot ISP, bursectomie and arthroscopic SSP repair			
Collagen count TEM patient / intra 15/6 / extra 15/8 10 / 1067 / 1375 714 / 936 1330 / 1131 1235 / 1218 mean / 1086,5 / 1165		Healthy and well organized, star-shaped cells	Rich in cells, less organized	Healthy and well organized, rich in cells

Addendum 4: European journal of anatomy: instructions for authors

Eur J Anat
ISSN: 1136-4890 (*print version*)
ISSN: 0000-0000 (*electronic version*)

INSTRUCTIONS FOR AUTHORS

General Information

The European Journal of Anatomy (Eur J Anat; ISSN 1136-4890) is the property of the Spanish Society of Anatomy and has been published continuously since 1996 (with a trimestral periodicity). It is a moderated journal that uses the system of external review (peer review) by experts in the subject areas researched and covering the research methodololgies. The journal adopts, and adheres, to the publication standards established in the *"Uniform requirements for manuscripts submitted to biomedical journals",* (Vancouver style) 6th edition, prepared by the "International Committee of Medical Journal Editors (ICMJE)", accessible on (http://www.icmje.org). The Spanish version of these standards may be obtained, among others, from the Spanish Journal of Public Health (*Revista Española de Salud Pública*) 2004; 78(3): 297-321, accessible on http://scielo.isciii.es/scielo.php?script=sci_issuetoc&pid=1135- 572720040003&lng=es&nrm=iso

Compliance with the Vancouver requirements (*or the corresponding reference Style Guide*) facilitates the indexing of the journal in the leading specialty databases and this, in turn, benefits the authors and their academic institutions because of the wider dissemination of the published works.

Scope and Coverage

The European Journal of Anatomy aims to disseminate results from original research into the following prioritized subject areas: descriptive human and experimental morphology (gross, embryological and microscopic anatomy); neurosciences, developmental biology, comparative morphology; educational research in the anatomical sciences. The works must be original, written in English, unpublished and not under consideration for publication in another journal. The author(s) alone is responsible for the claims made in the article.

The following **types of contributions** will be considered for publication: original articles, reviews, case reports, head-to-head articles, letters to the editor.

Original articles. They must have the following structure: summary, key words, text (introduction, materials and methods, results and discussion), acknowledgements and bibliography. The maximum length of the text will be 25 size DIN-A4 pages (*in Word format*), double-spaced with 2.5 cm. margins, font size 12, font Times New Roman or Arial, with figures and tables presented as separate sheets. The number of authors should not be greater than 10.

***Reviews*.** The maximum length of the text will be 30 size DIN-A4 pages, (*or Word format*) double-spaced, with 2.5 cm. margins and font size 12, font Times New Roman or Arial. The bibliography may not exceed 100 references. Optionally, the work may include tables and figures. Where included, the figures and tables must presented as separate sheets.

Case reports. These provide a summary description of rare variation cases or specific procedures of clinical interest. The maximum length will be 10 size DIN-A4 pages, with tables or figures included. The text must be double-spaced, with 2.5 cm. margins and font size 12, font Times New Roman or Arial.

Head-to-head articles. Such articles enable a discussion of controversial topics. Each topic includes an invited lead paper and comments from several contributors with different viewpoints. Suggestions for possible topics and offers of contributors are welcomed.

The journal also welcomes *Letters to the editor* and commissions ***Book review*** and publishes ***Abstracts*** from research meetings**.**

Manuscript submission

Manuscripts should be submitted online at http://www.vitjournals.com/home/default.aspx?ID=EJA Choose "Submit a new manuscript" option and follow the instructions. When you have completed the submission process, you will receive an e-mail with a manuscript identification number, your Username and your Password. When you want to check the status of your submission, go to the same page as above and access as Author with your Username and Password.

No charge is made for publication, but authors may be required to pay for extensive changes introduced after the manuscript has been set in press.

Presentation and structure of the works

Manuscripts should be presented on DIN-A4 size, double-spaced, wide margins (2.5 cm on all sides) and with the pages numbered sequentially in the upper right-hand corner. Submit manuscript and all figures as separate files (i.e. in MSWord format and the images in Tiff or JPEG format with a good resolution). You do not need to mail any paper copies of your manuscript. The manuscript shall be accompanied by a covering letter asking that the manuscript be taken into consideration, with the author explaining in 4 to 5 lines what is the original contribution of the work presented, a statement of non-simultaneous submission to other journals, and confirmation of the undersigned authors. This letter must also transfer the copyright to the publisher. The author(s) must keep a copy of the original manuscript to avoid irreparable loss or damage to the material.

