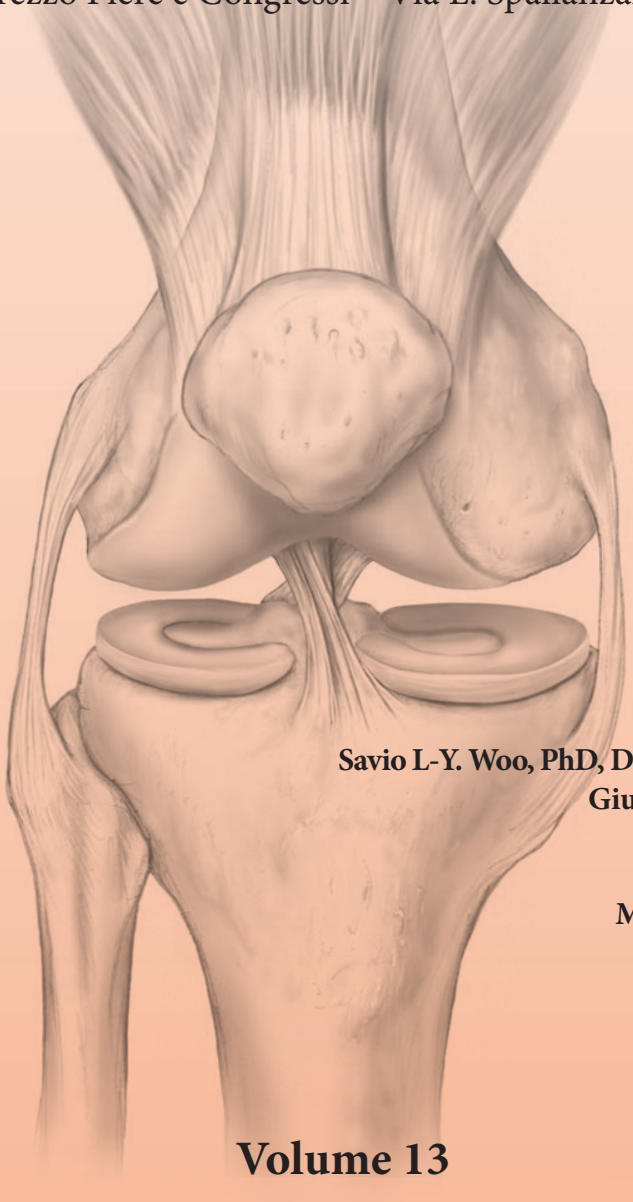
ISL&T

International Symposium on LIGAMENTS AND TENDONS XIII

Friday, 18th October 2013

Italy, Tuscany, Arezzo

Arezzo Fiere e Congressi – Via L. Spallanzani, 23



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Volume 13

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Volume 13

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Welcome by Professor Savio L-Y. Woo

Dear Friends and Colleagues,

On behalf of the International Advisory Committee, it is our distinct honor and pleasure to announce that the Thirteenth International Symposium on Ligaments and Tendons (ISL&T-XIII) will take place in Arezzo, Italy on October 18, 2013.

For thirteen years now, the ISL&T has brought together biologists, engineers, clinicians and surgeons that are ligament and tendon enthusiasts to present and to discuss new developments and important topics related to a subject dear to all of us. These gatherings have also facilitated graduate students as well as post-doctoral fellows to exchange ideas with senior level investigators freely and to learn from them. As a result, many new collaborative research projects have formed.

We would like to especially thank Professor Giuliano Cerulli for hosting ISL&T-XIII; and more importantly, for bringing the first ever ISL&T to Europe! Professor Cerulli and his organizing committee have done a wonderful job keeping with the traditions of ISL&T while adding their special flavor to the meeting. We are also pleased to have an International Program Committee Co-Chaired by Dr. Catherine Kuo, Professor Philippe Neyret and Professor Mahmut Nedim Doral. The international program committee has selected the following thematic topics:

- Biology and Biomechanics of Ligaments and Tendons
- Entheses
- Biological Augmentation (Stem Cells, PRP) for Tendon and Ligament Healing
- Tissue Engineering of Tendons and Ligaments
- MPFL

I am also pleased to let you know that there will be a number of awards for young investigators being offered at the ISL&T-XIII and encourage all to participate fully.

See you in beautiful Arezzo, Italy!

Savio L-Y. Woo, Ph.D., D.Sc. (Hon.), D.Eng. (Hon.)
Chair, International Advisory Committee

Welcome by Professor Giuliano Cerulli

The International Symposium on Ligaments and Tendons – XIII will be held for the first time in Europe. For all of us here at the Let People Move Research Institute it is a great honor and pleasure to be able to organize this very important scientific symposium.

Today, basic research and its clinical application is extremely enthralling, and the interest of all the professionals in this field is clear.

The natural beauty, the historical wealth present in Tuscany and Arezzo are the perfect setting for an unforgettable, scientifically-qualifying and socially-rich weekend.

We extend a warm welcome to you all and are looking forward to having you join us.

Giuliano Cerulli
Chair, ISL&T-XIII

ISL&T-XIII Committees

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Giacomo Placella, MD
Matteo Tei, MD
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Kazunori Yasuda, MD (Asia)

ISL&T Awards
Savio L-Y. Woo Young Researcher Award

ISL&T-X, Hong Kong, China (2010 Inaugural Year)

Biological Research – **Xiao Chen**
Advisor: H-W. Ouyang
Zhejiang University
Zhejiang, China

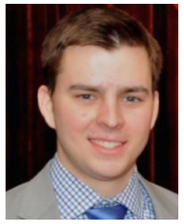
Clinical Research – **Saira Chaudhry**
Advisor: D. Morrissey
Queen Mary University of London
London, United Kingdom

ISL&T-XI, Irvine, CA

Biomechanical Research – **Joo H. Oh**
Advisor: T.Q. Lee
VA Long Beach Healthcare System
University of California
Irvine, CA

Biological Research – **Jeffrey P. Brown**
Advisor: C.K. Kuo
Tufts University
Medford, MA

ISL&T-XII, San Francisco, CA



Biological Research – **Jonathan P. Gumucio**
Advisor: C.L. Mendias
University of Michigan
Ann Arbor, MI

ISL&T-XIII Award Recipient

Biomechanical Research – Chauvanne T. Thorpe
Advisor: H.R. Screen
Queen Mary University of London
London, United Kingdom
Abstract Title: FATIGUE LOADING CAUSES A REDUCTION IN THE ABILITY OF TENDON FASCICLES TO RECOIL

Award

USD$1,000 and Certificate (up to 4)

Purpose

Professor Savio L-Y. Woo founded the International Symposium on Ligaments and Tendons (ISL&T) to promote awareness of the field, the exchange of information and collaboration nationally and internationally. The ISL&T has been a venue for lively discussion of current topics in connective tissue research and clinical applications. In addition to his leadership and significant scientific contributions to our field, Professor Woo has been an internationally recognized intellectual ambassador for training, mentoring and for aspiring students in the field of biomedical engineering and orthopaedic surgery. We are honored to present the Savio L-Y. Woo Young Researcher Award to individuals who perform the best research studies in three major areas, biomechanical, biological and clinical and have submitted their work to the ISL&T meeting.

The Award is intended to provide partial support (up to $1000) towards the applicant's research or for travel expenses to attend the ISL&T-XIII meeting. Up to four awards will be given.

Eligibility

Open to graduate students and postdoctoral fellows. Applicant must be the first author of the abstract and be present at the ISL&T-XIII meeting/banquet to accept the award. Advisor's verification of eligibility is required.

Application

Upon submission of the abstract by the regular submission deadline, applicant must indicate his/her intention to be considered for the award. Those selected will be invited to submit extended abstracts.

Award Categories

At least one award will be given in each of three main categories, namely:

- Biomechanical: Experimental studies involving biomechanics of ligaments and tendons, new methods for measurement of biomechanical properties, or computational analyses
- Biological: Basic science studies to characterize the cellular behavior of ligaments and tendons, as well as the extracellular matrix
- Clinical: Studies which compare existing surgical procedures or propose novel alternatives

Selection Committee

Applicant's abstract submitted for the ISL&T-XIII will be reviewed by the program committee through the regular evaluation process based on scientific merit and research quality.

Award Committee

Albert Banes, PhD *(Chair)*
Giuliano Cerulli, MD *(Chair, ISL&T-XIII)*
Thay Lee, PhD
Per Renstrom, MD
Graham Riley, PhD
Kwang Kim, BS (Assistant)

Acknowledgements

Sponsored by Flexcell International Corp. and the Asian♦American Institute for Research and Education (ASIAM).

I. Best Paper Award

Award: USD$200 and Certificate

Eligibility

Open to current graduate students. Applicant must be the first author of the abstract and be present at the ISL&T meeting/banquet to accept the award. Advisor's verification of eligibility is required.

Application

Upon submission of the abstract by the regular submission deadline, applicant must indicate his/her intention to be considered for this award.

Selection Criteria

Applicant's abstract submitted for the ISL&T-XIII will be reviewed by the program committee through the regular evaluation process based on scientific merit and research quality.

Selection Process

The Program Committee will select the best paper during the international meeting. Award winner will be announced at the banquet.

II. Best Fellow Paper Award

Award: USD$200 and Certificate

Eligibility

Open to clinical fellows or post-doctoral research fellows. Applicant must be the first author of the abstract and be present at the ISL&T meeting/banquet to accept the award.

Application

Upon submission of the abstract by the regular submission deadline, applicant must indicate his/her intention to be considered for this award.

Selection Criteria

Applicant's abstract submitted for the ISL&T-XIII will be reviewed by the program committee through the regular evaluation process based on scientific merit and research quality.

Selection Process

The Program Committee will select the best paper during the international meeting. Award winner will be announced at the banquet.

III. Best Poster Award

Award: USD$200 and Certificate

Eligibility

Open to clinical fellows or post-doctoral research fellows. Applicant must be the first author of the abstract and be present at the ISL&T meeting/banquet to accept the award.

Application

Upon submission of the abstract by the regular submission deadline, applicant must indicate his/her intention to be considered for this award.

Selection Criteria

Applicant's abstract submitted for the ISL&T-XIII will be reviewed by the program committee through the regular evaluation process based on scientific merit and research quality.

Selection Process

The Program Committee will select the best poster during the international meeting. Award winner will be announced at the banquet.

Acknowledgement

Awards sponsored by Flexcell International Corporation.

Instructions to Presenters

I. Podium Presenters

The time for presentations has been limited, in favor of discussion. Please see the presentation formats listed below. Your moderators have been asked to adhere to the time allotted for your presentation as well as the number of slides.

Important: All speakers are asked to check-in with the session moderators 15 minutes before the session in which they will present to meet the projectionist and the moderator.

Presentation Requirements

Keynote Presentations

- 10 min. presentations each immediately followed by a 5 min. discussion.
- Maximum **10 PowerPoint slides** for computer presentation.

Regular Presentations

- 6 min. presentations followed by a 15 min. group discussion of 4-5 papers.
- Maximum **6 PowerPoint slides** for computer presentation.

An Important Note on Slides

Kindly note that all speakers must be prepared to present their paper using PowerPoint projection. We ask that you send your PowerPoint presentation file to us by October 16, 2013 so that we can load all talks into a master computer prior to the symposium. Please make sure that you clearly label your file with the author's name and the title of your presentation.

Note: In view of time and the large number of talks, there will be no opportunity to use your personal computer or load your PowerPoint file during the symposium.

II. Poster Presenters

All posters should be no larger than 45 inches x 45 inches (114.3 cm x 114.3 cm). Poster boards will be available in the lobby prior to registration. Please set up your poster between 7:30 am - 8:00 am and leave the posters up throughout the day. Posters are to be taken down by 6:00 pm.

Note: An opportunity has been provided for you to present your posters during different breaks. Please be sure to attend to your poster at the assigned time (refer to the Program).

Program - Podium Sessions

07.00-08.00	Registration and Breakfast
08.00-08.15	Opening Ceremony ***S.L-Y. Woo, G. Cerulli***
08.15-09.09	**1st Session: Part I (Pages 18-22)** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***S.L-Y. Woo, M.N. Doral***
08.15-08.30	Clinical Lecture: ACL Graft Maturity with & without PRGF ***R. Cugat*** Discussion
08.30-08.36	Age-Specific Cleavage Patterns in Tendon Extracellular Matrix ***M.J. Peffers***
08.36-08.42	The Alpha 7 Nicotinic Acetylcholine Receptor is Expressed on Human Tenocytes and Inhibit Proliferation of Tenocytes in Culture ***G. Andersson***
08.42-08.48	Analysis of the Residual Strength of a Partially Cut Tendon ***M. Pensalfini***
08.48-08.54	Biochemical and Morphological Comparison of the Extracellular Matrix Composition of Tendons and Ligaments ***Y.A. Kharaz***
08.54-09.09	Discussion
09.09-10.09	**1st Session: Part II (Pages 23-28)** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***G. Cerulli, C. Kuo***
09.09-09.24	Keynote Lecture: Tendons Repair & Regeneration ***M. Marcacci*** Discussion
09.24-09.30	Collagen (I) Homotrimer in Age-Related Fibroses and Tissue Degeneration: Evaluation as a Stem Cell Biomarker ***K.A. Williamson***
09.30-09.36	TNF-α Induced Apoptosis in Human Tenocytes is Reduced by Substance p Through a NK-1 Receptor Specific Pathway ***L. Backman***
09.36-09.42	New Correlates of Damage in Rat Tail Tendons ***M. Thompson***
09.42-09.48	Elastosonography as Novel Follow-Up Method in Achilles Tendon Surgery: Pilot Study ***F. Fusini***
09.48-09.54	The Insertional Ligaments of the Menisci ***S.H.J. Andrews***
09.54-10.09	Discussion
10.09-10.39	**Coffee Break & Poster Session** Session Chairs: ***P. Renström, S. Zaffagnini***
10.39-11.48	**1st Session: Part III (Pages 29-33)** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***K. Shino, F. Vercillo***
10.39-10.54	Keynote Lecture: Tendon Biomarkers and Tendonopathy ***A. Banes*** Discussion
10.54-11.09	**Savio L-Y. Woo Young Researcher Award Winner (Pages 17)** Fatigue Loading Causes a Reduction in the Ability of Tendon Fascicles to Recoil ***C.T. Thorpe*** Discussion
11.09-11.15	ACL Reconstruction with a Fully Resorbable Vs. Partially Resorbable Tibial Fixation System: Comparative Results At A Mid-Term Follow-Up ***C. Carulli***
11.15-11.21	The Influence of Different Femoral Hamstring Graft Fixation Methods - Dependency on Dynamic Loading ***M. Handl***
11.21-11.27	Quasi-Linear Viscoelastic Properties of the Medial Patellofemoral Ligament ***G. Criscenti***
11.27-11.33	In-Vivo Anatomy-Based Patellar Tendon Tracking During Total Knee Arthroplasty ***C. Belvedere***
11.33-11.48	Discussion

11.48-12.48	**1st Session: Part IV (Pages 34-39)** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***R. Vanderby, G.M. Peretti***
11.48-12.03	Keynote Lecture: What is the Role of the Tendon and Muscle Properties in Hooping Performance ***M. Lamontagne*** Discussion
12.03-12.09	The Effect of Double-Row Repair on Rotator Cuff Tendon Healing ***M.H. Baums***
12.09-12.15	The Treatment of Rotator Cuff Tears with Two Different Patches ***P. Ciampi***
12.15-12.21	Identification of Collagen VI In The Pericellular Matrix of Human Anterior Cruciate Ligament ***F. Sardone***
12.21-12.27	Achilles Enthesis: a Privileged Site of Diabetic Damage? ***M. Abate***
12.27-12.33	Histological and Immunohistochemical Findings in the Collateral Ligaments of the Equine Metacarpo- and Metatarsophalangeal Joint ***F. Pohlin*** Discussion
12.48-13.50	*Light Lunch*
13.50-14.44	**2nd Session (Pages 40-43)** **Biological Augmentation (Stem Cells, PRP) for Tendon and Ligament Healing** Session Chairs: ***A. Banes, S. Bruè***
13.50-14.05	Keynote Lecture: Tendinopathy: a Treatable Disease? ***G. Riley*** Discussion
14.05-14.11	Evaluation of the Influence of Plasma Rich in Growth Factors on Tendon Healing in an Experimental Model of Divided Achilles Tendon in Sheep ***J.A. Fernandez-Sarmiento***
14.11-14.17	Isolation and Characterization of Adult Stem Cells Derived from Human Rotator Cuff Tendons ***P. Randelli***
14.17-14.23	Growth Factors Effects on Skeletal Muscle Lesions. Experimental Study ***M. Cianforlini***
14.23-14.29	Optimum and Formulation of Ascorbic Acid As a Supplementation for Human Tenocytes in Vitro ***O. Hakimi***
14.29-14.44	Discussion
14.44-15.44	**3rd Session: Part I (Pages 44-48)** **Tissue Engineering of Tendon and Ligament** Session Chairs: ***T. Wang, G. Zamarra***
14.44-14.59	Keynote Lecture The Shape and the Thickness of the ACL Along its Length in Relation to The PCL. A Cadaveric Study ***A. Georgoulis*** Discussion
14.59-15.05	A Tissue Engineering Experimental Approach for the Tendon Tissue ***G.M. Peretti***
15.05-15.11	Evaluation Of Knee Function After Repair Of The ACL With A Novel Magnesium-Based Ring – An In Vitro Study in Goats ***K.F. Farraro***
15.11-15.17	Evaluation of Ligament Fibroblast and Stem Cell Responsein Co-Culture ***D.R. Bogdanowicz***
15.17-15.23	Intra-Articular Injection of Tripeptide Copper Complex Ghk-Cu (ii) Improved Graft Healing in Anterior Cruciate Ligament Reconstruction ***S.C. Fu***
15.23-15.29	Healing Response of the Human Anterior Cruciate Ligament: a Histological Study of Reattached ACL Remnants ***D.T. Nguyen***
15.29-15.44	Discussion

15.44-16.23	**3rd Session: Part II (Pages 49-52)** **Tissue Engineering of Tendon and Ligament** Session Chairs: ***A. Georgoulis, B. Innocenti***
15.44-15.50	The Development of a Novel Magnesium-Based Interference Screw for ACL Reconstruction: A Time-Zero Study in a Goat Model ***K.E. Kim***
15.50-15.56	Genetically Engineered Kusabira-Orange Transgenic Pigs as in Vivo Model to Investigate Biological Remodeling After Anterior Cruciate Ligament Reconstruction ***H. Takeuchi***
15.56-16.02	The Blood - Tendon Barrier - A Novel Clue for Tendon Pathologies? ***H. Tempfer***
16.02-16.08	Establishment of Novel Tenogenic Differentiation Protocols ***O. Leupin***
16.08-16.23	Discussion
16.23-16.53	Coffee Break
16.53-17.32	**3rd Session: Part III (Pages 53-57)** **Tissue Engineering of Tendon and Ligament** Session Chairs: ***M. Handl, G. Riley***
16.53-16.59	Thyroid Hormones Receptors are Present and Effective on Healthy and Ruptured Rotator Cuff Tendons ***F. Oliva***
16.59-17.05	The Effect of Programmable Mechanical Stimulation on Tendon Homeostasis and Tissue Repair in a Bioreactor System ***T. Wang***
17.05-17.11	The Role of Interleukin-6 In The Response Of Human Hamstrings Tendon to Unloading, Loading And Overloading ***K. Legerlotz***
17.11-17.17	Contamination Rate During Allograft Procurement ***C. Terzaghi***
17.17-17.32	Discussion
17.32-17.47	Keynote Lecture: ACL Augmentation ***M. Ochi*** Session Chair: ***G. Cerulli*** Discussion
17.47-18.02	Closing Remarks ***G. Cerulli, S.L-Y. Woo***
20.00	Gala Dinner & Award Ceremony at Arezzo Fiere e Congressi

Posters

1. Considerations About PRP Injection Techniques in Tendinopathies ***M. Abate***
2. Bilaretal Re-rupture of Quadriceps Tendon Treated with a Syntethic Scaffold and PRP ***R. Magri***
3. Subacromial Infiltration with PRP: Clinical and NMR Evaluation at 0 and 3 Months after Treatment ***A. Picinotti***
4. Platelet-Rich Plasma-Related Complications in Orthopaedics ***A. Ventura***
5. Mechanical and Structural Properties of the Medial Patellofemoral Ligament ***M.M. Tei***
6. Identifying Key Proteoglycans in Different Regions of Canine Cranial Cruciate Ligament ***S. Allaith***
7. MRI Depiction of Triple-Bundle Structure in the Native Human acl ***H. Otsubo***
8. Surgical Treatment of Combined Postero Lateral Corner and Acl Injury ***U. Screpis***
9. Biomechanical Comparison Between Anatomical Rectangular Tunnel and Isometric Round Tunnel Acl Reconstructions With A Bone-Patella Tendon Bone Graft ***T. Suzuki***
10. Achilles Tendon Treated with LARS ***L. Corso***
11. Effect of the Matrix on Mechanical Regulation in Human Tenocytes ***G. Riley***
12. The Foot Biomechanics in the Aetiology of Achilles Tendinopathy ***M.G. Minicelli***
13. Fixation of Acute Distal Biceps Tendon Ruptures Using Mitek Anchors; a Retrospective Study ***M. Al-Taher***
14. Influence of Intramuscular Injection of Platelet-Rich Plasma in Serum Concentrations of IGF-I and CRP Concentrations in Dogs ***M. Rubio***
15. Scaffold and Growth Factors in the Treatment of the Acute Lesion of Anterior Talofibular Ligament ***A. Siclari***

Savio Woo Young Researcher Award Winner

FATIGUE LOADING CAUSES A REDUCTION IN THE ABILITY OF TENDON FASCICLES TO RECOIL

C T Thorpe[1,2]; S Chaudhry[1]; C P Udeze[1]; H L Birch[3]; P D Clegg[2] and H R C Screen[1]
[1]Queen Mary, University of London, London, UK, [2]University of Liverpool, Liverpool, UK, [3]UCL, London, UK.