Bibliographic references must be provided following the discussion section of the article (or acknowledgements, if any). The bibliographic references, which must be sufficient in number to relate the research to previous work, must be presented in alphabetical order. When a reference appears in a table or figure only, it should also be included in the reference list.

The manuscripts must be presented to the journal with sections in the following order:

Title Page. First page of the manuscript

As the cover of the manuscript, this must contain:

Title of the article (concise, yet informative), made up of the greatest number of significant terms possible taken from a controlled specialty glossary, such as the Medical Subject Headings (MesH). If necessary, a subtitle may be added, not to exceed 40 letters and spaces.

First name and last name of each of the authors, taking into account the signature format for indexing in international databases.

Full name of the institution/centre of each of the authors, which is referenced beside the name of the author with Arabic numerals in *superscript*.

Name and full address of the person responsible for the author responsible for correspondence, including the phone number and fax number, where appropriate, as well as the e-mail address.

Information on grants, aid or financial support provided (Research Projects) to subsidize the work and other specifications, where applicable.

To prepare this page, see the template (provided by the journal) attached to these instructions (see Guidelines for authors) on how to prepare the manuscript identification sheet.

Summary and Key-Words. Second page of the manuscript

A second, separate page, must contain the first and last names of the authors, the title of the article, article content summary and the list of key words.

The Summary of the work must be between 150 and 250 (*or between 250-300*) words. In the case of original articles, the content of the Summary must describe, concisely, the purpose and objective of the research, the methodology used, the most important results and the main conclusions. The novel and relevant aspects of the work must be emphasized.

Key words: beneath the summary, 5-10 key words or short phrases shall be specified to indicate the content of the work, for inclusion in collections and national and international databases. As many as possible should be provided, up to a maximum of ten. Controlled reference terms should be used (*based on each specialty, such as those in the Spanish Medical Index and the Medical SH (MeSH) for medicine, accessible on http://www.nlm.nih.gov/mesh/meshhome.html.*

Manuscript text. Third and following pages, where the manuscript text begins

The third and following pages are dedicated to the manuscript text (25 pages maximum if it is an original article, and 30 pages maximum if it is a review). In the case of case reports, the length shall be 10 pages, and for letters to the editor 2 pages. In the case of reviews, letters and other options, appropriate sections may be included at the discretion of the authors to facilitate comprehension. However, original articles must be divided into the following sections: Introduction, Materials and Methods, Results, Discussion, Acknowledgements (if any) and References.

Introduction: You must include the foundation and purpose of the study, using the bibliographic citations that are strictly necessary. Do not include data or conclusions of the work presented. Do not prove a detailed bibliographic review.

Materials and Methods: This must be presented with sufficient precision to enable the reader to understand and confirm the development of the research. Sources and methods published previously should be described just briefly, providing the corresponding references, except when they have been modified. The sample size and the sampling method used must be described, where appropriate. Reference must be made to the type of documentary, critical and statistical analysis, etc. used. If it is an original methodology, the reasons for using it must be explained, describing any possible limitations.

When dealing with experimental works in which groups of humans or animals have been used, indicate whether the ethical criteria approved by the commission corresponding to the institution/centre in which the study was carried out have been taken into account and, in any case, whether the agreements reached in the Declaration of Helsinki, revised in October 2000, drafted by the World Medical Association (http://www.wma.net/), have been respected. Neither the names nor the initials of the people participating in the study sample must be used. When describing substances or chemical products, indicate the generic name, the dosage and the administration method.

Results: These shall appear in a logic sequence in the text, tables or figures, without repeating the data included in the text. Try to highlight the important observations. Describe, without interpreting or judging, the observations made with the materials and methods used.

Discussion: Summarize the findings, relating the observations of this study with those of other studies of interest, highlighting the contributions and limitations of each. Do not repeat the data or other material already commented in other sections in detail. Mention the inferences from the findings and their limitations, including suggestions for future research. Link the conclusions to the study objectives, avoiding gratuitous affirmations and conclusions that are not fully supported by the study's data.

Acknowledgements: Thank only those who have made substantial contributions to the study, but who do not warrant the status of author; the author must obtain their consent in writing. Likewise, the Council Science Editors (CSE) recommend that authors, where appropriate, provide an explicit statement of the source of their research funding, placing this in the acknowledgements (CSE 2000)

(*Conflicts of Interest and the Peer Review Process. Draft for CSE member review, posted 3/31/00.* http://www.cbe.org/services_DraftPolicies.shtml).

References: The bibliography must be placed after the discussion section (or the acknowledgements, if any) in the prescribed format.