INTRODUCTION

The function of most tendons is to position the limb correctly for locomotion. Specific tendons, including the human Achilles and equine superficial digital flexor tendon (SDFT) also act as energy stores; to fulfil this role, these tendons are subjected to large, repetitive strains. The mechanisms that allow energy storing tendons to extend and recoil rapidly and efficiently in response to these large strains are poorly understood. However, it is well established that energy storing tendons are highly prone to injury, which is thought to occur due to an accumulation of microdamage within the tendon matrix[1]. In our previous work investigating structural specialisations within energy storing tendons, we have observed rotation within fascicles from the equine SDFT in response to applied strain, indicating the presence of helical sub-structures within this tendon[2,3]. Further, we have demonstrated that SDFT fascicles are able to recoil efficiently, which may be due to this helix structure[2]. Finally we have shown that fatigue loading results in decreased rotation within SDFT fascicles, suggesting alterations to this helix[4]. The aim of the current study was to compare the effect of preconditioning with that of fatigue loading on the microstructural strain response of SDFT fascicles and additionally investigate their ability to recoil. **We hypothesise that fatigue loading will decrease the ability of SDFT fascicles to recover following the application of strain.**

METHODS

Sample Preparation

Fascicles (n=6/tendon) were dissected from forelimb SDFTs (n=11) from young horses (age range: 3-6 years) and either imaged using scanning electron microscopy (SEM) (n=18) or subjected to mechanical loading followed by microstructural strain analysis using confocal microscopy (n=48).

Scanning Electron Microscopy (SEM)

Fascicles were fixed in 4% glutaraldehyde in phosphate buffer for 2hrs, then washed overnight in phosphate buffer. A graded dehydration was carried out first in ethanol and then in hexamethyldisilizane. Samples were dried overnight and then mounted on SEM stubs and sputter coated with a gold layer (10nm), before scanning at 10Kv.

Mechanical Loading

Fascicles were stained with 5-dicholorotriazynl fluorescein and then divided into 3 groups: control, preconditioned (PC) or fatigue loaded (FL). Samples in the control group remained unloaded, whereas PC and FL samples were subjected to cyclic testing as follows. PC: 30 cycles to 50% predicted failure stress at a frequency of 1Hz. FL: 1800 cycles to 50% predicted failure stress at a frequency of 1Hz. Hysteresis loss was assessed in the FL group at cycle 30 and cycle 1800.

Confocal Imaging

Each fascicle was then secured in a straining rig, viewed on a confocal microscope and a grid was photobleached onto the fascicle (Fig. 1a). Fascicle recoil was assessed by imaging grids through a series of loading and unloading strains (0% to 4% to 0% to 8% to 0%). Grid deformation was quantified by measuring local longitudinal strains ($x+\Delta x$), transverse strains ($y + \Delta y$), vertical gridline deviation (d_1+d_2) and horizontal gridline rotation (${}^{\theta}x$) (Fig.1b&c). In addition, the percent recovery of each grid parameter was calculated after both 4% and 8% applied strain. Significant differences between groups were determined using Kruskal-Wallis tests followed by Dunn's multiple comparison post-hoc analysis. Statistical significance was taken as $p<0.05$. Data are displayed as mean±SEM.

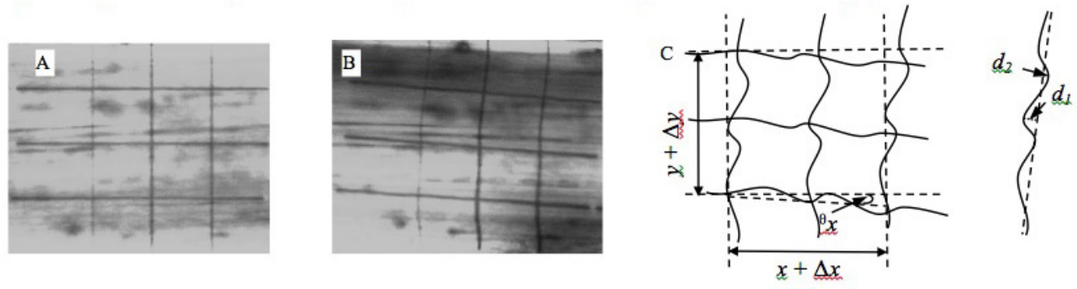


Fig 1. Images of grid at 0% (a) and at 8% (b) strain. Schematic showing calculations of grid deformations (c).

RESULTS

Fascicle Ultrastructure

SEM analysis demonstrated the presence of helix-like structures in fascicles from the SDFT, an example of which is shown in

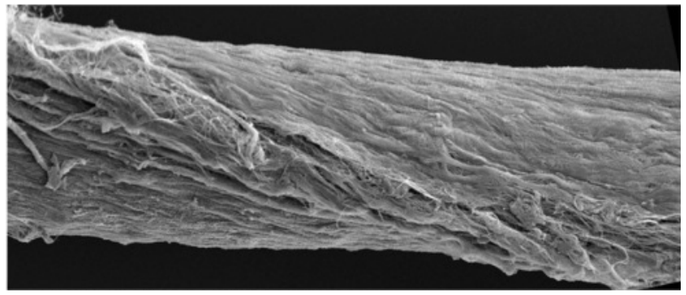


Figure 2.
Fig 2. SEM image showing presence of helix-like structure in an SDFT fascicle.

Microstructural Strain Response

In agreement with previous results[2,3], local longitudinal strains ($x+\Delta x$), representing fibre extension, were smaller than overall applied strain, and did not differ between test groups (Fig 3a). Large compressive strains ($y + \Delta y$) were observed perpendicular to the loading axis in unloaded samples; these strains were significantly reduced in both PC and FL groups at 8% applied strain ($p<0.01$, Fig 3b). A small amount of vertical gridline deviation (d_1+d_2), representing fibre sliding, was observed in all samples. There was a trend towards increased fibre sliding in FL samples, but this was not significant (Fig 3c). Horizontal gridline rotation (${}^{\theta}x$) was observed in both control and PC groups, but was decreased in FL samples at 8% applied strain ($p<0.05$, Fig 3d).

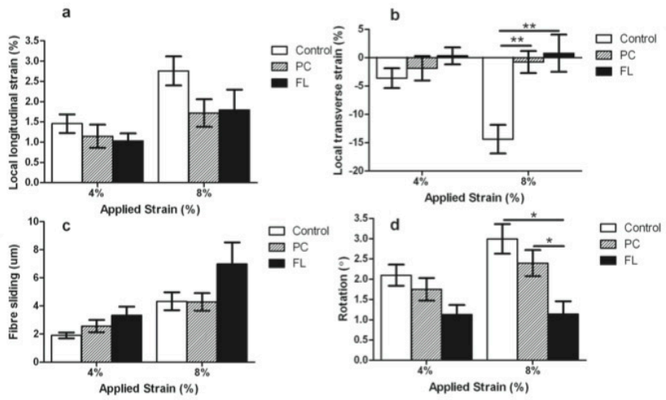


Fig. 3. Local longitudinal strains (a), transverse strains (b), fibre sliding (c) and grid rotation (d) at 4% and 8% applied strain in control, preconditioned (PC) and fatigue loaded (FL) groups. Statistical significance: * $p<0.05$, ** $p<0.01$.

Recovery of fibre extension was significantly lower in FL samples that in either control or PC samples (Fig. 4a, $p<0.05$). There was no difference in the percent recovery of transverse strain between test groups (Fig. 4b). Recovery of fibre sliding was significantly reduced in PC and FL samples compared to controls (Fig. 4c, $p<0.05$), whilst recovery of rotation was only significantly reduced after fatigue loading (Fig. 4d, $p<0.05$).

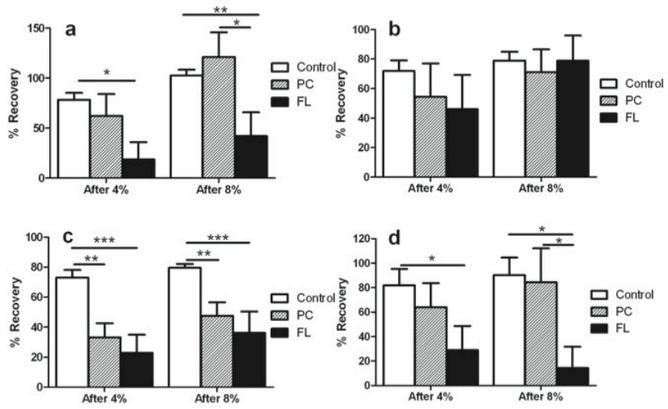
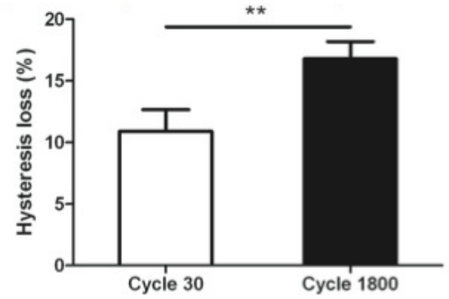


Fig.4. Percentage recovery of local longitudinal strains (a), transverse strains (b), fibre sliding (c) and grid rotation (d) after 4% and 8% applied strain in control, preconditioned (PC) and fatigue loaded (FL) groups. Statistical significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Hysteresis loss was significantly greater at cycle 1800 than at cycle 30, increasing from an average of 10.9±1.7% to 16.8±1.4% (Fig 5, $p<0.01$).

Fig 5. Percentage hysteresis loss in SDFT fascicles after 30 cycles and 1800 cycles of cyclic loading. Statistical significance: ** $p<0.01$.

DISCUSSION

Our previous data[2-4] indicate the presence of helices at the fascicle level within the SDFT, which we hypothesise act as springs, allowing the tendon to extend and recoil efficiently and rapidly. In the current study, we have provided visual evidence of this helix using SEM. In addition, using a combination of cyclic fatigue testing and confocal microscopy we have investigated SDFT fascicle micromechanics before and after loading, and assessed how samples recover from the loading conditions. Overall, fascicles appear to exhibit a gradual change from fibre extension to fibre sliding with FL, which is accompanied by a reduced recovery of both parameters. In addition, there is a large reduction in the compressive transverse strains, which occurs almost immediately once the sample is loaded, suggesting that water is moved out of the samples after a very small number of loading cycles.

Interestingly, we also observed a large decrease in rotation in response to applied strain in FL samples, indicating alterations to the helix structure. This is accompanied by a decreased ability to recover and increased hysteresis loss in FL fascicles. Combined, these results suggest that FL causes alterations to the helix substructure identified in fascicles from the energy storing SDFT, which appears to result in a decreased ability of these 'springs' to recoil efficiently. This may result in increased susceptibility to damage and subsequent injury during further bouts of loading. It is therefore important to fully characterise this helix structure within energy storing tendons, and determine how alterations in helix parameters affect tendon fatigue resistance to further understand the initiation and progression of tendon injury.

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ISL&T XIII Abstracts

ACL GRAFT MATURITY WITH & WITHOUT PRGF

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ACL ruptures have a high incidence in active soccer players. Repair is usually surgical and requires reconstructing the ligament with a graft which can be autologous B-PT-B which confers excellent stability, although it can be accompanied by anterior knee pain related to donor site (according to the literatura 16% of those undergoing this surgery).Therapy with plasma rich in growth factors (PRGF) has shown an accelerated rate and improvement of tendinous tissue, such as donor site of the patellar graft.

MATERIAL&METHOD

A prospective, randomized, double blind clinical trial was designed for the present study, in which two groups of patients with anterior cruciate ligament injuries are compared, operated with ligamentoplasty using patellar tendon. The first group of patients had an application of PRGF at the donor site after removing the graft, whereas the remaining group had the donor site without the application of any product in order to have a spontaneous wound healing.

Posteriorly a complementary study was carried out to assess the ACL Graft maturity grade at the same time periods as the previously mentioned study.

Results were obtained by evaluating:

1. Clinical progress of patients
2. Vascularization during regeneration of donor site with Doppler ultrasound at the patellar tendon.
3. X-Ray to assess the patellar anatomy after the application of PRGF.
4. Clinical and MRI examination assessing the appearance of the grafted tissue as new ACL.

The evaluation is performed in terms of local regeneration by means of seriated ultrasound evaluations over two years, developing a maturation classification, and thus classifying each ultrasound according to it. The ultrasound exam allows at the same time to evaluate the vascularization grade of the tendon using Doppler ultrasound. Pain at the donor site was collected over the two years follow-up, and in order to assess global functioning of the knee, general health and knee-specific tests were performed, as well as sports reincorporation, at 6, 12 and 24 months. MRI was used to determine graft maturation.

RESULTS:

1)PRGF group shows earlier donor site regeneration with statistically significant differences at 4 month follow-up. No statistically significant differences were found within tendinous maturation and regeneration both during first months and beyond 6th month.

2)PRGF group presents less pain at the donor site, with statistically significant differences during the first 4 months of follow-up, with a strong trend towards 6th month although without statistical significance.

3)PRGF group did not show differences in terms of functional return; in general quality of life index, as well as in specific, sports return or objective parameters such as KT-1000 or maturation grades of the graft measured with MRI.

4)More patients in the PRGF group attained higher stages of remodelling at month 4 (p=0.003), month 6 (p=0.0001), and month 12 (p=0.354)

5)PRGF and control groups did not differ significantly in terms of age, comorbidities, sex, height, weight, and postoperative swelling and complications.

CONCLUSION

PRGF group shows accelerated donor site maturation according to the study with ultrasound, and moreover, diminishes pain during the first months of evolution with statistically significant differences as well as statistically significant differences in ACL graft maturity. Several studies have shown how the use of PRGF can improve clinical outcomes, control tissue regeneration and decrease pain.

AGE-SPECIFIC CLEAVAGE PATTERNS IN TENDON EXTRACELLULAR MATRIX

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INTRODUCTION

Ageing represents a huge challenge for society as whilst lifespan increases the quality of life is often poor, in part due to a progressive loss of mobility. Alterations in tendon properties contribute to muscle weakness and thus reduced mobility in old age. In addition the risk of tendon injury increases with advancing age. Similar changes occur in the equine flexor tendons and the risk of injury increases significantly in older aged horses. The cellular and molecular mechanisms behind these changes in human and equine tendons are not well understood but are thought to result in altered matrix turnover. The aim of this study was to investigate age-specific cleavage patterns of extracellular matrix (ECM) proteins in young and old equine superficial digital flexor tendon.

METHODS

Fascicles were dissected from grossly normal equine SDF tendons from three young (< 4years) and three old (>20 years) donors. ECM proteins were extracted using 4M guanidinium chloride for 48 h at 4ºC in duplicate to give technical replicates. Following tryptic digestion the proteolysed products were identified using liquid chromatography mass spectrometry (LC-MS/MS) analysis performed using NanoAcquity™ Ultraperformance LC (Waters, Manchester, UK) on line to an LTQ-Orbitrap Velos (Thermo-Fisher Scientific, Hemel Hempstead). Data were analysed by searching against the Unihorse database using an in-house Mascot server (Matrix Science, London, UK). Complete sequences of cleavage patterns were determined for aggrecan, biglycan, decorin, fibromodulin, cartilage oligomeric matrix protein (COMP), lumican and collagens.

RESULTS

The range and abundance of proteins identified using mass spectrometry differed between young and old tendon extracts. The number of proteins identified in young tendon (107±39) was significantly greater than in old tendon (56±12.8) (p=0.05). Neopeptides were included in the analysis if they were identified in old tendon alone in ≥2 donors. This analysis resulted in the identification of a catalogue of age-related and potentially novel cleavage sites for aggrecan; 1, COMP; 4, decorin; 2, lumican; 2, collagen alpha-3(VI); 6, collagen alpha-1(XII); 13, collagen alpha-1(II); 2, based on none enzyme Mascot searches.

DISCUSSION

The results suggest a changing proteomic profile in ageing tendon as demonstrated by the difference in the abundance and types of proteins identified. The discovery of novel neopeptides, which relate to specific cleavage sites will contribute towards a better understanding of the basic biochemical processes underlying tendon ageing in human and equine species. Furthermore, the novel protein fragments identified represent potential biomarkers of tendon ageing in individuals. This study highlights the power of a proteomics technique to develop a list of potential ECM degradation sites and represents an approach that could be applied to other tissues and disease processes.

ACKNOWLEDGEMENTS

This study was funded by the Horserace Betting Levy Board. Mandy Peffers is funded by a Wellcome Veterinary Integrated Research Training Fellowship.

THE ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR IS EXPRESSED ON HUMAN TENOCYTES AND INHIBIT PROLIFERATION OF TENOCYTES IN CULTURE

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INTRODUCTION

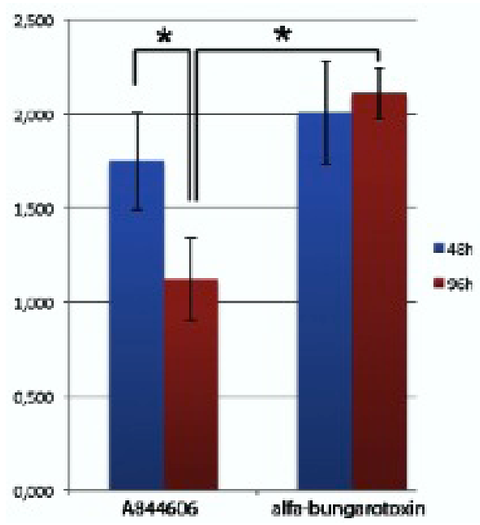
Tendons and ligaments are subjected to different kinds of disease and injury, and understanding the underlying mechanisms of pathology and healing are important both in the clinical setting, but also from a basic science perspective. Some of the afflictions known to affect tendons are characterized by hypercellularity among others, but the mechanism of this is still far from clearly described. It has been shown that neuropeptides such as Substance P can cause tendon cells to proliferate, and other neuronally derived have similar effects. These signaling systems are likely to be of great importance in the normal healing of tendon tissues, but when over-expressed they may lead to pathologies such as tendinosis. It is important to find the conditions under which tendon cells are proliferative and under which they are halted, in order to fully understand the tendon biology. One such counteracting pathway could be through the α7 nicotinic acetylcholine receptor (α7nAChR). This receptor has been shown to inhibit inflammatory responses in various tissues when activated and its existence and effects in tenocytes could prove highly interesting considering its potential as a therapeutic target.

METHODS

Tendon biopsies from healthy controls and patients suffering of Achilles tendinosis were collected for immunohistochemical staining of the α7nAChR. From theses biopsies primary cultures were grown. Cells from passage 3 were used to investigate the effects of stimulation and blocking of α7nAChR using A844606 (an α7nAChR agonist) and alfa-bungarotoxin (α7nAChR antagonist) respectively. The cellular activity after incubation was measured using MTS at 48 and 72 hours.

RESULTS

α7nAChR was expressed in tenocytes both in the tendon tissue biopsies, and in cultured cells. The tenocytes showing a more rounded shape had a higher level of granular reactions for α7nAChR. Some tendon cells did not show any reactions at all. No difference in expression patterns was seen in healthy controls as compared to tendinosis patients, when examining the tissues by immuno-fluorescence microscopy. When incubated with the agonist and antagonist, the tenocytes showed a lower level of cellular metabolism as measured by MTS in the samples incubated with the agonist as compared with the antagonist.



FFig. 1: MTS of tendon cell culture
The α7nAChR-agonist A844606 inhibit tenocyte proliferation *in vitro*

DISCUSSION

Many factors have been shown to stimulate the proliferation of tendon cells *in vivo* and *in vitro* but here we have found preliminary evidence that tenocytes express the α7 nicotinic acetylcholine receptor and that stimulation of this receptor leads to a decrease in cellular metabolism in tendon cell cultures. This is interpreted as an indirect measurement of proliferating cells. Smoking is known to decrease the healing rate of tendons, and these findings indicate that this may in part be due to a direct effect of the nicotine on the α7nAChR in tenocytes.

ANALYSIS OF THE RESIDUAL STRENGTH OF A PARTIALLY CUT TENDON

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INTRODUCTION

Partial lacerations of tendon diminish strength; however details of structural compromise and potential for further damage remain poorly understood. Clinical practice often considers surgical repair only after 50% of tendon cross-sectional area is lacerated (the "50% rule" [1]), but few studies provide mechanical insight into this empirical rule. Experimental observations have shown that load that can be borne by lacerated tendon decreases in a non-proportional way with increasing cut depth [2]. This indicates that mechanical mechanisms are more complex than an equal redistribution of load in the residual fibers.

METHODS

This study explores an analytical description of the decay of bearable load by comparing three models which are quite popular in composite materials fracture mechanics: one model based on a decay proportional to cut fibers, global load sharing (GLS), and two models based on a shear lag approach providing non-linear curves, Hedgepeth's and Wagner's models. This analytical study considered 100 aligned fibers in a 2D structure. The general predicted behaviors were interpreted in the context of experimental results from a study by Kondratko, et al. (2013) [2].

DISCUSSION OF RESULTS

The GLS model proved generally unsuitable to describe the experimentally observed behavior. Predictions given by the two shear lag approaches better described the non-proportional decay of bearable loads, with Wagner's closer to experimental results (Fig. 1), confirmed by the analysis of the slope of the obtained load curves (Fig. 2), with a near-plateau region beginning at about 20% cut depth. The shear-lag based model fails when deep cuts are considered; in this case a better description is given by GLS which, however, has only been considered as the best approximation available and not as a definitive model for said situation. This result further confirms the existence of more complex phenomena in the load redistribution when more than the 50% of its cross-sectional area is cut, providing a theoretical support to the cited 50% rule.

The analytical curve did not exactly reproduce the experimental one, but Wagner's shear lag model was sensitive to absolute number of fibers as a result of fiber interactions; this effect became smaller as fiber number was increased. The theoretical curve approached the experimental one as fiber number was increased. These two observations, along with the computed results for curve shape and slope, are promising. They suggest that this model should be expanded to analyze a full size tendon, allowing the near-plateau region (Fig. 1) to be investigated more precisely. A generalization of the model prior to its application to a full size tendon should also consider some of the more complex phenomena such as crimping, fiber recruitment, fiber interconnections, and higher level structures.

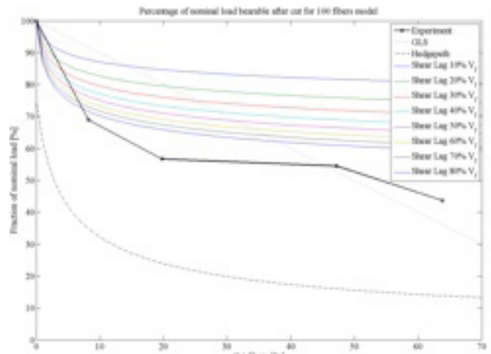

Figure 1. Theoretical models and experimental results

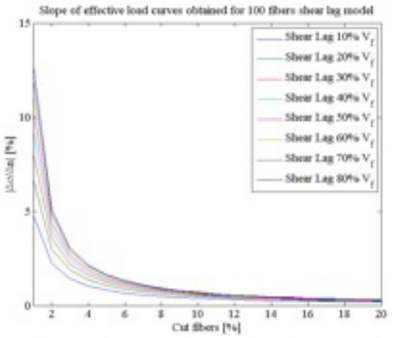

Figure 2. Slopes of curves obtained with Wagner's model for 100 aligned fibers.

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2. Kondratko, J., et al., 2013, J. Biomech. Eng., **135**(1), pp. 1–8

BIOCHEMICAL AND MORPHOLOGICAL COMPARISON OF THE EXTRACELLULAR MATRIX COMPOSITION OF TENDONS AND LIGAMENTS

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INTRODUCTION

Tendons and ligaments (TLs) play key roles in the musculoskeletal system attaching muscle to bone and bone to bone, respectively. However, they are commonly damaged due to age-related wear and tear or torn in traumatic/sport related incidents resulting in pain and immobility.

TLs contain cells and extracellular matrix (ECM) comprising of collagen, elastin, glycoproteins and proteoglycans. Although TLs are composed of similar components, their precise composition and arrangement of matrix macromolecules differ to provide specific mechanical properties and functions. Increased knowledge of the basic structure of TLs is important in understanding their pathology and potential for regeneration/replacement. In this study, therefore, our aim was to compare the ECM architecture and composition of ligaments and tendons with regard to location (extra- and intra-articular) and function (extensor and flexor). We hypothesised that tendons and ligaments are significantly different in terms of ECM architecture and composition.

METHODS

The anterior cruciate ligament (ACL), medial collateral ligament (MCL), long digital extensor tendon (LDET) and superficial flexor tendon (SDFT) were harvested from five paired canine hindlimbs. TLs tissues were divided for biochemical and histological analysis. The ECM composition was assayed for total collagen, DNA, sulphated glycosaminosglycans (sGAG) and elastin content. Architectural differences were compared using H&E, alcian blue-PAS and Millers staining and scored blind sighted to each sample by two different observers.

RESULTS

Collagen content ranged between 40.6% and 85.43% (dry weight tissue), with SDFT containing the lowest collagen content ($p=0.001$). sGAG was an average of 13μg/mg (dry weight tissue) in ligaments and 10μg/mg in tendons. sGAG in ACL was significantly higher than LDET ($p=0.00$), MCL ($p=0.00$) and SDFT ($p=0.001$). Elastin was also significantly higer in ACL 5% (dry weight tissue) than MCL (1.9%, $p=0.00$), LDET (2.4%, $p=0.00$) and SDFT (2.8%, $p=0.00$). H&E scoring analysis showed that tendons had more of a spindle shaped cell nuclei and were more vascularised with more compact dense collagen bundles. The ligaments contained a reduced density of collagen bundles that was in part loose and in part compact with more rounded, heterogenous cell nuclei. The ACL in particular was less vascularised with a more heterogenous arrangement of cells compared to the other tissues. Alcian blue-Pas and Millers staining scoring supported the biochemical results, as more staining was observed with ACL than MCL, LDET and SDFT. The kappa statistical scoring for all the histological scoring was above 0.6, indicative of good of agreement between observers.

DISCUSSION

The result of this study shows that TLs are composed of different proportions of matrix macromolecules. Collagen provides the major resistance to mechanical forces, whereby its content and organisation would be reflected in the mechanical properties of the structure. Higher sGAGs in ACL might allow for more slippage between fibrils and fascicles, allowing greater deformation. The higher elastin content in ACL might be due to its required stretch and recoil mechanism.

TENDONS REPAIR & REGENERATION

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Tissue repair in musculoskeletal injuries is often a slow and sometimes incomplete process. Tendon pathologies, traumatic or degenerative, are a troublesome condition which affects a high number of athletes in every kind of sport. Pain and limited function often become a chronic problem, which can hinder performance and even contribute to athletes deciding to quit their career. The optimal treatment for these injuries should aim to restore patients to their pre-injury status in a safe, cost-effective way and as quickly as possible. Basic science studies have shown that healing tendon is responsive to local the application of growth factors, and describe the role of many of the growth factors contained in the platelet alpha granules in tendon regeneration. Therefore, the use of growth factors is thought to be useful in clinical practice, in order to promote rapid healing with a high quality tissue and an early and safe return to unrestricted activity. Platelet Rich Plasma (PRP) is a simple, low cost and minimally-invasive way to obtain a natural concentration of autologous growth factors and is currently being widely experimented in different fields of medicine for its ability to aid the regeneration of tissue with a low healing potential. In particular, for tendon regeneration releasate from PRP has been seen to stimulate gene expression of the matrix molecules and tendon cell proliferation and promote the synthesis of angiogenic and other growth factors, and also activate circulation-derived cells, that also play an important role in the tissue healing process. Other than in vitro studies, also animal studies have shown the usefulness of platelet concentrates. However, results of clinical trials are still controversial. In fact, many aspects still need to be studied, to define the optimal formulation, the proper dosage and timing of application, and to determine which patients, type of tendinopathy and phase of pathology may better respond to this biological minimally-invasive approach. The evidence base for the indication for this use of PRP is still in its infancy, but several clinical and surgical trials are ongoing worldwide to support the preliminary data.

COLLAGEN (I) HOMOTRIMER IN AGE-RELATED FIBROSES AND TISSUE DEGENERATION: EVALUATION AS A STEM CELL BIOMARKER

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INTRODUCTION

Type I collagen is the most abundant structural protein in vertebrates, providing mechanical stability and strength to bone, skin and other connective tissues. The predominant form of type I collagen is a heterotrimer consisting of two α1(I) chains and one α2(I) chain, encoded by the *COL1A1* and *COL1A2* genes respectively. However, cancer cells have been found to synthesise a homotrimeric isoform ($\alpha1_3$) of type I collagen, containing three α1(I) chains, which is resistant to matrix metalloproteinase (MMP) degradation, thereby creating MMP-resistant pathways for metastatic tissue invasion. This homotrimeric isoform of type I collagen has also been detected in embryonic cells, embryonic tendon and bone, and fetal cell populations derived from amniotic fluid, suggesting that stem and/or progenitor cells could be a source of α1(I) homotrimers. In adults, the production of collagen (I) homotrimers has been associated with an array of fibrotic and connective tissue pathologies by genetic linkage, RNA and protein analysis. Tendon and ligament degeneration are poorly understood pathological processes that result in impaired musculoskeletal function. We aim to determine whether these degenerative pathologies share a common stem cell-mediated molecular mechanism involving the aberrant production of type I collagen homotrimers.

METHODS

Ruptured canine cranial cruciate ligaments (CCLs) (n=14) were harvested during reconstructive surgery and normal CCLs (n=9) were removed from canines which had died for unrelated reasons. The relative expression of *COL1A1* and *COL1A2* in normal and diseased ligament samples was determined by reverse transcription quantitative real-time-PCR (RT-qPCR). Canine mammary tumour samples were used as a positive control expected to produce collagen (I) homotrimers.

RESULTS

Initial results show that expression of the *COL1A1* and *COL1A2* genes is significantly increased in ruptured canine CCL samples as compared to normal CCL samples (p=<0.001and p=<0.001 respectively). Interestingly, the *COL1A1:COL1A2* gene expression ratio is significantly increased in ruptured canine CCL samples as compared to normal CCL samples (p=<0.01).

DISCUSSION

The significant increase in the gene expression ratio of *COL1A1:COL1A2* in diseased ligament suggests that collagen (I) homotrimers may be produced during the pathological process of tendon degeneration. To corroborate the findings the α1(I):α2(I) chain ratio and proportion of homotrimers present in normal and ruptured CCL samples is currently being assessed by metabolic labelling. Colonies of putative stem cells have also been isolated from these tissues and collagen (I) homotrimer production in these cells will be examined after confirming their clonogenic, self-renewal and multi-lineage differentiation capacity. Collagen (I) homotrimers have been reported to possess a reduced mechanical strength and thus the production of this collagen (I) isoform in diseased ligament may comprise the structural integrity of the tissue, thereby predisposing to rupture. The data from this study will be important for understanding how to harness stem cells for tissue repair and regeneration, without adverse effects on tissue function.

TNF-α INDUCED APOPTOSIS IN HUMAN TENOCYTES IS REDUCED BY SUBSTANCE P THROUGH A NK-1 RECEPTOR SPECIFIC PATHWAY

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INTRODUCTION

The up-regulation of the neuropeptide substance P (SP) and its preferred receptor, the neurokinin-1 receptor (NK-1 R), in chronically painful tendons, has been hypothesized to be a causative factor in inducing tenocyte hypercellularity, a characteristic of tendinosis, through both proliferative and anti-apoptotic stimuli. We have earlier demonstrated that SP stimulates proliferation of human tenocytes in culture. The aim of this study was to investigate if SP can mediate an anti-apoptotic effect in Tumour Necrosis Factor-alpha (TNF-α) induced apoptosis of human tenocytes *in vitro*.

METHODS

Immunocytochemistry was used to confirm presence of Tumour Necrosis Factor Receptor-1 and -2 (TNF-R1, TNF-R2). Cell death was measured with Lactate dehydrogenase (LDH) assay and cell viability with a total protein stain, crystal violet staining. Cell signalling was analysed using RT-qPCR (for mRNA) and Western blot (for protein).

RESULTS

TNF-R1 and TNF-R2 was expressed in a majority of tenocytes in culture. Exposure of the cells to TNF-α significantly decreased cell viability. TNF-α furthermore significantly increased the amount of caspase-10 and caspase-3 mRNA, as well as both BID and cleaved-PARP protein. Incubation of SP together with TNF-α resulted in a decreased amount of BID and cleaved-PARP, and in a reduced lactate dehydrogenase release, as compared to incubation with TNF-α alone. The SP effect was blocked with a NK-1 R inhibitor. See figure 1.

DISCUSSION

SP, via stimulation of the NK-1 R, was in this study shown to have the ability to reduce TNF-α induced apoptosis of human tenocytes. Considering that SP has previously been shown to stimulate tenocyte proliferation, the study confirms SP as a potent regulator of cell-turnover in tendon tissue, capable of stimulating hypercellularity through different mechanisms. This gives further support for the theory that the up-regulated amount of SP seen in response to load of tendons/tenocytes, could be a causative factor for the hypercellularity of tendinosis.

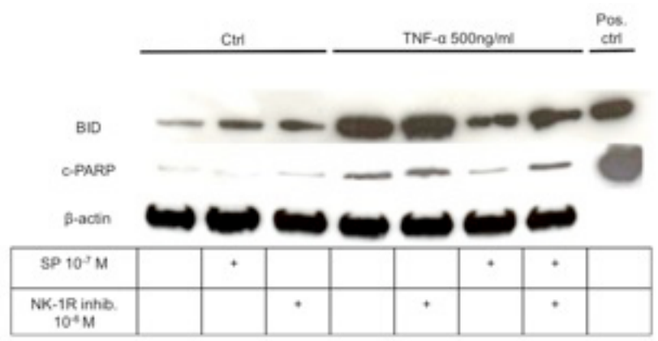

Fig. 1: SP reduces the TNF-α induced apoptosis in human tenocytes. The increase in expression of BID and c-PARP seen in cultured human tenocytes after incubation with TNF-α as compared to control (Ctrl), was clearly reduced when cells were pre-treated with SP. Blocking of the SP receptor with an NK-1 R inhibitor (NK-1 R inhib.) reduced this effect of SP. β-actin was used as a loading control and jurkat apoptosis cell lysate was used as a positive control (Pos. ctrl). 12 hours of incubations.

NEW CORRELATES OF DAMAGE IN RAT TAIL TENDONS

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INTRODUCTION

Tendon mechanical damage under creep loading has been modelled previously by curve-fitting an exponential to the change in stiffness, assuming that the effective load-bearing cross sectional area of the material decreases continuously [Wang 1995]. However, more recent work [Fung 2009] has found that strain measures characterise damage more closely and can be related to microscopy evidence of fibril derangement.

Study of the mechanisms of collagenolysis using an inactivated MMP1 (E200A) mutant has shown that before peptide bonds may be hydrolysed, the triple helix must be mechanically unwound [Chung 2004]. Fibril damage is likely to expose cryptic sites predisposing to collagenolysis.

The aim of this study was to define a strain-based damage model for tendon creep and to investigate the use of an inactivated fluorescent labelled MMP1 (E200A) mutant as a novel sensitive measure of damage in tendon.

METHODS

Tendon fascicles, carefully dissected from mature Sprague-Dawley rat tails, killed as part of unrelated experiments and frozen at -20 °C within 2 hours, were thawed and equilibrated in PBS at room temperature on the day of testing. The diameter of the fascicle was measured at 6 points using an optical micrometer (Keyence Milton Keynes, UK; precision 1×10^{-5} mm) immediately before testing in a custom temperature controlled (± 0.5 °C) PBS bath at 37 °C using a 20 N frame (Bose Electroforce) under a constant creep stress of 10 MPa.

Defining the total strain $\varepsilon_{total}(t) = \varepsilon_{visc}(t) + \varepsilon_{damage}(t)$ and assigning a linear viscoelastic model for ε_{visc}, two models for the creep strain damage, were investigated:

(1) $\varepsilon_{damage}(t) = C_1 + C_2 \exp(t / \tau_1)$ and (2) $\varepsilon_{damage}(t) = C_3 + C_4 t + C_5 \exp(t / \tau_2)$

Viscoelastic and damage models were least squares fitted to the creep curves.

Further creep tests were carried out at 37 °C, stopping the tests at the 0.2, 0.4, 0.6 and 0.8 of creep lifetime. The unclamped section of each tendon fascicle were then incubated with 25 nM MMP-1(E200A) AlexaFluor 568 in TNC buffer at 37 °C overnight. Tendons were examined using a Zeiss 710 confocal scanning laser multiphoton microscope.

RESULTS

Preliminary analysis of creep curves (Fig 1) for 16 tendons provided a mean fitting parameter (squared two norm of the residual) for damage model (1) of 1.4×10^{-4}, which was significantly larger ($p = 0.0164$) than that for damage model (2), 5.9×10^{-5}.

Preliminary multiphoton microscopy (Fig 2) showed kinked fibril regions in the creeped specimens.

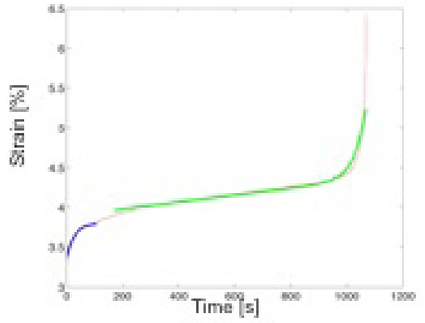

Figure 1: Creep curve (red) fitted: viscoelastic (blue) and damage model (1) (green)

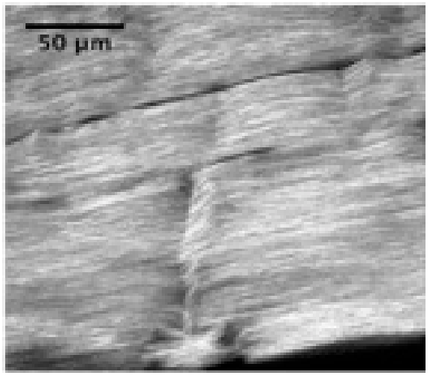

Figure 2: Multiphoton image showing kinked fibrils in creeped tendon

DISCUSSION

These results confirm the utility of a strain-based model for quantifying tendon damage. The use of a fluorescently labelled inactivated enzyme as a damage marker is a novel method which may provide more sensitive detection of lower levels of damage in tendon tissue. Further investigation using this method will determine the rates of damage accumulation in the different sections of the creep curve.

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ELASTOSONOGRAPHY AS NOVEL FOLLOW-UP METHOD IN ACHILLES TENDON SURGERY: PILOT STUDY

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BACKGROUND

After surgical repair, by the biomechanical point of view, it is not possible to assess in vivo if and when the treated tendon reaches the "restitutio ad integrum" stage. Recently, several pilot studies identified the elastosonography, an evolution of the conventional ultrasonography, as a tool to analyze the deformation of biological tissues[1-3]. Aim of this pilot study was to evaluate the elastosonographic features of the Achilles tendon after percutaneous surgical repair.

PATIENTS AND METHODS

The study was set up as a "pilot" prospective study. During 2012, according to exclusion criteria, patients with traumatic rupture of the Achilles tendon were recruited. Percutaneous tenorrhaphy was performed in all the cases using "BIO-Tex Para-Pé®" system. The elastosonographic evaluation was performed using the Philips iU22 machine with a 5-12 MHz probe. The tendon thicknesses were evaluated at the myotendinous junction, tendon body / lesion site, and osteotendinous junction, both on the operated side than on the contralateral unoperated side.

These evaluations were performed at 40 days, 6 months and 1 year after the treatment. Using standard windows of 4x1 mm the "strain index" ratio was calculated, that is the index of deformability of the tendon under the ultrasounds source. In the same time clinical outcome was assessed by the ATRS (Achilles Tendon Rupture Score)[4], which was correlated with elastosonographic findings. As control group, thicknesses and elastosonographic indices of 60 healthy adult tendons were calculated.

RESULTS

According to exclusion criteria, 25 patients (22M, 3F; Mean Age 42.1±9.0) have been recruited. From the elastosonographic point of view treated tendons showed a progressive stiffness during the follow up, especially at the level of myotendinous junction and at the site of lesion. They were significantly stiffer than contralateral untreated tendons and than healthy ones. At 6 months, treated tendons showed a thickness peak , with a tendency to reduce at one year, but without reproduce physiological values. The area of injury is the one that showed the greatest remodeling with the reduction of the thickness. Also the contralateral tendon showed a thickening at the level of the myotendinous and osteotendinous junction, due to a possible functional overload, and in particular at the site of lesion. The strain index of the contralateral untreated tendon was lower than the physiological value, however it was still more rigid or anelastic if compared with a healthy tendons. The surgical outcome, investigated with ATRS assessment, improved significantly between 6 months and one year, while they were negatively correlated with the Strain Index.