The recommended style for the references is indicated below in the examples; it is based on the Vancouver standards (*for the areas of Biomedicine and Health Sciences, accessible on http://www.icmje.org).*

- The names of the journals must be abbreviated in accordance with the generally accepted practices in the area of knowledge, whenever there is a reference list (*for example, the Spanish Medical Index list (*http://www.uv.es/~fresquet/TEXTOS/) or the *Index Medicus "List of Journals Indexed", which publishes the Index Medicus each year in the January issue, and in the Journal Browser section of Medline (*http://www.ncbi.nlm.nih.gov/entrez/jrbrowser.cgi).

The bibliography must be corrected by the author(s), correcting it with the copy in their possession. Avoid using vague phrases as bibliographical quotations. Do not use those that require clarifications such as "unpublished observations" or "personnel correspondence", although they may be cited in the text in parentheses. Works that have been accepted, but not yet published, shall be included in the bibliography, specifying the name of the journal, followed by the expression "pending publication".

The bibliographic references must be taken from the original documents, always indicating the starting and ending page numbers of the work from which they are taken. To ensure consistency, at any time during the publishing process, the journal editors may require authors to send the first page (photocopy) of each of the articles quoted in the bibliography.

For journals, given their importance for quotation indexes and Impact Factor calculations, the following shall be cited: a) all the author(s), with their last name(s) and initial(s) of the first name(s), in versal type without period or comma separators. If there is more than one author, they must be separated by a comma, but not the conjunction "and". b) Year of publication in parentheses. c) Title of the article in the original language, with the appropriate spelling and accents. Place a period after the title. d) Indexed or correct abbreviated name of the journal in *italics*. Do not use a period after each abbreviation in the name of the journal, placing a comma between the final abbreviation and section e) issue number. The separation between this section and section f) shall be a colon followed by a space: f) first and last pages, separated by a hyphen. Place a period at the end of the quotation.

The following are examples of properly referenced bibliographic references: 1.

Journal article.

Standard article.- DOWNES GB, GRANATO M (2004) Acetylcholinesterase function is dispensable for sensory neurite growth but is critical for neuromuscular synapse stability. *Dev Biol,* 270: 232-245.

The author is not mentioned.- Cancer in South Africa [editorial] (1994) *S Afr Med J*, 84: 15.

Volume supplement.- SHEN HM, ZHANG KF (1994) Risk assessment of nickel carcinogenicity and occupational lung cancer. *Environ Health Perspect*, 102 Supl 1: 275-282.

Part of a volume.- OZBEN T, NACITARHAN S, TUNCER N (1995) Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. *Ann Clin Biochem*, 32 (Pt 3): 303-306.

Part of an issue. - POOLE GH, MILLS SM (1994) One hundred consecutive cases of flap lacerations of the leg in aging patients. *N Z Med J*, 107 (986 Pt 1): 377-378.

Issue without volume. - TURAN I, WREDMARK T, FELLANDER-TSAI L (1995) Arthroscopic ankle arthrodesis in reumathoid arthritis. *Clin Orthop*, (320): 110-114.

No issue or volume. - BROWELL DA, LENNARD TW (1993) Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. *Curr Opin Gen Surg*, 325-333.

2. Books, monographs and others

Individual author.- CARRANZA FA Jr (1984) *Glickman's Clinical Periodontology*. Saunders, Philadelphia.

Chapter of a book.- TAKEY H, CARRANZA FA Jr (1984) Treatment of furcation involvement and combined periodontal endodontic therapy. In: Carranza FA Jr (ed). *Glickman's Clinical Periodontology*. Saunders, Philadelphia.

Editor(s) or compiler(s) as author.- NORMAN IJ, REDFERN SJ, editors (1996) Mental health care for elderly people. Churchill Livingstone, New York.

Conference minutes.- KIMURA J, SHIBASAKI H, editors (1996) Recent advances in clinical neurophisiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Elsevier, Amsterdam.

Conference article.- BENGTSSON S, SOLHEIM BG (1992) Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienholf O (eds). MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. North-Holland, Amsterdam, pp 1561-1565.

Scientific and technical report.- SMITH P, GOLLADAY K (1994) Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections. Report No.: HHSIGOE169200860.

Doctoral thesis.- KAPLAN SJ (1995) Post-hospital home health-care: the elderly's access and utilization [doctoral thesis]. St Louis (MO), Washington Univ.

Patent.- LARSEN CE, TRIP R, JOHNSON CR, inventors; Novoste Corporation, assignee. Methods for procedures related to the electrophisiology of the heart. US patent 5,529,067. 1995 Jun 25.