CONCLUSIONS

Through the elastosonography we observed how the treated tendon becomes progressively more "stiff" in all its structure during follow-up, while the ATRS score progressively improved. Furthermore, the excellent ATRS demonstrated that percutaneous Achilles tendon repair is an effective surgical technique with several advantages and low rate of complications. In our opinion, the inverse correlation between the increased stifness in the tendon scar and the excellent clinical outcome is due to some compensatory mechanisms not yet explained by sonoelastography and probably not only related to the tendon itself.

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THE INSERTIONAL LIGAMENTS OF THE MENISCI

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INTRODUCTION

The insertional ligaments, or roots, of the menisci are integral to load bearing in the menisci; their strong attachments to the tibia are key to resisting meniscal extrusion. Injury to the insertional ligaments results in rapid degeneration in the knee [1]. How these ligaments transition from a tensile load bearing ligament into the meniscal fibrocartilage is unknown. Understanding this transition will be critical in attempts to tissue engineer menisci capable of resisting the extrusive forces generated in the knee joint. Therefore, the purpose of this study was to complete a structural analysis of the tissue organization as it transitions from the roots into the body of the menisci.

METHODS

Bovine menisci were obtained from a local abattoir and harvested within 48 hours of slaughter. The menisci were dissected with careful attention paid to retaining the insertional ligaments. Menisci were then fixed in 100% methanol at -20˚C for 72 hours. The insertional ligaments were then cut in cross-section, starting from the bony insertion. Serial sections were then cut (~1 mm thick) until the body of the meniscus was reached. Sections were washed in PBS for 1 hour and stained with fast green (0.02% w/v) and safranin o (0.1% w/v) for collagen and proteoglycan (PG) respectively. These whole-mount sections were imaged using a dissection microscope and digital camera (Zeiss Stemi SV8 Microscope with moticam 5.0M Pixel camera).

RESULTS

Serial sections of the insertional ligaments identified common structural features between the ligaments and meniscus. Tie-fibers were observed in the sections of the ligaments furthest from the meniscal body (1-2 cm) (Figure 1). Diffuse vascularization was observed in the sections. Blood vessels were situated in the peri-fascicular space and were oriented along the length of the ligaments. Blood vessels were observed to be surrounded by tie-fibers and a PG rich region within the ligaments. Sections farthest from the meniscus were ligament-like morphologically, containing large collagen fascicles. Near the meniscus, the ligament increased in size and its shape began to resemble the meniscal body. Closer to the meniscus, there was an increase in tie-fiber size and density. Small tie-fibers extended into the ligament from the epiligament in the outermost sections of the meniscus; while large tie-fiber bundles were apparent at the meniscus transition. The staining pattern of the tissue indicate that the roots may continue into the outer portion of the meniscus where it then blends with the more fibrocartilage-like inner portions of the tissue (Figure 2).

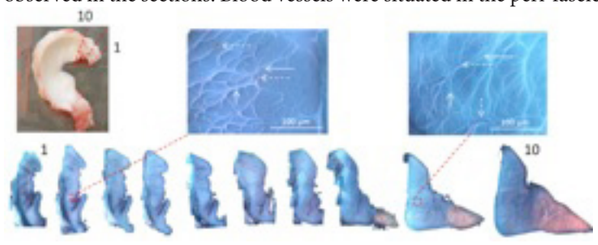

Figure 1. Top left: photo of a medial meniscus (dashed red lines: orientation of sections). Bottom: serial sections of the anterior insertion showing the transition in shape and staining pattern from the ligament to the meniscus. Breakout images: identify blood vessels (dashed arrows) and tie-fibers (solid arrows) in sections.

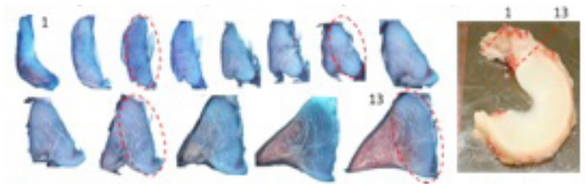

Figure 2. Right: photo of a lateral meniscus. Left: serial sections of the anterior insertion. Note the increase in PG staining as sections approach the meniscus. Portions of the ligament appear to be preserved as it approaches the meniscus (dashed ellipses).

DISCUSSION

The insertional ligaments of the menisci contain structural similarities with the main body of the menisci. Tie-fibers, originating from the epiligament were observed in the ligament. These fibers were observed to surround blood vessels and associated PG rich region. It appears that the insertional ligament may persist into the meniscal body and blend with the fibrocartilage of the meniscus. This finding may indicate that the tensile load bearing mechanism is predominantly born by the outer edge of the meniscus through insertional ligaments. This is the first study of the structural transition between in the insertional ligaments and the menisci. These new structural details are important to understanding meniscal load bearing mechanisms for future development of tissue engineered menisci.

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TENOMODULIN TRANSLOCATES TO THE NUCLEUS AND ASSOCIATES WITH CHROMATIN AT MITOSIS IN TENOCYTES AND HELA CELLS

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INTRODUCTION

Tenomodulin (Tnmd) is a reputed tenocyte biomarker that exists as three isoforms (3). Results from a knockout mouse model of isoforms I and II, as well as RNAi experiments that knocked down all three isoforms, reduced cell proliferation (1,2,3). Tenocytes subjected to strain for 4 days in vitro localized Tnmd immunochemically to the nucleus. Given that Tnmd translocates to the nucleus in response to strain and persists there, has a nuclear involvement when cells are stimulated with cytokines and when Tnmd is knocked down with RNAi, decreases cell proliferation, we hypothesized that Tnmd is involved in regulation of the cell cycle.

METHODS

Tenocytes were isolated from the flexor carpi radialis or biceps tendons of patients at surgery or from equine superficial flexor digitorum profundus tendons at necropsy by a combination of trypsin followed by collagenase digestion and the internal fibroblast population isolated and cultured in growth medium. HeLa cells were obtained from the UNC Cell Culture repository. GFP-tenomodulin isoforms I, II, and III constructs in plasmid pEGFP-C1 vector were constructed and transfected into tenocytes or HeLa cells with lipofectamine. Cells at passage 0-3 were sub-cultured onto collagen-coated glass coverslips at 10k cells/cm^2, grown for 48 h, then washed and treated for 16h with 10% serum-containing medium with 100-300nM nocodazole to block cells at prometaphase. Cells were washed and released from the block with fresh serum-containing medium and collected at t_0, 0.5, 1, 2, 3, 4 h post-block. Cells were fixed, washed, then treated with antibodies specific to the C-terminus of Tnmd, lamin B for the nuclear envelope, -tubulin for spindle fibers, and DAPI for DNA. Appropriate secondary antibodies were used to bind to primary antibodies and images taken.

RESULTS

Prometaphase cells from tenocytes or HeLa cells showed robust Tnmd staining throughout the cytoplasm (Figure 1). Cells released at 16h (t_0) from the nocodazole block, paused cells in prometaphase, showed Tnmd localization in the peri-chromatin boundary. One h post-block, cells were in metaphase and showed robust Tnmd staining in a punctate border at the periphery of the chromosomes and in the cytoplasm. At two h post-block, at anaphase, Tnmd localization was peri-chromosomal at the segregating chromosomes and in dual puncta at poles in the cell where centrioles locate. At 2.5h post-block, at telophase, Tnmd signal was less robust, still in a peri-chromosomal localization with polar puncta. GFP isoforms one and two were cytoplasmic, showing globules of GFP-Tnmd, with a focus around the nuclear envelope (Figure 2). GFP-isoform I had a clear overlap with anti-Tnmd antibody signal as did isoform II. GFP-isoform I was cytoplasmic at prometaphase and in a distinct halo around the chromatin, then tracked with the segregating chromosomes throughout mitosis (Figure 3). HeLa cells expressed tenomodulin isoforms and post-nocodazole block showed the same association as did tenocytes. **Discussion:** Tenomodulin is a reputed tenocyte biomarker, however, its distribution in tissues is broad, from brain to connective tissues. We report its expression in immortalized HeLa cells derived from a cervical carcinoma, therefore precluding Tnmd exclusivity to connective tissue. It is a member of the BRICHOS domain proteins, some of which are involved in cancer. Moreover, Tnmd, isoform I associates with chromatin during mitosis. This association is dynamic as Tnmd accumulates at the nuclear envelope, binds in a peri-chromatin localization, then peri-chromosomal at anaphase and appears to release from chromosomes at late anaphase or telophase. **Significance:** This observation may place Tnmd isoform I at the checkpoint complex in anaphase as a novel but potentially dispensable regulatory protein in the cell cycle, since RNAi results demonstrated reduced, but ongoing, cell proliferation. **Acknowledgement:** AJB is president of Flexcell International Corp and receives compensation as such. This work was supported by Flexcell Intl. Corp and the Hunt Foundation of Pittsburgh, PA.

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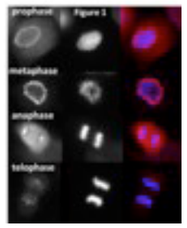

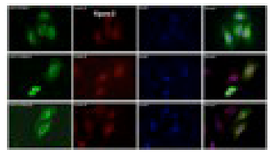

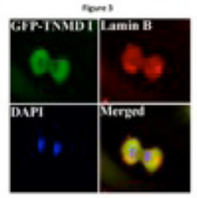

ACL RECONSTRUCTION WITH A FULLY RESORBABLE Vs. PARTIALLY RESORBABLE TIBIAL FIXATION SYSTEM: COMPARATIVE RESULTS AT A MID-TERM FOLLOW-UP

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INTRODUCTION

Over the last decades, several fixation systems have been proposed for ACL recostructions with ST and G tendons. Particularly in the tibial side, new devices (metallic or not metallic) have been recently released: among the not metallic devices, the Biointrafix® (Mitek, DePuy, Johnson & Johnson), showed a wide diffusion. However, very few papers report its use and advantages, mainly from a biomechanical and biological point of view. Purpose of this experience is to show the clinical and instrumental results of the reconstruction of injured ACLs in patients treated by Endobutton® + Biointrafix comparing the outcomes to a homogeneous population of 65 subjects treated by our previous fixation combination (Endobutton® + two Washers + Bioresorbable interference screw).

METHODS

In the period between July 2009 and October 2011, 75 patients were enrolled for a ACL reconstruction with quadruple ST and G tendons fixed proximally with the Endobutton device and distally with the Biointrafix system. All patients gave their consent to the procedure, study and follow-up. And the Institutional Borad of our Institution approved the study, based on the Helsinki declaration. All subjects underwent a preoperative analysis by Knee Injury and Osteoartrhitis Outcome Score (KOOS), International Knee Documentation Committee (IKDC) score, and evaluation with con KT-2000. Moreover, a x-rays study was conduted to evaluate any case of radiologic alteration in the tibial side, given the recent reports regarding osteolysis around resorbable devices and tunnel enlargements. Any complications was recorded. Follow-up study was conducted by the same scores and evaluations, at 6, 12, and after yearly. All results were compared to a homogeneous group of 65 patients treated between January 2007 and June 2009 with Endobutton on the femoral side and fixation by two metallic washers (Citieffe, Italy) and a bioresorbable interference screw (BioRCi, Smith & Nephew). All procedures were performed by the same three Surgeons.

RESULTS

All patients completed a minimum follow-up of two-years. No intraoperative complications were recorded either in the study and control group. Similar early complications were recorded for both groups in the postoperative period: five superficial wound infections (two for the control group, three for the study group) managed by advanced wound care and oral antibiotics; postoperative effusion and persistent pain (two cases, each for both groups), managed by physical therapy. Comparable results with no significant differences ($p<0.001$) were recorded for both groups, with general high satisfaction and performance of the operated knee of alla subjects. However, we recorded a significant ($p< 0.001$) difference in late complications: we observed 7 cases of late infection around the metallic implants (washers), or intolerance with irritation of pes anserinus tendon in the group treated by aour previous fixation method. Five of these subjects underwent a surgical removal of the washers (3 cases) associated with local debridement and prolonged antibiotic administration (2 cases). No similar complication was recorded in the study gorup. From a radiologic point of view, we observed 6 cases of tunnel widening in the control group, and similar 10 cases in the Biointrafix group: in both cases, the patients remained asymptomatic.

DISCUSSION

Similar clinical and functional outcomes and no significative difference in early postoperative complications were recorded for both groups. However, at mid-term follow-up, no case of local infections or intolerance to the devices was recorded in the group of patients treated by the fully resorbable tibial system with respect to the partially resorbable one. From a biomechanical point of view, recent in vitro studies demonstrated the significant superiority of Biointrafix with respect to other similar metallic or not metallic fixation system in term of strenght and load failure stresses. We feel that a fully resorbable fixation as Biointrafix has to be strongly considered nowadays in the management of the ACL reconstructions, given the similar outcomes and the lower rate of late complications: however, long-term studies will furtherly prove its validity.

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THE INFLUENCE OF DIFFERENT FEMORAL HAMSTRING GRAFT METHODS – DEPENDENCY ON DYNAMIC LOADING

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BACKGROUND

Hamstring grafts are commonly used for ACL reconstruction at our clinic.

HYPOTHESIS

We assume that the best fixation method should be a suspension technique that does not interfere with the graft and does not damage its structure. The purpose of our study is to determine the effects of suspension fixation compared to graft cross-pinning transfixation.

STUDY DESIGN

The design of the study is a descriptive laboratory study - a cadaveric biomechanical study.

MATERIALS AND METHODS

38 fresh frozen human hamstring specimens from 19 cadaveric donors were used. The grafts were tested for their loading properties. One half of each specimen was suspended over a 3.3mm pin, the other half was cross-pinned by a 3.3mm pin to simulate the graft cross-pinning technique. Single impact testing was performed and the failure force, elongation and acceleration/deceleration of each graft was recorded and the loading force vs. elongation of the graft specimens was calculated. Results for suspended and cross-pinned grafts were analyzed, comparing the grafts from each donor.

RESULTS

The ultimate strength of a double-strand gracilis graft was 1287±134N when suspended over a pin, the strength of a cross-pinned graft was 833±111N. For double-strand semitendinosus grafts the strengths were 1883±198N and 997±234N, respectively. Thus, the failure load for the cross-pinning method is only 64.7% or respectively 52.9% of the suspension method.

CONCLUSION

Femoral fixation methods using suspension of the graft showed superior strength over graft cross-pinning transfixation methods. **Clinical relevance:** Suspension techniques appear to be more suitable for femoral hamstring graft fixation, because of their superior loading properties. Use of suspension techniques should be used for hamstring graft fixation in athletes.

KEY WORDS

ACL reconstruction, hamstring graft, femoral fixation, failure load, dynamic loading

QUASI-LINEAR VISCOELASTIC PROPERTIES OF THE MEDIAL PATELLOFEMORAL LIGAMENT

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INTRODUCTION

The Medial Patellofemoral Ligament (MPFL) displays time- and history-dependent viscoelastic behavior, and is nonlinear in its stress-strain response. This behavior results from the complex interaction among collagen, elastin, proteoglycans and water and it is usually described by the quasi-linear viscoelastic theory (QLV) proposed by Fung [1] (fig.1). In order to describe the viscoelastic behavior of the MPFL, the objective of this study was to determine and validate the five constants used by the QLV theory to describe the instantaneous elastic response and reduced relaxation function on experiments with finite ramp times followed by stress relaxation to equilibrium[2].

METHODS

Fifteen human fresh frozen MPFL were dissected to obtain the correct length-to-width aspect ratio (4:1) and a constant cross-sectional area. The specimens were left in a saline bath at 37°C for 30 minutes and then were fixed with cyanoacrilate and sandpaper in standard clamps and aligned to the 5kN load cell of an Instron 5965 materials-testing machine. In order to reduce tissue hysteresis, the specimens were preloaded with a force of 1N and preconditioned by a series of ten cycles until reaching the strain of 3% with a strain rate of 0.1%/s. Then a stress relaxation test was performed by elongating the MPFL to 6% strain and held for 60 minutes. Strain at the midsubstance was measured using a custom-made optical system. These experimental data were used in combination with the quasi-linear viscoelastic theory (QLV) to characterize the tissue's reduced relaxation function, G(t), (described by constants C, $\tau_1\tau_1$, and $\tau_2\tau_2$) and its elastic response, $\sigma^e(\varepsilon\sigma^e(\varepsilon)$ (described by constants A and B). For validation, the constants obtained were used to predict the results of a separate cyclic stress relaxation experiment [3]. In particular, 10 cycles of deformation between 4% and 6% at 0.3%/s and the corresponding peak stresses were recorded.

RESULTS

The obtained constants are summarized in table 1

$$T(t: 0 < t < t_0, \theta) = \frac{A B \gamma}{1 + C\, ln\,(\tau_2/\tau_1)} \int_0^t \{1 + C(E_1[(t-\tau)/\tau_2] - E_1[(t-\tau)/\tau_1])\} e^{B\gamma\tau} \partial\tau$$

$$T(t: t_0 \leq t, \theta) = \frac{A B \gamma}{1 + C\, ln\,(\tau_2/\tau_1)} \int_0^{t_0} \{1 + C(E_1[(t-\tau)/\tau_2] - E_1[(t-\tau)/\tau_1])\} e^{B\gamma\tau} \partial\tau$$

Fig.1 – QLV Theory equations

	A (MPa)	B	C	τ_1 (sec)	τ_2 (sec)	t_0 (sec)
Mean	1,21	26,03	0,11	6,32	903,47	19,99
SD	0,96	4,16	0,02	1,76	504,73	-

Table 1 – Five constants of the QLV theory

At the end of the loading phase, the corresponding stress was 4.1 ± 3.2 MPa and presented a non linear trend. Most of the relaxation occurs within the first 20 minutes and, after 60 minutes, the total stress relaxation was 32,7 ± 4,7%. The QLV theory allows a proper fit of the experimental data for each sample evaluated with a $R^2 = 0{,}993$.

For validation, the constants A, B, C, $\tau_1\tau_1$, and $\tau_2\tau_2$ obtained from the strain history approach could accurately describe the experimental data of the cyclic stress relaxation test for each specimen. Error between the prediction and experimental data ranged from 0.5% to 3.1% for the best prediction and 9.7% to 17.4% for the worst one. In general, the prediction of the initial peak stress was the most erred for all specimens and the average error for this peak measured 6.5 ± 7.1% across all specimens.

DISCUSSION

The obtained results demonstrate that the QLV theory could be successfully used to describe the viscoelastic behavior of the human Medial Patello-Femoral Ligament. This is foundamental to understand its contribution as stabilizer and for the selection of the methods of repair and reconstruction.

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IN-VIVO ANATOMY-BASED PATELLAR TENDON TRACKING DURING TOTAL KNEE ARTHROPLASTY

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INTRODUCTION

The mobility and the stability of the human knee are controlled by a synergic interaction between tibiofemoral (TFJ) and patello-femoral joint (PFJ) surfaces and a number of important soft tissues. Among these, the patellar tendon (PT) plays an important role in transmitting tensile forces within the extensor apparatus [1]. A thorough anatomy-based mapping of PT attachments is essential for a reliable assessment of fibres location and deformation during knee motion, to understand its contribution to knee function, and to restore physiological knee kinematics after total knee arthroplasty (TKA). Several methodologies have been used to analyze PT fibre deformation both in-vitro and in-vivo. However, these did not allow the acquisition of accurate, natural and continuous movements over a large range of flexion. Furthermore, previous studies did not report correlations between PT tracking and PFJ kinematics before and after TKA. Additionally, the inaccessibility of important anatomical landmarks prevented three-dimension PT mapping and robust anatomical reference definitions. These limitations can result in unreliable patterns of PT fibre deformations and orientations. Among possible measuring devices, current knee surgical navigation systems used for prosthesis component positioning enable direct digitization of bony landmarks and surfaces at the lower limb. These systems make possible anatomy-based tracking of bony segments together with the relevant attachment areas for PT [1]. The aim of the present study was to assess in-vivo variations in length and orientation of a number of PT fibres at the intact knee and after TKA. A thorough description of slackening/tightening and orientation patterns via careful anatomy-based PT fibre mapping is here provided together with PFJ kinematics.

METHODS

Six patients affected by primary gonarthrosis were implanted with a fixed bearing posterior-stabilized prosthesis (Triathlon® TKA system, Stryker®-Orthopaedics, Mahwah, NJ-USA) with patellar resurfacing, using a knee surgical navigation system (Stryker®-Leibinger, Freiburg, Germany; 0.5°/0.5mm accuracy). Before TKA, marker clusters were pinned onto the femur and the tibia. An additional lighter and specially-designed cluster was fixed onto patellar anterior aspect by four mono-cortical metal screws. A pointer was used for system control and landmark digitization. Anatomical and articular definitions were according to recommendations [1,2,3]. Series of 3 trials of manually driven knee flexion-extension in a 0°-140° arc were recorded with the intact and replaced knee. For PT fibre recruitment analysis, point strips along the bone-to-fibre PT proximal attachment on the patella were digitized together with PT distal attachment on the tibial tuberosity. Point strips were also collected along the most medial, central and lateral PT fibres. Proximal and distal attachment centroids and the strips extremities were assumed as origins and insertions. Corresponding distances were calculated over flexion and reported as % of the corresponding maximum length at the intact knee. Location of the most isometric fibres (ISO) was also investigated. Fibre orientation was calculated in tibial frontal and sagittal planes with respect to the proximo-distal axis.

RESULTS

Repeatable patterns of TFJ, PFJ and PT kinematics were observed versus flexion within each knee, standard deviation over trials being smaller than 1.0 mm and 1.0°, both at the intact and replaced knees. The corresponding values over specimens were larger due to the different status of the diseased knees, these being about 7 mm and 9°. At the natural knees, PT lengthening occurred with different extents mainly in the initial 30°-40° of flexion for all fibres. This was observed also after TKA, although at near full extension all fibres were about 15% tighter than by in the intact knee. All fibres in the intact knee inclined laterally and posteriorly of about 11° and 30°, respectively; after TKA, an extra lateral inclination of 4° was observed. ISO had no anatomical consistency. PT kinematics was always correlated to TFJ flexion. After TKA, fibre frontal orientation was always correlated to medio-lateral PFJ shift, but this was not observed at the intact knees, likely due to the eroded status of the native patellae.

DISCUSSION

The adopted methodology enabled reliable tracking of PT kinematics throughout the passive flexion arc and careful mapping of fibres recruitment. Complete information about fibre deformation and orientation was provided in consistent anatomy-based reference frames before, i.e. at the intact knee, and after TKA. The present study was aimed at contributing to the much controversial knowledge on PT tracking in the normal and replaced knee over the flexion range. Complete re-establishment of natural tendon behaviour is fundamental for restoring normal knee function. Currently, this pilot study is being extended to larger patient cohorts to produce more statistically robust results.

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WHAT IS ROLE OF TENDON AND MUSCLE PROPERTIES IN HOOPING PERFORMANCE

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Repetitive vertical hopping is a simple and relatively controlled task useful for studying basic neuromuscular properties and tissue mechanics. However, several biomechanical and physiological factors are involved. This article provides an overview of muscle and tendon properties, and how these interact during vertical hopping. Muscle properties discussed are force-velocity and force-length relationships, electromechanical delay, muscle fibre type, stretch induced contraction amplification and muscle spindle afferent feedback. Tendon properties include storage and reuse of elastic energy, tendon stiffness, afferent information from Golgi tendon organs and failure points. These muscle and tendon properties interact to generate vertical hopping force and power. In addition to these basic properties, there are other more complicated factors to consider when analysing vertical hopping such as balance and coordination. A wealth of information can be gathered by studying vertical hopping. However, caution should be taken to prevent inappropriate conclusions being drawn about hop performance due to oversimplification.

THE EFFECT OF DOUBLE-ROW REPAIR ON ROTATOR CUFF TENDON HEALING

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INTRODUCTION

Aiming at a sufficient restoration of the anatomical configuration as a condition for mechanical strength double-row techniques for rotator cuff repair gain more interest during the past years. Previous studies have shown high initial mechanical and morphological properties especially compared to different single-row techniques. However, a controversy exists regarding the optimal fixation technique in rotator cuff repair because of heterogeneous clinical results. The aim of the study on hand was to investigate the time-dependent cell biological and biomechanical properties using a double-row technique for rotator cuff repair in an in-vivo sheep model. Additionally, specimens were evaluated by MRI using a standardized investigation protocol.

METHODS

Thirty-six female mature sheep were randomly assigned to either a single-row group using arthroscopic Mason-Allen stitches, or a double-row group using a combination of arthroscopic Mason-Allen (lateral) and mattress stitches (medial). Each group was analysed at one of six survival points postoperatively. The integrity of tendon repair was evaluated using MRI. Biomechanical properties were analysed using a mechanical testing machine detecting maximum load-to-failure loads. Moreover, expression of collagen types I, II, and III using histological, ultra structural and molecular biological methods was determined.

RESULTS

The mean load-to-failure was significantly higher in the double-row group compared to the single-row group at 6 and 12 weeks. At 26 weeks, the differences were not significant and adapted each other. Nevertheless, the double-row repair achieved a mean load-to-failure similar to that of a healthy infraspinatus tendon at final follow-up. In contrast, the single-row group reached 70% of the load of a healthy infraspinatus tendon. Conversely, no significant differences were observed based on MRI investigation.
Collagen type III expression remained positive until six weeks postoperatively in the double-row group, whereas it was detectable for 12 weeks in the single-row group. Additionally, collagen Type II expression was increased in the cartilage zone after 12 weeks in the double-row compared to the single-row group.

DISCUSSION

The results of the study confirm that double-row repair may simultaneously enhance as well the speed of mechanical as histological recovery of the tendon-bone complex. This phenomenon could be explained due to faster tissue revitalization in using this technique as a condition for biomechanical properties. By contrast, the used single-row technique reached 70% of the load of a healthy infraspinatus tendon. Conversely, the MRI results did not show any significant differences in using both techniques. In summary, double-row repair enables higher mechanical strength that is especially sustained during the early recovery period and may therefore improve clinical outcome.

THE TREATMENT OF ROTATOR CUFF TEARS WITH TWO DIFFERENT PATCHES

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INTRODUCTION

The treatment of massive rotator cuff tears presents a challenging problem in shoulder surgery. Traditional repair techniques are associated with high rupture rates due to excessive tension on the repair and the presence of degenerated tendon tissue. These factors have led to attempts to reconstruct the rotator cuff with grafts, using synthetic materials or biologic tissues. The purpose of this study was to compare the efficacy of the use of pericardium patch with the use of prolene patch in the repair of extensive rotator cuff tears.

MATERIALS & METHODS

A retrospective series of 180 patients, 115 men and 65 women with a mean age of 66.8 years treated for a massive rotator cuff tear from 1997 to 2008 is reported. The inclusion criteria were: patients symptomatic with pain, deficit of elevation, not responsive to the physiotherapy, tear size (massive: 2 or more tendons), minimum follow-up of 2 years since surgery, active and motivated patients. Patients were divided into three groups according to the type of treatment received: group 1 was treated with Pericardium patch, group 2 with Prolene patch, group 3 with simple suture. All groups were homogeneous. Plain radiographs, ultrasound and MRI were performed preoperatively and at 3 years. Patients were clinically evaluated using the UCLA score before surgery and at 2 months and 3 years after surgery (mean follow-up 2,6 years). Pain was assessed by use of VAS scale, strength by the use of dynamometer. The surgical procedure (mini-open technique) was similar in all groups. Statistical analysis was conducted by one-way ANOVA between groups of treatment with Dunnett's C post-hoc correction for multiple comparisons. P-values of 0.05 or less were considered as statistically significant.

RESULTS

After 2 months the mean VAS was 6.85±1.11, 6.45±1.01, 4.9±0.9 while the mean UCLA was 11.28±1.43, 13.35±14.21, 20.85±12.77, respectively for Control, Collagen and Prolene group. After 36 months the mean VAS was 3.7±1.01, 4.05±0.98, 3.23±1.07, while the mean UCLA was 14.73±1.96, 14.86±2.08, 24.6±3.3 respectively for Control, Collagen and Prolene group. In addition, after 36 months elevation on the scapular plane was 140.75°±10.48, 141.58°±11.87, 174.75°±8.1 and abduction strength was 8.57kg±0.63, 8.82kg±0.7, 13.61kg±0.84, respectively for Control, Collagen and Prolene group. Retear rate after 12 months was 40% (24/60) for Control group, 48.33% (29/60) for Collagen group, 15% (9/60) for Prolene group.

CONCLUSION

The use of Prolene patch as an augmentation graft in the treatment of massive rotator cuff tears is safe and, in most patients, can give a significant pain relief and improvement of range of motion and strength with few complications.

IDENTIFICATION OF COLLAGEN VI IN THE PERICELLULAR MATRIX OF HUMAN ANTERIOR CRUCIATE LIGAMENT

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INTRODUCTION

Cell-extracellular matrix interaction plays important functions in maintaining the structural integrity of connective tissues and sensing changes in the biomechanical environment of cells[1]. Collagen VI is a widely expressed non-fibrillar collagen which regulates tissues homeostasis. The objective of the present investigation was to extend our understanding to the role of collagen VI in human anterior cruciate ligament (ACL).

METHODS

Human ACL biopsies were harvested from healthy donors subjected to standard orthopedic surgery. Collagen VI localization on ACL sections was performed by immunofluorescence and immunogold techniques[2]. ACL cultures[3] were characterized for the expression of collagen VI and NG2 by immunofluorescence, rotary shadowing and western blot analysis. After equibiaxial cyclic mechanical tension, ACL cultures were evaluated by immunofluorescence and scanning electron microscopy[4].

RESULTS

Here we report that collagen VI is associated to the cell membrane of knee ACL fibroblasts, forming a microfibrillar extra-cellular scaffold. The cell membrane localization of collagen VI correlated with the expression of NG2 proteoglycan, a trans-membrane collagen VI receptor. The treatment of ACL fibroblast cultures with anti-NG2 neutralizing antibody abolished the localization of collagen VI at the plasma membrane and prevented the experimental stretching induced cell alignment.

DISCUSSION

These data indicate that the NG2 proteoglycan plays a critical role in mediating the collagen VI pericellular matrix organization in ligaments; the inhibition of NG2-collagen VI interaction alters the response of cell to mechanical stress and may influence the biomechanical properties of ligaments.

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ACHILLES ENTHESIS: A PRIVILEGED SITE OF DIABETIC DAMAGE?

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INTRODUCTION

The prevalence of tendinopathies is increased in subjects with diabetes mellitus. However, there are few data on the presence and distribution of structural abnormalities of Achilles tendon (AT) in asymptomatic diabetic patients (**1**). Aims of the study were to assess the morphologic characteristics of AT in subjects with diabetes in comparison with controls without diabetes and their preferential location in the tendon.

METHODS

Participants were consecutively recruited from an outpatients population. Ultrasound (US) scans of AT were performed: degenerative features (*abnormal fibrillar pattern, hypo-hyperechoic areas*), signs of enthesitis (*bony erosions, enthesophytes, and bursitis*), and neovascularisation were then acquired.

RESULTS

Sixty tendons with asymptomatic sonographic abnormalities (ASA), observed in 136 diabetic patients, were compared with 45 tendons with ASA, observed in a larger series of 273 controls. The prevalence of midportion ASA was higher in controls, whereas the prevalence of signs of enthesitis was higher in the study group (**Figure 1**). Neovessels were scarcely represented, without any difference between the experimental groups (**Table 1**).

	Diabetic patients	*Controls*	*p*
Tendon with ASA	60/272 (22%)	45/546 (8.2%)	0.0000
Age	64.6 ± 6.1	63.9 ± 6.5	ns
Midportion ASA	36 (60%)	38 (84.4%)	0.006
Enthesitis	32 (53.3%)	9 (20%)	0.000
Neovessels *(grade 1 and 2)*	7 (11.6%)	5 (11.1%)	ns

Table 1. Asymptomatic sonographic abnormalities (ASA)

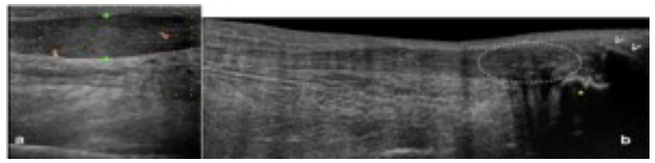

Figure 1. Longitudinal scans of AT: in panel a) thickening of AT (calipers), hypoechoic echotexture and few neovessels are expression of degeneration; in panel b) bony erosion (*), entesophyte (arrowheads) and degeneration (dot circle) are signs of calcific entesopathy

DISCUSSION

These observations confirm that diabetes may predispose to AT structural abnormalities, as shown by the higher percentage of ASA observed in subjects with diabetes compared to controls; the new finding is that in these subjects the enthesis is the privileged site where these abnormalities are localized (**1, 2**). Longitudinal studies, evaluating the progression of the lesions not only in the tendon but also in the structures of the "enthesis organ", are needed to support this conclusion.

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HISTOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS IN THE COLLATERAL LIGAMENTS OF THE EQUINE METACARPO- AND METATARSOPHALANGEAL JOINT

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INTRODUCTION

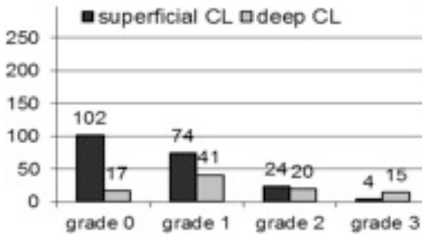
Of all joints in the horse, the metacarpo- or metatarsophalangeal (MCP/MTP) joint experiences the highest joint powers. It is extended in the normal standing position, and hyperextension occurs when loads are applied. Even though movement is limited to the sagittal plane, uneven foot bearing or imbalanced feet lead to asymmetric loading. It has been shown that mechanical strain is an important component in cell activation, leading to cell signaling, production of biochemical mediators and reconstruction of the extracellular matrix (ECM). The purpose of this study was to describe histopathological abnormalities in the collateral ligaments (CL) of the fetlock joint in horses without clinical signs of lameness. The CL of MCP/MTP joint consists of a long superficial and a shorter deep part which were compared in our study. It was hypothesized that due to the high loads put on this joint, degenerative abnormalities in the collateral ligaments would be frequent and that in response to potential hyperextension and medial-lateral instability changes in cellular and ECM components could be detected.

METHODS

26 limbs of nine adult horses (3 stallions, 4 mares, 2 geldings; age 3- 28 y; mean age 13 ± SD 8 y; mean weight 521 ± SD 85 kg) were included in the study. Each of the 52 MCP or MTP CL was divided into six parts (4 long superficial and 2 deep parts) and embedded in paraffin. Subsequently specimens were sectioned sagittaly and stained with H&E, Safranin O, Alcian blue and van Gieson's staining. Immunohistochemistry was performed for detection of MMP-2 and 9, Collagen type I and II and Substance P (SP) with subsequent DAB or Alexa Fluor 488 Ab staining. Evaluation was performed according to a histological grading by Dyson et al. (2008): 0= normal, 1= variations in cellular shape and density + variations in collagen fiber structure and orientation, 2= presence of chondroid cells indicating moderate fibrocartilaginous metaplasia, 3= severe fibrocartilaginous metaplasia.

RESULTS

Only 40% of the CL showed normal histology characterized by elongated cells embedded in a collagen-rich ECM, organized longitudinal to the axis. Mild histopathological abnormalities were observed in approximately 39%. 15% showed moderate fibrocartilaginous metaplasia whereas severe fibro-cartilaginous metaplasia was only observed in 6% (Fig 1). Distal CL parts were involved more frequently, and the deep CL was affected more severely. These changes coincide with an upregulation of MMP 2 and 9 as well as SP that increase with the degree of histopatholocial abnormalities detected.



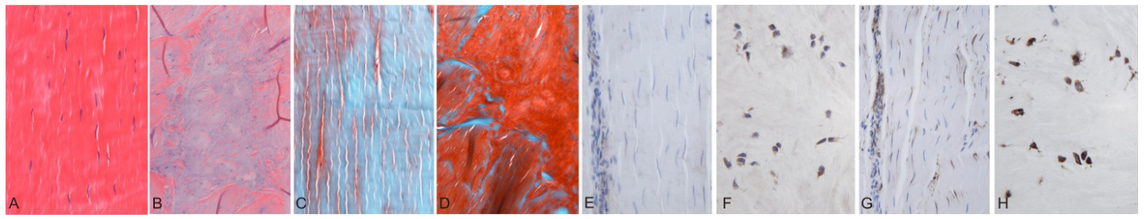


Fig 2: A H&E grade 0; B H&E grade 3; C Saf O grade 2; D Saf O grade 3; E MMP-2 grade 0; F MMP-2 grade 3; G MMP-9 grade 0; H MMP-9 grade 3

DISCUSSION

In the present study we determined the general histological appearance including histopathological changes of the equine MCP/MTP CL in a random set of horses. With the limitation of a small number of horses studied and a heterogenic population we nevertheless conclude that degenerative abnormalities in the MCP/MTP CL are fairly common and vary from mild changes in cellular density and collagen fiber orientation to severe fibrocartilaginous metaplasia. Fibrocartilage is known to be present in tendons or ligaments subjected to compression. The distal third of the superficial and the deep CL are subjected to high compressive forces when increasing loads are applied, and might therefore be predisposed for adaptive fibrocartilaginous metaplasia. Moreover, increasing levels of MMPs as well as SP that is known to be produced by fibroblasts and tendon cells in response to mechanical signals indicate adaptive processes within the different parts of the normal CL.

EVALUATION OF THE INFLUENCE OF PLASMA RICH IN GROWTH FACTORS ON TENDON HEALING IN AN EXPERIMENTAL MODEL OF DIVIDED ACHILLES TENDON IN SHEEP

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INTRODUCTION

The acute rupture of the Achilles tendon is probably the most dramatic lesion that affects this tendon, and the management of this injury may result challenging. Recovery from a tendon rupture is a slow process because the poor-healing capacity of tendon compared to other tissues, mainly associated with a reduced blood supply and a reduced metabolism of the tenocytes. The use of Platelet-Rich in Growth Factors (PRGF) has been proposed to improve the healing of Achilles tendon injuries. Several authors have reported that applications of PRGF for improve the healing response after tendon injuries but there is debate about the effectiveness of this biological therapy. The objective of this study was to evaluate the effects of PRGF injections on Achilles tendon healing, using an experimental model in sheep.

METHODS

The Achilles tendons of twenty adult sheep were divided surgically in a standardized way. After the tenotomy, the Achilles tendon was sutured using a three loop pulley pattern. Animals were randomly divided into four groups of five animals each. The repaired tendons of two groups received an infiltration of PRGF intraoperatively and every week for the following three weeks under ultrasound guidance. The other two groups received injections with saline as described previously.

The animals in one PRGF-treated group and one saline group were euthanized at four weeks, and the animals in the remaining two groups were euthanized at eight weeks. During the postoperative period, weekly general health examinations were performed, and the operated tendons were evaluated by mean of ultrasound in order to monitor the healing process in tendons. After euthanasia, the Achilles tendons were harvested and examined macroscopically. Histological examinations were also performed. The morphometry of fibroblast nuclei, fibroblast density, and vascular response were evaluated with the aid of image analysis software. Other histological parameters as the arrangement of collagen fibers, or inflammatory cell infiltration were evaluated using a semiquantitative grade scale. Immunohistochemical techniques were performed to evaluate the collagen type I and type III.

RESULTS

All animals used in the design of this experimental model reached the end of the study without any casualties. After the statistical analysis of the data, a significant improvements related to ultrasonographic appearance of the Achilles tendon was observed in PRGF-treated tendons at eight weeks after surgery. Regarding histological study, fibroblast nuclei of PRGF-treated tendons were significantly more elongated and more parallel to the tendon axis than the fibroblast nuclei of the saline-injected group at eight weeks. PRGF-treated tendons also showed more packed and better oriented collagen bundles, both at four and eight weeks. In addition to an increased maturation of the collagen structure, fibroblast density was significantly lower in tendons that had been infiltrated with PRGF. PRGF-treated tendons exhibited faster vascular regression than tendons in the control groups at eight weeks. PRGF-treated group showed a lower degree of inflammation than saline group, both at four and eight week after surgery. Tendons infiltrated with PRGF showed significantly less collagen type III four weeks after surgery; and more collagen type I at eight weeks, compared to tendons injected with saline.

DISCUSSION

The findings of this study suggest that PRGF injections accelerated the healing process in repaired tendons after experimental disruption of Achilles tendons in sheep. PRGF-treated tendons showed improvements in morphometric features of fibroblast nuclei, suggesting a more advanced stage of healing. Histological examinations at eight weeks of tendons treated with PRGF revealed a more mature organization of collagen bundles, lower vascular density and lower fibroblast density, producing more advanced histological appearances of the healing process than in tendons infiltrated with saline. Ultrasonographic evaluations also showed an improvement in tendon healing in PRGF-injected tendons at eight weeks. These results suggest that PRGF infiltration may be an interesting coadjuvant treatment during the surgical management of acute rupture of Achilles tendons.

ISOLATION AND CHARACTERIZATION OF ADULT STEM CELLS DERIVED FROM HUMAN ROTATOR CUFF TENDONS

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INTRODUCTION

Rotator cuff (RC) tendons are often prone to lesions, as 30 to 50% of the population over fifty suffers of partial- and full-thickness RC tears. Several approaches have been developed over the years, including the use of growth factors, bone morphogenetic proteins and, more recently, stem cells. Among adult stem cells, bone marrow mesenchymal stromal cells (BMSCs) are by far the most studied, although tendon-derived stem cells (TDSCs) have been found in several animal species, including humans (1,2). However, the isolation of a cell population with stem cell characteristics from the human RC has yet to be reported.

The purpose of this study was to isolate and characterize two new adult stem cell populations from the supraspinatus (SS) and from the long head of the biceps (LHB) tendons.