Newspaper article.- LEE G (1996) Hospitalizations tied to ozone pollution: study estimates 50,000 admissions annually. *The Washington Post*, Jun 21. Sec. A:3 (col. 5).

Computer file.- Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

(For most Natural and Experimental Sciences areas, the Harvard System format system for bibliographical references may also be used, where the bibliography is sorted by last name, citing the text by author-year (e.g. Sanz 1996). All details on this system are accessible on http://libweb.anglia.ac.uk/referencing/harvard.htm.)

Tables: These must be presented on separate pages, numbered according to their order of appearance in the text with Arabic numerals. They are used to clarify important points; double/repeated documentation by means of tables and figures shall not be accepted. The titles or footers that accompany them must well explain their content.

Figures: All types of photographs, graphs or drawings are considered figures. They are assigned an Arabic numeral according to their order of appearance in the text, identified by the abbreviated term fig.(s). The footers or legends of each one must be typed, with the corresponding number on a separate sheet. The text in the figures must be in uppercase. They must be designed to fit the format of 81 mm wide (1 column), although they may also be accepted with 172 mm (one full page), presented with good contrast so that quality is not lost in the reduction. Micro-photographs will be accepted in these proportions for publication without reduction; they must include a linear scale. Photographs shall be sent unframed, only when authors wish to publish several photographs in a block must they be sent framed.

The figures must clarify the text in a major way. They must thus be used sparingly and only wherever necessary.

Abbreviations: Only standard, universally accepted abbreviations shall be used. See Units, Symbols and Abbreviations. The Royal Society of Medicine London. When a term that is used frequently in the text is to be shortened, the corresponding abbreviation must accompany it (in parentheses) the first time it appears. No Roman numerals shall be used in the text; periods shall be used to the right of the zero for decimals, not commas. Trademarks shall not be used except as needed, in which case the first time they are used they shall be accompanied by the symbol.

Editorial process

The works shall be submitted with a covering letter that requests evaluation of the work for publication in one of the sections of the Journal, expressly indicating that it is a work that has not been disseminated or published previously, sent only to the European Journal of Anatomy for evaluation and publication, if appropriate, as well as the contributions regarding originality and novelty that, according to the authors, the work contains.

The covering letter must be signed by all authors. It shall include written authorization from all persons appearing in the section of thanks or from those studied during the research whose identification is essential in presenting the results. It shall also include a statement, where appropriate, accepting the introduction of changes to the manuscript by the journal editors.

The Journal's editorial staff shall acknowledge receipt to the authors of the works they receive, subsequently notifying them of their acceptance or rejection.

The editorial staff shall pass the work under consideration for publication to the Editorial Board, verifying that it suits the journal's coverage and complies with the publication standards. If it does, it will be reviewed externally.

The manuscripts shall be reviewed anonymously (*blind or double blind*) by two experts in the work's subject area and/or according to the methodology used. Based on the external reports, the Journal's editorial staff reserves the right to accept/reject the articles for publication, as well as to make style modifications and/or shorten texts that surpass the established limit, committing to respect the original content.

In the event of differences of opinion between the two expert reviewers, the manuscript shall be sent to a third reviewer. External paired review will be applied to original articles, short reports, reviews, etc. Works that are reviewed and considered for publication following modification shall be returned within a period of 2 months, regardless of whether minor or major corrections are requested. The authors will receive the evaluation reports from the reviewers, anonymously, so that they may make (where appropriate) any necessary corrections or replies.

In general, once the external reports have been seen, the factors on which the Journal's editorial staff bases its decision on accepting/rejecting the works are the following: a) originality: completely original, valuable information, repetition of known results; b) timeliness and novelty; c) relevance: applicability of the results in resolving specific problems; d) significance: advancement of scientific knowledge; e) reliability and scientific validity: verified methodological quality; f) presentation: good writing, organization (logical coherence and material presentation).

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It is the responsibility and duty of the editorial staff of the European Journal of Anatomy to remind its collaborators of the following:

– When describing the experiments carried out on human beings, indicate whether the procedures followed are in compliance with the ethical standards of the responsible human experimentation committee (institutional or regional) and the 1975 Declaration of Helsinki, revised in 2000. No names, initials or hospital numbers shall be used, especially in the figures. When describing experiments on animals, indicate whether guidelines from an international research institution or council or a national law regulating the care and use of laboratory animals were followed.

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– The journal does not accept previously published material. The authors are responsible for obtaining the appropriate permission for partially reproducing material (text, tables or figures) from other publications and for citing their source correctly.

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**

Richting: **master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij neurologische aandoeningen**
Jaar: **2014**

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Cox, Jolien **Proesmans, Michael**