METHODS

Twenty-six patients who underwent arthroscopic RC repair were enrolled in this study. Cells were isolated from fragments of discarded tissue from surgeries, cultured, expanded *in vitro* and phenotypically characterized. The differentiation potential was tested by inducing differentiation toward adipose, condrogenic and osteogenic lineages. Finally, these new cell populations were compared to human BMSCs and to human dermal fibroblasts (DFs).

RESULTS

Two new adult stem cell populations from SS and LHB tendons were isolated, characterized and cultured *in vitro*. Cells showed adult stem cell characteristics, *i.e.* they are self-renewing *in vitro*, clonogenic and multipotent, as they could be induced to differentiate *in vitro* into different cell types, including osteoblasts, adipocytes and skeletal muscle cells.

DISCUSSION

This work demonstrates that adult stem cells from SS and LHB tendons can be easily isolated and induced to differentiate toward several cell types, indicating their intrinsically appropriate cell plasticity, which is comparable with that of BMSCs. These results will allow to study their potential role in RC tendon pathology and their possible application in tissue engineering.

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GROWTH FACTORS EFFECTS ON SKELETAL MUSCLE LESIONS. EXPERIMENTAL STUDY

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INTRODUCTION

Even though muscle injuries are very common, few scientific data on their effective treatment exist. Growth Factors (GFs) may have a role in accelerating muscle repair processes and a currently available strategy for their delivery into the lesion site is the use of autologous platelet-rich plasma (PRP).

METHODS

The present study is focused on the use of Platelet Rich Plasma (PRP), as a source of GFs. Unilateral muscle lesions were created on the longissimus dorsi muscle of Wistar rats. The lesion was filled with a PRP intramuscular injection at different concentrations after 24 hours from the surgical trauma. A group of rats was left untreated (controls). Animals were sacrificed at 3, 15, 60 days from surgery. Histological, immunohistochemical and histomorphometric analyses were performed to evaluate muscle regeneration, neovascularization, fibrosis and inflammation. The presence of metaplasic zones, calcifications and heterotopic ossification were also assessed.

RESULTS

PRP treated muscles exhibited an improved muscular regeneration, an increase in neovascularization, and a slight reduction of fibrosis compared with controls. No differences were detected between the groups treated with different concentrations of PRP. Metaplasia, ossification and heterotopic calcification were not detected.

DISCUSSION

This preliminary morphological experimental study shows that PRP use can improve muscle regeneration and long-term vascularization. The different PRP concentrations seem not determine a significative modification in histological analyses. Since autologous blood products are safe, PRP may be a useful and handily product in clinical treatment of muscle injuries.

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OPTIMUM AND FORMULATION OF ASCORBIC ACID AS A SUPPLEMENTATION FOR HUMAN TECNOCYTES IN VITRO

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INTRODUCTION

There is evidence to suggest that ascorbic acid could help tendon healing by improving the rate and quality of rat Achilles repair, as well as protecting Hamstring-derived tenocytes from oxidative stress [1, 2]. However, there is no consensus about which formulation and dose are most beneficial. Thus, the objective of this study was to determine the effect of a gradient of concentrations of L-ascrobic acid (Figure 1A, Vit C), the natural form of Vitamin C, and magnesium ascorbyl phosphate (Figure 1B, Mg Vit C), a relatively new, stable derivative of vitamin C on cell growth and collagen synthesis by human tenocytes in vitro.

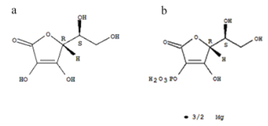

Figure 1: The formulae of L-Ascribc acid (Vit C, a) and magnesium ascorbyl phosphate (Mg Vit C, b)

METHODS

Human tendon cells were extracted from rotator cuff tissue resected during surgical repair, with appropriate ethical approval. $5\,'10^3$ cells per well were seeded in 96 well plates. Cells were fed DMEM-F12 media supplemented with 5% FBS, 1% pen/strep and different concentrations: 0, 0.01, 0.1, 1, 10, 100, 1000 or 2000mM of either VitC or MgVitC (both Sigma-Aldrich, UK). For pulsed-supplementation, media was changed every 2 days. AlamarBlue (AbD Serotec, UK) was used to monitor changes in cell growth. The pH of the growth medium supplemented with vitamins was measured using a Seveneasy pH meter (Mettler Toledo). A hydroxyproline assay [3] was used to quantify the total collagen content of populations in 10cm dishes. Multiphoton microscopy (MPM) was used to image cells and collagen fibrils.

RESULTS

As can be seen in Figure 2, Mg VitC consistently improved cell growth with increased concentrations. Effect was stronger in short term (Fig 2a) and pulsed release (Fig 2d). Vit C was toxic in concentrations over 10mM. pH mesurements (Fig 3b) showed pH with media supplemnted with Vit C started to drop at concentrations over 1mM, helping to explain the toxic effect. Good cell growth of cells growth with Mg Vit C supplemnetation also showed higher total collagen quantities.

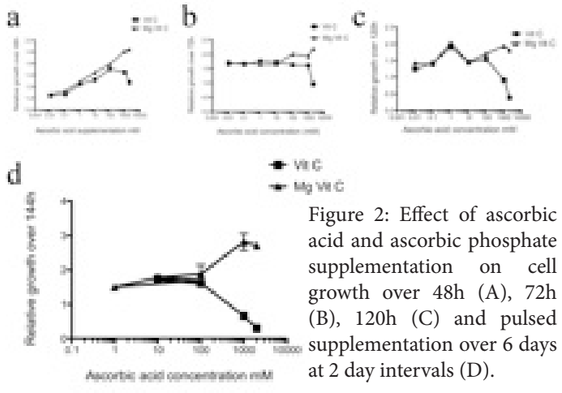

Figure 2: Effect of ascorbic acid and ascorbic phosphate supplementation on cell growth over 48h (A), 72h (B), 120h (C) and pulsed supplementation over 6 days at 2 day intervals (D).

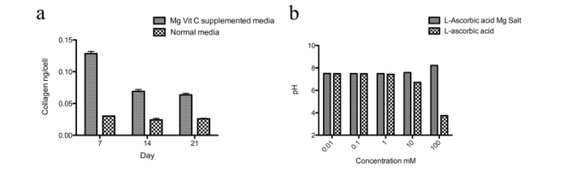

Figure 3: Total collagen content of plates supplemented with 1mM MgVitC over 21 days compared to normal culture conditions (A) and the pH of media supplemented with Vitamin C (B).

Finally, using Second Harmonic Generation (SHG), collagen fibrils could be clearly identified in plates supplemented with 1mM MgVitC (Fig 3b) but no collagen at all at non-supplemented plates (Fig 3a).

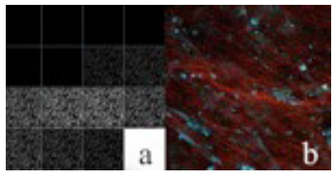

Figure 3: Whilst no collagen was detected in control plates (A), clear collagen fibril were seen by MPM in plates supplemnted with 1mM Mg VitC.

Vitamin C has been proposed as a tendon culture supplementation in the past, but no optimum or formulation has been indicated. Here, we investigated the effect of two vitamin C formulations and various concentrations on tendon-derived cells. An unexpected finding of this study is the complete absence of collagen fibrils in tenoctye cultures non- supplemented with Vitamin C. Supplementation with the phosphate derivative of ascorbic acid (Mg VitC) resulted in clear collagen fibril formation, an increase in total collagen content, and a concentration-dependent increase in cell growth. We thus propose Mg VitC to be regularly added to tendon cells cultures, especially when collagen fibril formation is desired. However, it should be noted that high doses of plain L-ascorbic acid (VitC) were toxic *in vitro*, due to a pH drop of the media.

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THE SHAPE AND THE THICKNESS OF TEH ACL ALONG ITS LENGTH IN RELATION TO THE PCL. A CADAVERIC STUDY

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ABSTRACT PURPOSE
The purpose of this study was to evaluate the shape of the native anterior cruciate ligament (ACL) along its length and to compare it with the size of the three commonly used autografts (Bone Patellar Tendon Bone, (BPTB), hamstrings: single or double bundle (SB or DB).
METHODS
With the knee in extension, the intercondylar notch was filled with paraffin, fixing the cruciate ligaments in their natural position in eight cadaveric specimens. The ACL-PCL tissue specimen, embedded in paraffin, was removed en-bloc. Gross sections were prepared in coronal plane and were evaluated histologically. The dimensions of the ligaments were determined, estimating the width and thickness of each section. The dimensions of Semitendinosus Tendon (ST) and gracilis tendon (GT) and BPTB grafts were measured and compared with the dimensions of the native ACL.
RESULTS
The PCL occupies the biggest part of the intercondylar area leaving only a small space for the ACL at knee extension. The ACL at the mid-substance has a width of 5mm, resembling to a band shape. Only before its tibial insertion the ACL fans out in order to take finally the form of the tibial attachment. BPTB has a thickness of 5.8 mm while ST and GT graft have a thickness of 6.25 mm and 4.5 mm respectively. A ST-G graft has a diameter of approximately 8mm The BPTB as a SB and the ST ,G grafts, as a DB graft, are comparable to the mid-substance of the ACL, but undersized in the tibial insertion ($p<0.05$). In contrary, the quadrupled SB graft is oversized in the mid- substance, but it fits better in the tibial attachment .

A TISSUE ENGINEERING EXPERIMENTAL APPROACH FOR THE TENDON TISSUE

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INTRODUCTION
The inability of tendons to spontaneously self-repair have suggested the development of tissue-engineering strategies for tendon repair and regeneration. In particular, this study was focused on a model based on a collagen I scaffold that was combined to fibrin glue and to a bioreactor in order to develop a tissue with tendon-like properties.
EXPERIMENTAL METHODS
1st part: tenocytes were seeded into the collagen I scaffold in presence or absence of fibrin glue, cultured in static condition for 0, 4, 7, and 10 days in order to analyze the effects of fibrin glue on cell survival and matrix deposition; 2nd part: tenocytes were seeded into the collagen I scaffold in presence of fibrin glue, cultured both in static and dynamic condition (bioreactor) for 1 and 2 weeks in order to highlight the effects of the bioreactor on tissue organization; 3rd part: tenocytes, dermal fibroblasts and adipose-derived mesenchymal stem cells (ASCs) were seeded into the collagen I scaffold in presence of fibrin glue, cultured in the bioreactor for 1 and 2 weeks in order to compare the contribution of different cell sources to tissue formation and organization.
RESULTS
1st part: fibrin glue promoted cell survival at longer time culture (10 days). 2nd part: the cellular scaffold cultured in static conditions showed matrix deposition and reduced survival at longer time points; cells were randomly distributed within the scaffolds. The cellular scaffolds cultured in the bioreactor showed cell and fibers alignment along the direction of the mechanical stimulus; in some areas, the matrix was organized in bundle-like fashion; however, cells showed reduced survival at longer time points. 3rd part: dermal fibroblasts and ASCs showed similar results with respect to tenocytes: they align along the tension direction and produced matrix; however, tenocytes showed higher response to the mechanical stimulus.
DISCUSSION AND CONCLUSION
These data suggest an interesting combination between collagen I scaffold and fibrin glue in supporting cell survival and matrix production of the engineered composite; the potential of this model in tendon engineering is further ameliorated by the introduction of a basic mechanical stimulus that induces a specific cell and matrix organization. Finally, this model can be successfully combined also with dermal fibroblast and ASCs.
In conclusion, this work presents a novel model that, by combining a collagen I scaffold with fibrin glue and a mechanical stimulation, is able to develop into a tissue with preliminary tendon-like properties.

EVALUATION OF KNEE FUNCTION AFTER REPAIR OF THE ACL WITH A NOVEL MAGNESIUM-BASED RING – An *In Vitro* Study in Goats

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INTRODUCTION

Functional tissue engineering involving the use of cells, growth factors, and scaffolds has renewed interest in healing a torn ACL [1,2]. In our research center, we have used an extracellular matrix sheet and hydrogel from the porcine small intestinal submucosa to successfully heal the ACL by 12 weeks in a goat model [3]. However, the healing ACL became stronger than its attachment at the femur by 26 weeks. As such, mechanical augmentation of the healing ACL is also necessary to restore initial joint stability and prevent disuse atrophy of its insertion sites throughout the healing process [4,5]. Thus, we have designed a bioresorbable magnesium (Mg)-based ring to bridge the gap between the two ends of an injured ACL. We hypothesized that the ring could restore knee kinematics as well as keep the repaired ACL (and its insertion sites) loaded and reduce in-situ forces in the medial meniscus (MM). To test this, we employed a robotic/UFS testing system to quantitatively evaluate knee function in the Mg-based ring-repaired joint.

METHODS

8 cadaveric goat stifle joints were tested in 3 knee states: intact, ACL-deficient, and after repair with the Mg-based ring. The knee kinematics and in-situ forces in the ACL and MM were obtained in each state using a robotic/UFS testing system at 3 flexion angles (30°, 60°, and 90°) under 2 externally applied loading conditions: (1) a 67 N anterior tibial load (ATL) and (2) a 67 N ATL with 100 N axial compression. These loading conditions simulated those used in clinical exams to test ACL function [6]. The anterior tibial translation (ATT) and in-situ forces in the ACL and MM were compared to another group of stifle joints treated with suture repair alone using independent t-tests with significance set at $p \leq 0.05$.

RESULTS

To eliminate animal-to-animal variation, all data were normalized by the corresponding value in the intact joint. Under the 67 N ATL, normalized ATT (ATTn) with Mg-based ring repair was 2.1 ± 0.9, 2.5 ± 1.2, and 2.9 ± 1.2 at 30, 60, and 90° of flexion, respectively. These values were significantly lower than those for suture repair obtained from a previous study [5]: i.e., 5.5 ± 2.5, 5.3 ± 2.1, and 6.2 ± 1.9, respectively ($p < 0.05$). With the added compression, ATTn with Mg-based ring repair remained 50-60% lower than with suture repair ($p < 0.05$, Fig. 1A). The normalized in-situ forces in the Mg-based ring-repaired ACL were up to 3.6 times those of the suture-repaired ACL under both loading conditions and were close to those in the intact ACL ($p < 0.05$, Table 1, Fig. 1B). Under the ATL, normalized in-situ forces in the MM were 39-86% lower with Mg-based ring repair than with suture repair ($p < 0.05$ at 60°, Table 1). With the added compressive load, normalized in-situ forces remained 85-88% lower with Mg-based ring repair ($p < 0.05$, Table 1).

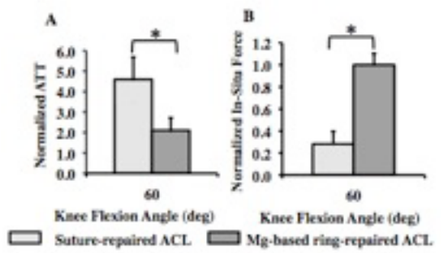

Figure 1: Normalized ATT (A) and in-situ force in the ACL (B) under a 67 N ATL with 100 N axial compression (*significantly different, $p < 0.05$)

DISCUSSION

Mg-based ring repair was able to restore stifle joint stability and load the ACL, as well as reduce in-situ forces in the MM, confirming our hypothesis. Improvements in ATT and in-situ forces compared to suture repair were particularly notable under combined anterior tibial and compressive loading. Thus, the Mg-based ring would be an effective tool for mechanical augmentation of the healing ACL and is suitable for *in vivo* testing.

ACKNOWLEDGEMENTS

Financial support from an NSF Engineering Research Center Grant (#0812348) and Biomechanics in Regenerative Medicine training grant (NIH T32) is gratefully acknowledged.

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Table 1: Normalized in-situ forces in the repaired ACL and MM (mean ± SD) (*significantly different versus ring repair)

	Flexion angle	A. 67 N ATL			B. 67 N ATL + 100 N Comp.		
		30°	60°	90°	30°	60°	90°
I.	**Repaired ACL**						
	Ring-repaired ACL	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.3
	Suture-repaired ACL	0.8 ± 0.1*	0.8 ± 0.2*	0.6 ± 0.3*	0.3 ± 0.2*	0.3 ± 0.1*	0.5 ± 0.3*
II.	**Medial meniscus**						
	Ring repair	1.8 ± 1.2	1.9 ± 1.5	4.4 ± 4.7	2.0 ± 1.5	4.5 ± 3.9	2.8 ± 1.0
	Suture repair	4.7 ± 3.9	13.3 ± 9.5*	7.2 ± 6.5	13.3 ± 10.0*	34.6 ± 32.1*	22.7 ± 22.4*

INTRA-ARTICULAR INJECTION OF TRIPEPTIDE COPPER COMPLEX GHK-CU (II) IMPROVED GRAFT HEALING IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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INTRODUCTION

Anterior cruciate ligament reconstruction (ACLR) is the standard treatment to restore knee function. However, the biological healing of the graft is often slow and poor healing can lead to ACLR failure and excessive knee laxity. If this process can be further enhanced, it will reduce the time of maturation of the graft, and hence earlier returns to full activities and sports. In the present study, the possibilities of biological augmentation of graft healing in ACLR were investigated. Recent findings show that bioactive small molecules such as matrikines may also take part in tissue remodeling. Matrikines are small peptides liberated by partial proteolysis of extracellular matrix macromolecules, which are able to regulate cell activities. Among different classes of matrikines, Glycyl-Histidyl-Lysine (GHK) tripeptide and its copper (II) chelated form (GHK-Cu) exhibit profound involvements in the tissue remodeling processes. GHK have been extensively used in the cosmetic industry for skin tissue remodeling for years with its high safety profile in humans. GHK-Cu can simultaneously activate the *in vivo* synthesis of matrix components, and the *in vivo* production of degradative enzymes and the inhibitors. GHK-Cu can promote bone healing and promote implant attachment to bony tissues. It can also act as chemoattractant for repair cells and induce wound angiogenesis. It is possible that GHK-Cu can promote graft healing in ACLR.

METHODS

The animal experiments in this study were approved by the Animal Experimentation Ethics Committee in authors' institution (Ref. no.: 11/054/GRF and 460611). The procedures of ACLR were performed on the right knee according to our previous study. Seventy-two male Sprague Dawley rats (12 weeks old, 400-450g) were used. Intra-articular injection (50µl per injection) of saline or GHK-Cu solution (0.3 or 3 mg/ml) was performed weekly from 2nd week to 5th week post operation. At 6 or 12 weeks post-operation, the rats were euthanized to harvest knee specimens for static anterior-posterior (AP) knee laxity test and graft pull-out test (n=8). Histological scoring on H&E stained sections was performed (n=4) with polarization microscopy. A 2-way ANOVA was used to analyze the effect of time and treatment on the outcome measures. Statistical significance was accepted at $\alpha=0.05$.

RESULTS

At 6 weeks post operation, rats treated with 0.3 or 3 mg/ml GHK-Cu resulted in a significantly smaller side-to-side difference in AP-knee laxity as compared to saline group, but no difference in AP laxity was detected at 12 weeks post operation ($p=0.531$) (Figure 1). There was no significant difference in pull-out strength of the graft complex between GHK-Cu groups and saline group at 6 and 12 weeks post operation ($p=0.301$, 0.834), however, the stiffness of the graft complex was significantly higher in 0.3 mg/ml GHK-Cu as compared to saline group at 6 weeks post operation ($p=0.007$). All grafts failed at mid-substance during the pull-out test. Histological examination showed that graft incorporation and bone healing inside tunnels was significantly better in the GHK-Cu treated groups. Graft degeneration as shown by decreased collagen birefringence was less severe in the 0.3 mg/ml GHK-Cu group as compared to saline group, but significantly increased cell recruitment to graft mid-substance in 3 mg/ml GHK-Cu group also rendered poor graft integrity (Figure 2).

DISCUSSION

Our study suggests that GHK-Cu may improve graft healing in ACLR. GHK-Cu may improve tissue remodeling in graft mid-substance and graft tunnel interface, but extensive tissue remodeling may not be beneficial as shown in 3 mg/ml GHK-Cu group. Further studies are necessary to investigate the underlying mechanisms for the observed effects of GHK-Cu. As restoration of A-P knee laxity after ACLR was improved at earlier time point, biological enhancement in graft healing by GHK-Cu may imply earlier return to normal activity after operation. However, as the pull-out strength was not improved, high demand activities may not be warranted safe. There are still plenty of rooms for biological augmentation of graft healing in ACLR.

Figure 1

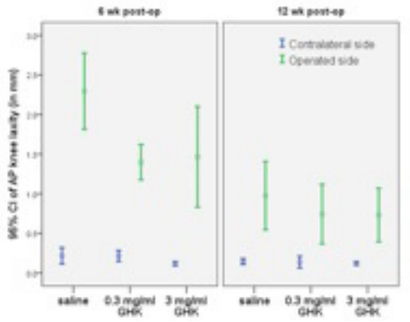
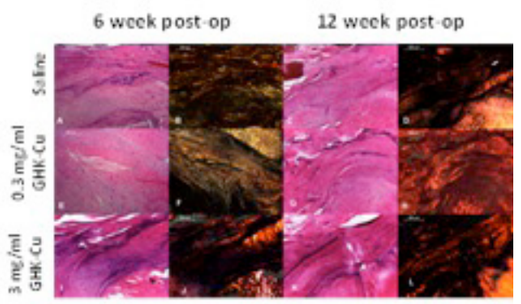


Figure 2



HEALING RESPONSE OF THE HUMAN ANTERIOR CRUCIATE LIGAMENT: A HISTOLOGICAL STUDY OF REATTACHED ACL REMNANTS

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INTRODUCTION

A reattachment of the tibial remnant of the torn anterior cruciate ligament (ACL) to the posterior cruciate ligament (PCL) is sometimes observed during surgery and apparently implies that the human ACL does have a healing response. The aim of this study was to investigate whether this reattachment tissue has similar histological characteristics of a healing response as the medial collateral ligament (MCL) which can heal spontaneously. These characteristics include: disorganized collagen fiber orientation, increased neovascularization, voids (e.g. lipid vacuoles), increased number of myofibroblasts and elevated content of collagen type 3. (1) The hypothesis is that these typical histological characteristics are also present in the reattached ACL tissue.

METHODS

Informed consent was obtained from nine patients who underwent ACL reconstruction surgery. The reattached ACLs, which are normally discarded as surgical waste, were collected from five patients The tissue samples were embedded in Tissue-Tek, and directly frozen. 5µm thick sections were cut and stained with H&E and Lendrum Masson's trichrome to evaluate cell morphology, extracellular matrix and collagen fiber orientation. An AEC labeling immunohistochemical technique was used with the following antibodies: monoclonal mouse anti-human alpha-smooth muscle actin (α-SMA) antibody as a marker for myofibroblasts and monoclonal mouse anti-human collagen type 3 antibody to identify collagen type 3. Quantification of α-SMA expressing cells in the reattached tissue was performed with digital image analysis (DIA), as previously described. (2)

RESULTS

ACL remnants attached to the PCL were identified arthroscopically (n=5) (Figure 1). Microscopy showed that the collagen fibers in all 5 reattached tissue samples were disorganized with no preferred orientation (figures 2-3). All tissue samples showed areas of neovascularization and lipid vacuoles (figure 2). The cell nuclei tended to be more round than normal fibroblasts with inhomogeneous staining, suggesting that the cells were metabolically more active (figures 3-4). The mean number of cells in the tissue biopsies was 631 (± 269 s.d.) per mm^2; 68% (± 20% s.d.) was expressing α-SMA. Semi-quantitative analysis of collagen type 3 within the reattached tissue showed that collagen type 3 had an abundant expression (figure 5) with an average score of 3 (range 1-4).

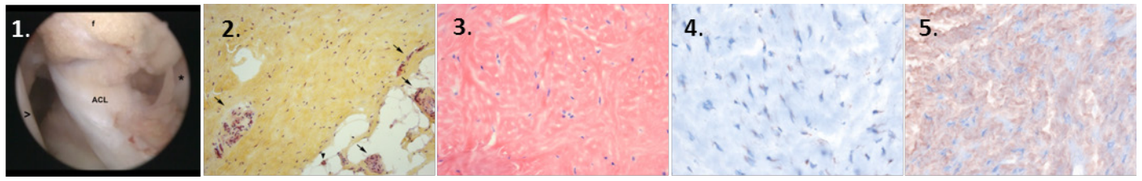

Figure 1. Arthroscopic view of the tibial ACL remnant reattached to the PCL; ">" indicates the area on the medial wall of the lateral condyle where normally the ACL inserts on the femur; "*"indicates the medial condyle. Figure 2. Example of Lendrum Masson Trichrome staining. The collagen fibers (stained in yellow) are disorganized. Arrows indicate capillaries. The arrowhead indicates an adipocyte. Also note the presence of lipid vacuoles, right bottom side. (x200) Figure 3. Example of H&E staining shows the scattered cells within the disorganized extracellular matrix. (x400) Figure 4. Example of the inhomogeneous haematoxylin staining and the presence of nucleoli. (x400) Figure 5. Example of collagen type 3 staining. High magnification shows the identification of red-brown staining of collagen type 3. (x400)

DISCUSSION

It can be concluded that the human ACL has a healing response with typical characteristics similar to that of the MCL. The results confirm the observational study of Lo et al. (3) and extend the conclusion by the new histological findings. With this finding and the recent findings of ACL healing in animal models, future translational studies are warranted with the aim to develop methods to repair the torn ACL in the acute phase.

ACKNOWLEDGEMENTS

The authors are grateful for the technical assistance of Maarten de Boer and the financial support of the Netherlands Organization for Scientific Research and the Marti-Keuning Eckhart foundation.

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THE DEVELOPMENT OF A NOVEL MAGNESIUM-BASED INTERFERENCE SCREW FOR ACL RECONSTRUCTION: A TIME-ZERO STUDY IN A GOAT MODEL

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INTRODUCTION

Recently, bioresorbable polymer interference screws have been favored to fix a replacement graft in the bone tunnel during ACL reconstruction in order to avoid issues associated with metallic interference screws. However, they could fracture during implantation [1], and they have poor osteointegration [3] as well as unpredictable degradation rates [2]. Thus, the objective of the current study was to develop a biodegradable metallic screw using a novel magnesium(Mg)-based alloy since it possesses superior mechanical properties and can promote osteointegration [4]. We hypothesized that the Mg-based interference screw could provide good fixation of the graft and thereby restore joint stability and graft function comparable to levels of the intact joint. To test this, we evaluated effectiveness of the Mg-based interference screw in maintaining the joint stability and graft function after being used to fix the ACL replacement graft at time zero.

METHODS

Six cadaveric goat stifle joints were tested. Using the robotic-UFS testing system, a 67 N anterior-posterior tibial load was applied to the joint in three states: intact, ACL-deficient, as well as after reconstruction with a Mg-based interference screw. The resulting joint stability as represented by anterior-posterior tibial translation (APTT) was recorded at 30, 60, and 90 degrees of flexion. Further, the in-situ forces in the ACL and the replacement graft were also obtained at these flexion angles [6].

Six additional stifle joints were used for tensile testing to evaluate structural properties of graft fixation. Following ACL reconstruction with a Mg-based interference screw (Mg screw group), all the soft tissues around the joint were removed, leaving a femur-graft-tibia complexe (FGTC). The FGTC was subjected to a load-to-failure test, and the stiffness, ultimate load and elongation at failure, and energy absorbed were obtained. As a control, a titanium screw was used to perform identical ACL reconstruction on six stifle joints (Ti screw group).

RESULTS

The APTT in the intact joint was between 4.8 mm and 5.5 mm at 30, 60, and 90 degrees of flexion (See **Figure 1A & Table 1**). This value increased by as much as 16 mm for the ACL-deficient joint. But, after reconstruction with a Mg-based interference screw, the APTT was reduced to within 1 mm of that of the intact joint. The in-situ force in the replacement graft was also found to be within 5 N of that of the intact ACL at all tested angles (See **Figure 1B &Table 1**, $p > 0.05$).

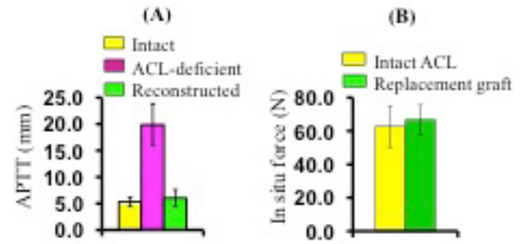

Figure 1. (A) APTT and (B) in-situ force in response to 67 N A-P tibial load at 30 degrees of flexion

The stiffness of the FGTC was 52 ± 6 N/mm for the Mg screw group and 50 ± 10 N/mm for the Ti screw group ($p > 0.05$). The ultimate load was 400 ± 135 N and 440 ± 109 N/mm, respectively ($p > 0.05$). Neither ultimate elongation nor energy absorbed was significantly different between the two groups ($p > 0.05$).

Table 1. Anterior-posterior tibial translation and in-situ force in response to 67 N anterior-posterior tibial load (*statistical significance from both intact and reconstructed)

Flexion angle (degree)	APTT (mm)			In-Situ Force (N)	
	Intact	ACL-deficient	Reconstructed	ACL	Graft
30	5.5 ± 0.9	17.9 ± 4.9*	6.1 ± 2.1	63 ± 13	67 ± 9
60	5.5 ± 0.9	17.5 ± 3.9*	6.2 ± 2.3	57 ± 6	60 ± 9
90	4.8 ± 0.8	15.6 ± 2.6*	5.5 ± 1.7	51 ± 6	51 ± 9

DISCUSSION

Results of the current study demonstrated that the novel magnesium-based interference screw could restore knee stability and ACL function close to levels of the intact knee, as well as provide good initial fixation of the replacement graft in a goat stifle joint, confirming our hypothesis. With these promising time zero results, *in vivo* studies are ongoing to evaluate its biomechanical performance as well as biocompatibility. It hope that a new class of novel biodegradable metallic materials could have clinical application in the future.

ACKNOWLEDGEMENTS

Financial support from NSF Engineering Research Center Grant (#0812348) and NIH T32 Biomechanics in Regenerative Medicine Predoctoral Fellowship is gratefully acknowledged.

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GENETICALLY ENGINEERED KUSABIRA-ORANGE TRANSGENIC PIGS AS IN *VIVO* MODEL TO INVESTIGATE BIOLOGICAL REMODELLING AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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INTRODUCTION

After ACL reconstruction, recruited cells from surrounding tissues play a crucial role in the graft remodeling processes for ligamentization. To allow athletes to return to their previous sports activity sooner, these processes should be elucidated and be accelerated. However, in conventional animal models, it has been difficult to differentiate donor and recipient cells. Here we introduce the transgenic Kusabira-Orange (KO) pigs, in which cells express fluorescence systemically, as a unique *in vivo* model to investigate the remodeling after ACL reconstruction.

METHODS

KO (n=22) and wild type (WT) pigs were allotted as a recipient and a donor, respectively. To validate KO pigs as an appropriate *in vivo* model of ACL reconstruction, the histology, mitogenic and migration cell activities were compared to those of WT pigs. The sensitivity and specificity of KO fluorescence were analyzed. The length change pattern in our ACL reconstruction was evaluated to validate the procedure. After allograft ACL reconstruction with fresh-frozen flexor digital tendon from WT pigs, histological analyses (HE, Masson trichrome, fluorescence, DAPI) were conducted at 3, 6, 12, and 24 weeks postoperatively.

RESULTS

The histology and mitogenic/migration assays did not demonstrate any statistically significant differences between KO and WT pigs. The sensitivity and specificity of KO fluorescence was 98%. Maximal length change of the reconstructed ACL was less than 3.5 mm. Three weeks postoperatively, host cells producing KO fluorescence repopulated mainly at the peripheral part of the graft, while cells also located in inter-territorial space of collagen fascicles interestingly. More cells migrated towards the mid of the graft in 6-12 w. Cell distribution became homogeneous 12-24 w. On the contrary, the donor graft matrix remained even 12 w postoperatively.

DISCUSSION

Here we demonstrated that KO pigs were efficacious *in vivo* model to examine biological remodeling after ACL reconstruction focusing on cell invasion and recruitment. The recipient cells could be easily differentiated from donor cells especially 3 weeks postoperatively, at which it has been occasionally confusing to differentiate necrotic nuclei from donor cells. Cells migrated into the inter-territorial region among collagen fascicles earlier than we expected. On the contrary, the matrix remodeling was rather slow compared with the cell recruitment and migration. We are going to investigate structural properties in parallel to these remodeling processes.

THE BLOOD- TENDON BARRIER- A NOVEL CLUE FOR TENDON PATHOLOGIES?

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INTRODUCTION

It is well known that tendons harbour a population of cells with a large differentiation potential[1]. These cells can differentiate into chondrocytes, osteoblasts and adipocytes. Degenerated tendons are not only characterized by a loss of fibre orientation, but also by the appearance of ectopic bone or cartilage as well as fatty infiltrations.
Therefore, a major challenge for understanding tendon pathologies such as tendinopathy is to obtain insight into the mechanisms that keep tendon cells in their differentiation status in intact tissue.
Along our attempts to characterize tendon cells we observed a population of perivascular cells expressing tendon- and neuronal stem cell associated markers[2]. Moreover we observed that tendon endothelial cells express the tight junction (TJ) associated marker Occludin. We therefore hypothesise that tendon vessels form a barrier between blood and tendon tissue, thus establishing a privileged microenvironment.

MATERIALS AND MEHODS

By immunohistochemistry, Lasermicrodissection, qRT-PCR and transmission electron microscopy (TEM) we examined human biceps and Palmaris longus tendons and mouse Achilles tendons for TJ associated markers and structures. In order to asses the functional integrity of a potential blood tendon barrier, mice were injected a biotinylated 10kD Dextran tracer.

RESULTS

The tight junction associated proteins ZO1, Occludin, Caudin3 and Claudin5 localize to the cell boundaries of tendon endothelial cells, as confirmed by confocal laser microscopy of isolated tendon vessels. We also show that these cells express mRNA encoding for Occludin, Claudin3 Claudin5 and Claudin12.
TEM revealed that tendon endothelial cells are non fenestrated and form tight junction-like structures. The dextran tracer was found to be trapped inside the tendon vessels 15 min after injection with no tracer diffusing into the tissue, whereas in the heart, a tissue with leaky vessels, the tracer leaks out.

DISCUSSION

With this work we show for the first time the existence of a blood-tendon barrier with remarkable similarities to other endothelial barriers such as the blood-brain barrier (BBB) or the blood-retina barrier. So far, in endothelial cells Claudin3 had only been described in brain and retina. Claudin-3 is a central component determining the integrity of the BBB, preventing immune cells from entering the tissue[3]. The observation that tendon endothelium is impermeable to a 10kD tracer proves the functionality of this barrier.
The finding of a blood-tendon barrier sheds new light on the composition of the tendon niche. Now the question arises, which blood borne factors really reach tendon cells in intact tendons. The role of this barrier in tendon de- and regeneration as well as well as its role for a potential immunoprivilege will be addressed by further studies.

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ESTABLISHMENT OF NOVEL TENOGENIC DIFFERENTIATION PROTOCOLS

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INTRODUCTION

The understanding of tendon growth, maintenance, and pathogenesis is to date incomplete thus hampering the development of effective treatments for chronic tendinopathy or acute tendon injury. Here we aimed at the establishment of a cellular tenogenic differentiation protocol, which allows for the elucidation of the molecular mechanisms governing tendon cell fate.

METHODS

We isolated primary cells by enzymatic digestion from mouse and rat tendons of different age, gender and anatomical sites. The cells were cultured in a series of cell culture media, mRNA was extracted and the induction of tenogenic differentiation was analyzed by real-time PCR. In addition, we investigated the impact of different growth factors, cell culture matrix/substrates and loading as potential tenogenic stimuli.

RESULTS

Under nearly all test conditions tendon-derived cells displayed a rapid decline in tendon marker expression compared to whole tendon tissue. Interestingly we observed however a robust induction of tendon gene expression upon usage of a chemically defined cell culture medium. Importantly we found that the induction of tendon markers was comparable between cells isolated from adult rat Achilles tendons and rat and mouse tail tendons of both genders. Only cells isolated from the mouse tail tendon postnatal stage (P8) were not responsive. Taken together our data indicate that we have established a relevant cellular tenogenic differentiation assay.

DISCUSSION

We are using this robust and relatively simple assay system now for the identification of novel tendon markers and for the elucidation of pathways that are relevant to tendon cell fate.

THYROID HORMONES RECEPTORS ARE PRESENT AND EFFECTIVE ON HEALTY AND RUPTURED ROTATOR CUFF TENDONS

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INTRODUCTION

Thyroid hormones (THs), T3 and T4, play an essential role in the development and metabolism of many tissues and organs, and have profound metabolic effects in adult life. Thyroid hormone receptors (TRs) seem to be ubiquitous, but, to our knowledge, their presence in tenocytes has not been investigated.

METHODS

We therefore evaluated the expression pattern of TRs isoforms in tenocytes from three groups of patients: one with rotator cuff tendons tears and with thyroid diseases, one with rotator cuff tendons tears without thyroid diseases, and one with healthy rotator cuff tendons and no thyroid disease. In addition, we evaluated the action of THs on growth and apoptosis of primary tenocyte cultures.

RESULTS

The α/β nuclear receptor isoforms are present in healthy and pathologic rotator cuff tendons. THs enhance the growth and counteract apoptosis in primary tenocyte cultures in a dose and time dependent manner.

CONCLUSIONS

This study confirm the increasing recognition of the prevalence of autoimmune thyroid diseases in patients with connective tissue disorder, highlighting a common mechanism for the pathogenesis of shoulder rotator cuff tears.

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THE EFFECT OF PROGRAMMABLE MECHANICAL STIMULATION ON TENDON HOMEOSTASIS AND TISSUE REPAIR IN A BIOREACTOR SYSTEM

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INTRODUCTION

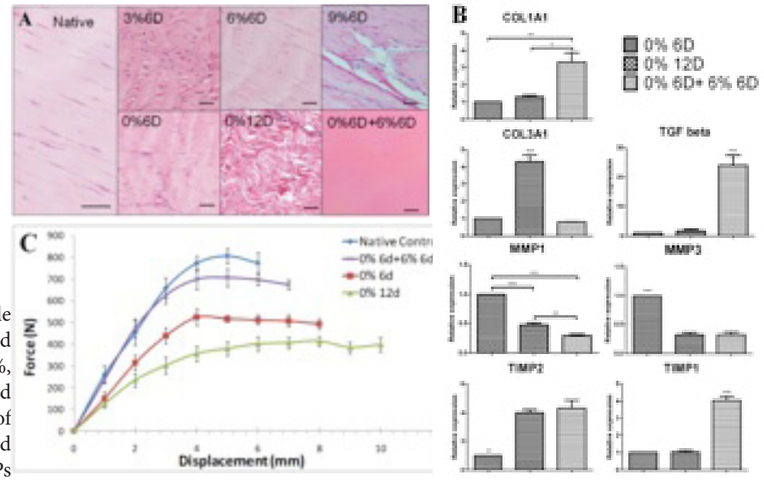
Mechanical stimulation has been identified as an essential factor for maintaining tendon homeostasis. Loading deprivation caused tendon tissue exhibiting pathological changes, including extracellular matrix degeneration, turnover and cell apoptosis. A bioreactor system with programmable mechanical stimulation (PMS) has been designed to mimic the *in vivo* loading conditions, and to define the impact of different cyclic tensile strain on tendon. We hypothesized that an optimal tensile loading regimen would be required to maintain the tendon homeostasis and promote tendon tissue repairing.

METHODS

To identify the optimal tensile loading regimen, full-length rabbit Achilles tendons were loaded in the bioreactor under different cyclic tensile loading program (0.25Hz for 8h/day, 0, 3, 6, or 9% for 6 days). Histological assessment, type III collagen immunohistochemistry, TUNEL assay, real-time PCR and biomechanical testing were preformed to evaluate the tendon structure and cell function. Furthermore, to examine the repairing effect of mechanical loading, tendons destructed by loading deprivation (0%, 6 days) were subjected to the optimal loading condition.

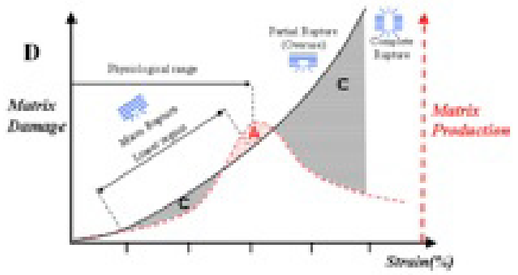
RESULTS

Tendons displayed various morphological changes when exposed to different mechanical loading conditions. Loading deprivation (0%), 3% and 9% cyclic tensile loading led to diverse degree of pathological changes such as disorientated collagen fiber (Fig. A) and cell apoptosis. However, 6% of cyclic tensile strain was able to maintain the structural integrity and cellular function, and thus identified as the optimal loading regimen for tendon homeostasis. Further experiments also revealed that 6% cyclic tensile loading (6 days) could repair the destructed tendon caused by loading deprivation (0%, 6 days), as evidenced by histology and mechanical testing (Fig. A, C). Expression of COL1A1, TGFb and TIMPs was upregulated by the 6% cyclic tensile loading, whilst MMPs was downregulated (Fig. C).



DISCUSSION

Our data indicated that there is only a narrow range of tensile strain could induce the anabolic action on tendon (Fig. D). Optimal PMS could maintain the tendon homeostasis, as well as regaining the tendon normality after damage. The finding suggested that precise mechanical stimulation is able to reverse early-stage tendon degradation, and thus have a therapeutic application on tendon disease. Clinically, Achilles tendon weakness after a period of immobilization has been reported, but no clinical rehabilitation guideline was found. Our study suggested that mechanical stimulation is able to improve tendon strength after immobilization. Optimized eccentric training program is needed to achieve maximum beneficial effects on chronic tendinopathy management



THE ROLE OF INTERLEUKIN-6 IN THE RESPONSE OF HUMAN HAMSTRINGS TENDON TO UNLOADING, LOADING AND OVERLOADING

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INTRODUCTION

Biological materials such as tendons must be able to maintain and adapt their matrix structure and mechanical properties in order to remain healthy and functional. While loading is known to be required for tendon tissue homeostasis, overloading has been thought to contribute to the initiation of tendinopathies. The aim of our study was to investigate the effect of defined loading or overloading regimes on the mechanical properties and gene expression of healthy human hamstrings tendon fascicles, with a particular focus on the inflammatory cytokine Interleukin-6 (IL6).

METHODS

Tendon fascicles were isolated from healthy human hamstrings tendon, harvested from patellar stabilisation surgery, and secured in custom made loading chambers (Legerlotz et al. 2013). Fascicles were subjected to a variety of different loading conditions, including unloading, different durations of moderate to heavy loading, static and cyclic strain. This was followed by either mechanical testing, or gene expression analysis by Taqman low density array (TLDA) and qRT-PCR.

RESULTS

Human hamstrings tendon fascicles seemed to be very fatigue resistant; even very high loads (60% of the strain at failure) did not induce a significant reduction in failure stress during 1800 loading cycles (Figure 1). The IL6 loading response was induced early, peaking at 3h of static strain (33-fold induction) and was shadowed by COX2 expression (14-fold induction) (Figure 2). Even very low levels of strain, such as securing the fascicle in the loading chamber without imposing any additional strain, were sufficient to induce IL6 expression compared to freshly dissected tissue (Figure 2 and 3). However, unloading and overloading (16% strain) both induced IL6 expression to a great extent. IL6 expression was lower under some loading (9% strain) than under unloading or overloading (Figure 3).

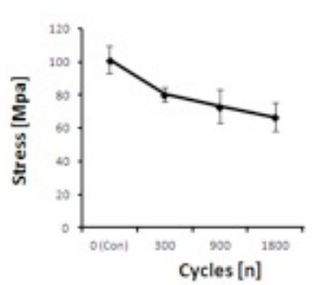

Figure 1: Failure stress of human hamstrings fascicles subjected quasi-static tests to failure after 0, 300, 900 or 1800 cycles to 14% strain at 1 Hz. Data are expressed as means±SE.

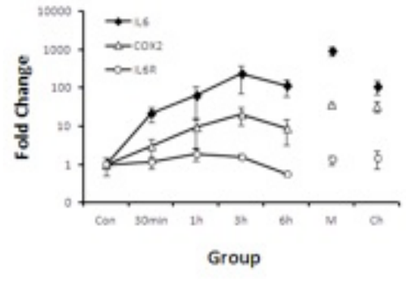

Figure 2: Time course of IL6, COX2 and IL6-receptor gene expression after 30min, 1h, 3h and 6h static strain to 9% compared to samples kept in medium for 6h (M) or secured in a loading chamber without applying any additional strain (Ch). Data are expressed as means±SE of fold change in relation to directly frozen control (Con)

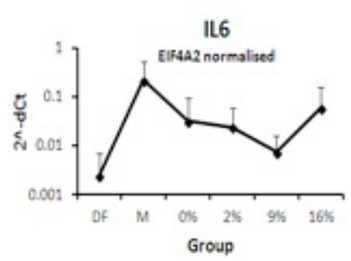

Figure 3: IL6 expression in samples directly frozen after dissection (DF), kept unloaded in medium for 3 h (M), or statically strained to 0%, 2%, 9% or 16% for 3h. Data are expressed as means±SE

DISCUSSION

We have shown that IL6 gene expression is upregulated with loading, and that only low levels of strain are needed to induce this response. Since IL6 is known to stimulate the synthesis of collagen, the main component of tendon matrix, IL6 might play an important role in tendon adaptation to exercise. However, high levels of IL6 experienced over an extended period of time could also be harmful for the tendon.

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CONTAMINATION RATE DURING ALLOGRAFT PROCUREMENT

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INTRODUCTION

Multiorgan and tissue donors offer a large quantity and high quality of allografts, but sterile recovery in an operating theatre is required for the collection of musculoskeletal allograft1,2,3. The aim of this study was to analyse factors contributing to bacteriological contamination of bone and tendon allograft and propose a recovery procedure protocol that can be used to reduce contamination and discarding allograft.

METHODS

Between January 2005 and December 2011 the bacteriological cultures of 3384 segments obtained from 218 multiorgan or tissue donors were analysed. For data interpretation multiple factors were taken into account: cause of death, time interval between death and tissue procurement, duration of the procurement procedure, number of team members, associated surgical procedures, positivity to haemoculture.

RESULTS

The incidence of graft contamination was 23% (SD 3.2) No micro-organism was found in 2143 donors (77%). Micro-organisms of low pathogenicity were cultured from 608 (27%) samples and micro-organism of high pathogenicity from 27 (1%). The most commonly isolated organism were Coagulase Negative Staphylococcus (60%), Propionibacterium spp (30%), Corynebacterium spp (10%). The iliac crest, the femoral head, and the achilles tendon presented the highest contamination incidence.

DISCUSSION

Significant difference in contamination incidence was observed with number of staff members performing the procurement. The cause of death, the procurement time, the duration of procurement, the associated surgical procedures were not associated with increased risk of contamination. Bone grafts were significantly more often contaminated compared to tendon grafts. Extensive handling, multiple incision might be affect the contamination rate. To overcome the risks of contamination, allograft procurement should be performed by a small and experienced surgical team.

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AUGMENTATION PROCEDURE FOR ANTERIOR CRUCIATE LIGAMENT INJURY

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Interest in double-bundle anterior cruciate ligament (ACL) reconstruction has been growing because of this technique's greater potential to restore normal knee kinematics. This method aims to reconstruct both the anteromedial (AM) and posterolateral (PL) bundles of the ACL to mimic the native ACL and its insertion sites. Several recent studies have shown that central anatomic single-bundle ACL reconstruction can also restore normal knee function. In this method, the openings of the femoral and tibial bone tunnels are carefully placed in the centers of their respective ACL footprints. Several clinical studies have been performed to compare the single-bundle and double-bundle techniques but have obtained conflicting results.

Restoration of normal biomechanical function is an essential goal of ACL reconstruction. However, early biological healing of the graft is vital to obtaining satisfactory clinical results. Accelerated biological healing of the graft is necessary not only for early return to sports activities but also for reliable remodeling of the grafted tendon. An augmentation technique for treatment of ACL injury has recently received attention from orthopedic surgeons because preservation of the ACL remnant has several potential advantages:

1. The ACL remnant may contribute to knee stability and guarantee mechanical strength in the early postoperative period.
2. With respect to the proprioceptive function of the knee, nerve fibers may originate from the preserved ACL remnant and regenerate mechanoreceptors around the augmented graft.
3. The ACL remnant may accelerate cellular proliferation and revascularization of the grafted tendon.

In 2000, we reported that the joint stability and proprioceptive function in 40 patients who underwent the ACL augmentation procedure were superior to those of 40 patients who underwent standard ACL reconstruction during the same period [4]. However, the early procedures needed 2 incisions and were not true reconstructions that restored normal ACL anatomy. Therefore, I started to perform the ACL augmentation one-incision technique for the partial rupture of the AM or PL bundle of the ACL, publishing first report in 2006 [1]. Later, we also showed that ACL augmentation using the one-incision technique significantly improved joint stability, the joint position sense, and the Lysholm score postoperatively in cases of partial ACL rupture [2]. In 2008, we started performing the ACL augmentation procedure even for patients with continuity of the ACL remnant between the tibia and the femur after complete rupture of the ACL. Central single-bundle or double-bundle ACL reconstruction with the remnant preserving technique was carried out for patients with a complete rupture [3, 5].

Although longer follow-up is necessary before a definitive conclusion can be reached, we believe that this technique is a valuable procedure.

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Poster Session

CONSIDERATIONS ABOUT PRP INJECTION TECHNIQUES IN TENDINOPATHIES

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INTRODUCTION

Several techniques have been suggested for PRP infiltration in tendinopathies (**1**). The use of local anesthetics is controversial, because PRP is sensitive to changes in pH and its addition may alter PRP effects by decreasing tenocyte proliferation and cell viability (**1**). PRP may be injected barely in the injured site, or with the peppering technique: in the first instance PRP is concentrated in the diseased area, whereas in the latter a more expansive zone of delivery can be achieved (**2**). Real-time ultrasound imaging is mandatory to visualize the proper needle position, and to display the precise anatomical location of PRP. Aim of this communication is to report the infiltration technique currently used in our laboratory.

METHODS

The technique, performed under sterile ultrasound control, is characterized by the following phases: **a)** a small amount of saline solution (8 mL), added to anesthetic (mepivacaine 2%), is injected between tendon and peritenon and in the subcutaneous tissue; **b)** after gentle needling, the injection of PRP (3-4 mL) is performed in the pathologic areas.

RESULTS

Phase **a)** Achilles tendon (**Figure 1**): a 22 gauges needle is positioned in the subcutaneous tissue and then between tendon and peritenon, which is unstuck by the solution.

Phase **b)** Achilles tendon (**Figure 2**): the needle is positioned in the hypoechoic areas and PRP is here delivered; it is a common occurrence the PRP spreading along the interfibrillary spaces.

Figure 1. Transverse scan: the solution injected (*) unsticks tendon and peritenon. AT= Achilles Tendon; arrows indicate the needle position.

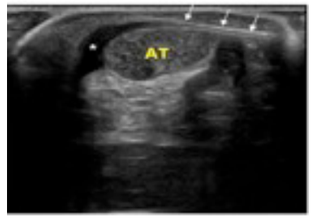
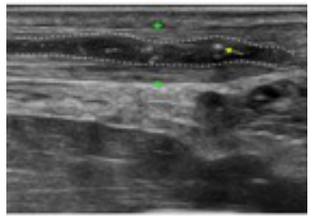


Figure 2. Longitudinal scan**:** PRP spreading is observed as hypo/hyperechoic material (dot lines). The tip of the needle is clearly seen (arrow).



DISCUSSION

This technique allows to minimize pain, without changing the milieu where PRP is administered, to disrupt the peritendinous adhesions, to damage pathological neovessels and accompanying nerves (**3**), and to reach the optimal concentration in the site of lesion. Moreover, it associates the PRP administration to needling, which disrupts fibrils and causes internal bleeding; this could reinforce its therapeutic activity. Obviously, the above quoted benefits are merely theoretical, and so further studies are necessary to establish whether this technique could allow better therapeutic responses.

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BILARETAL RE-RUPTURE OF QUADRICEPS TENDON TREATED WITH A SYNTETHIC SCAFFOLD AND PRP

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INTRODUCTION

The rupture and the re-rupture of the quadriceps tendon are quite rare occurances. This is a case-report of a bilateral re-rupture of the quadriceps tendon in a 47 year old man; he was a professional sportsman (squat) from 18 through 27, ten years later he underwent a bilateral rupture of the quadriceps tendon caused by a mild stress and treated with termino-termila suture, ten years later, at the age of 47 he underwent a bilateral re-rupture of the quadriceps tendon caused by a mild stress.

METHODS

We treated the rerupture with a termino terminal suture, reinforced with a mesh scaffold made of readily available synthetic degradable poly(urethane urea) material and stimulated the tissue formation with platelet-rich plasma gel.

RESULTS

The patient was asked to file the Lysholm scale and the VAS at 45, 60 and 90 days after surgery. The results are good, showing an optimal improvement in the function of the knee and a reduction of the pain that in fact was never distressing.

DISCUSSION

The termino terminal suture was made in full extension and quite difficult, making a reinforcement of the fragile resulting structure mandatory; that is the reason why we used the poly-urethane urea mesh. The degenerative nature of the first and second failure of both tendons is clearly deduced by the mild stresses that brought to the ruptures and the absence of symptoms before the events; that is the reason why we used the PRP gel.

The clinical safety and the biomechanical propertyes of the poly-urethane urea mesh are well documented in literature about tendon ruptures, mainly Achilles tendon ruptures, but we didn't find any reference regarding the use of this kind of augment in the treatment of the quadriceps tendon rupture.

Despite the literature uncertainty regarding the use of PRP, our experience in the treatment of other tendons rupture and tendinopathy, so as the well documented potential of PRP in tendon regeneration led us to stimulate with it the regeneration of a tissue that had proven to be unable to repair conveniently.

The good results reported are purely clinical and the follow-up is still short, but we are confident that the long term results, planned clinical and RM comparison, will be good too.

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SUBACROMIAL INFILTRATION WITH PRP: CLINICAL AND NMR EVALUATION AT 0 AND 3 MONTHS AFTER TREATMENT

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INTRODUCTION

An increasing number of people around the 5/6 decades of life presents partial tears (usually type b2 <50% the thickness of the tendon within the classification Sneyder) of the rotator cuff of the shoulder because of both work and sports. However, these persons generally need to continue with these activities, because the retirement age is far and not want to give up their favorite hobbies. Often an arthroscopic treatment to have only a debridement of the lesion or the completion of the same with subsequent suture to the bone often appear as an overtreatment and force the patient to a long period of inactivity for prolonged rehabilitation. Therefore was born the idea of subacromialis infiltrations with autologous PRP obtained from apheresis to reduce or eliminate the inflammation of tendons or, perhaps, to give a stimulus to tissue regeneration through angiogenesis that you get with growth factors.
In fact, despite the fact that the headset has in itself a valid arterial supply, the Over - stress resulting from use and the phenomenon of Wringing out (for which you leads to a reduction of the functional portion of the vasculature posterior superior tendon in adduction) cause their progressive rupture. The aim then was to check whether with simple infiltration PRP could improve both the clinical results and nmr results in rotator cuff partial lesions.

MATERIAL AND METHODS

Between the July 2012 and May 2013 we have treated 15 people (9 females and 6 males) aged under 60 years (to exclude phenomena of age-related degeneration) with 2 infiltration of autologous PRP on a weekly basis. We evaluated them with ASES score and CONSTANT score and with NMR, before and at 3 months after treatment.

RESULTS

Results of the two groups were then compared. They were statistically significant for: reduction of the level of inflammation, reduction of the level of pain, increased functionality.

CONCLUSIONS

PRP reduces pain after about 10 days, compared with pre-treatment values, accelerates a more rapid functional recovery and probably, promotes healing in the rotator cuff with minor injuries, most predisposed toward a biological therapy, rather than surgery.

PLATELET-RICH PLASMA-RELATED COMPLICATIONS IN ORTHOPAEDICS

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INTRODUCTION

Platelet-rich plasma (PRP) has been successfully applied in orthopaedics as bone and soft-tissue stimulating agent to promote positive tissue healing responses for the treatment of several orthopaedic conditions. However, controversy exists whether it should be routinely used in clinical practice.

METHODS

Since 2000 in our department PRP have been used in selected patients for the treatment of cartilage lesions and to shorten post-operative rehabilitation period after autologous hamstring ACL reconstruction. In addition PRP has been used for the treatment of delayed healing or the non-union of long bone fractures. We reported that PRP may accelerate the integration of the new ACL in the femoral and tibial tunnels, and patients were able to return to sport after a period of 3 to 4 months following ACL surgical reconstruction. Satisfying subjective and objective results were also reported in the treatment of cartilage lesions of the femoral condyles and the talar dome. Similarly, the use of PRP for the treatment of bone non-unions led to complete fracture healing.

RESULTS

Two patients which underwent ACL reconstruction complained symptoms of persistent swelling. They were further investigated and were diagnosed with synovitis in the knee and ankle joint. On patient which was treated for clavicle non-union cysts in the connective tissue surrounding long-bone. Histologic examination documented presence of hyperthrophic tissue reaction in all three cases.

DISCUSSION

PRP has demonstrated to promote positive tissue healing responses such as promotion of collagen production and enhancement of vascularity. However, PRP is not specific for one type of tissue, and can influence several cell types and organs in the body. Its local delivery, dosage and concentration should be strictly controlled. Further clinical trials are required to investigate on the efficacy, efficiency, and on the safety of PRP applications.

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MECHANICAL AND STRUCTURAL PROPERTIES OF THE MEDIAL PATELLOFEMORAL LIGAMENT

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INTRODUCTION

The Medial Patello-femoral Ligament (MPFL) is the primary static stabilizer of the patellofemoral joint. The MPFL restrains the patella during early flexion, and it's commonly injured during a first time lateral patellar dislocation [1,2]. The study of the biomechanical behavior of this ligament is essential to understand its role as joint stabilizer. For this reason the objective of this work is the description of the mechanical properties of the ligamentous tissue itself and the structural properties of femur-medial patellofemoral ligament-patella complex (FMPC) [3]. First, we hypothesized that the MPFL has a typical ligament's mechanical behavior, second that the anatomical orientation for tensile testing could influence the structural properties of the complex.

METHODS

Fifteen human fresh frozen isolated ligaments and fifteen femur-MPFL-patella complex were tensile tested. All the samples were left in a saline bath at 37°C for 30 minutes before the tensile test. The isolated ligaments were dissected to achieve the correct length-to-width aspect ratio (4:1) and a constant cross sectional area. The specimens were fixed with cyanoacrilate and sandpaper in a standard clamps and aligned to the 5kN load cell of an Instron 5965 materials testing machine. Four markers were used to evaluate the displacement with Vicon optical system. In order to reduce tissue hysteresis, the specimens were preloaded with a force of 1N and preconditioned by a series of ten cycles until reaching the strain of 3% with a strain rate of 0.1%/s. Then a tensile test was performed by extending the MPFL at 0.3%/s until failure. For the realization of the FMPC tensile test, the patella was mounted in anatomical position in a custom-made clamp attached to the load cell and the femur was rotated internally so that the line of posterior femoral condyle was 37±2° to the orizontal[4]. In order to reduce tissue hysteresis, the specimens were preloaded with a force of 2N and preconditioned by ten cycles between 0-2 mm of extension and then extended at 10 mm/min until failure. The anatomical orientation of the patella was achieved placing the MPFL and the patella with an angle of 90°.

RESULTS

The ultimate stress of the ligament was 16 MPa (SD 11), the ultimate strain was 24,3 % (SD 6,8) and the Young Modulus was 116 MPa (SD 95). All the isolated ligaments failed at the midsubstance.
The ultimate load of the FMPC was 145 N (SD 68), the ultimate elogantion was 6,4 mm (SD 1,6) and the linear stiffness was 36,3 N/mm (SD 14,1). Fourteen failure occurred at femoral attachment and only one at midsubstance.

DISCUSSION

This study evaluated the mechanical and structural properties of the medial patellofemoral ligament. The isolated ligament tests showed the typical stress-strain curve of a ligament.
The bone-ligament-bone complex tensile tests demonstrated that the orientation of the MPFL respect to the patella may affect the results of tensile testing. In the anatomical orientation, the fibers were uniformly loaded and the femoral attachment became the weakest link, which explains why it failed consistently at this position where the cross sectional area is thinnest. The study of the tensile behavior of the Medial Patello-femoral Ligament is fundamental to understand its contribution as primary stabilizer and for the selection of the methods of repair and reconstruction.

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IDENTIFYING KEY PROTEOGLYCANS IN DIFFERENT REGIONS OF CANINE CRANIAL CRUCIATE LIGAMENT

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INTRODUCTION

Cranial cruciate ligament disease (CCLD) and rupture is one of the most common orthopaedic conditions to affect dogs [1]. Once the cranial cruciate ligament (CCL) is damaged, complications can occur such as osteoarthritis [2]. The extracellular matrix (ECM) of ligament is composed of complex proteins such as collagen, elastin and proteoglycans (PGs) [3]. The small leucine rich proteoglycans (SLRPs) are one of the unique groups of PGs which are characterised by a leucine rich repeat structure. Decorin, biglycan, lumican, fibromodulin, keratocan and osteoglycin are all members of SLRPs that play an important role in tissue repair and development by regulating the ECM [4]. Our aim was to identify the expression of SLRPs in different regions in healthy CCL using SDS-PAGE and western blot analysis.

METHODS

Six paired CCL samples were divided into three sections (origin, middle and insertion), frozen in liquid nitrogen and stored at -80 C˚ for western blot analysis. Proteins were extracted twice with an extraction buffer of 4M Guanidine/HCL and dialysed against 0.1 M sodium acetate buffer, pH 6.8, consisting of proteinase inhibitors. Some dialysed extracts were further dialysed in ultrapure water, freeze dried and reconstituted in deglycosylation buffer (0.1 M Tris/ acetate, pH 8.0). These extracts were then treated with chondroitinase ABC (ChABC) (Sigma; 0.01 units/ 10 µg of GAG) to remove the glycosaminoglycan chains.

RESULTS

Analysis of SLRPs in CCL: All proteins were expressed in each region. Decorin and biglycan were visible at a distinct band around 50 kDa with evidence of a range of higher molecular weight isoforms 55-260 kDa. Single bands of fibromodulin and lumican were shown at the expected sizes of 59 kDa and 60 kDa. Lastly, keratocan and osteoglycin molecular weights were difficult to estimate due to their production of nonspecific multiple bands. **Effect of ChABC digestion on migration of decorin and biglycan in western blots:** Decorin and biglycan pre-digestion with chondroitinase ABC resulted in altered migration with biglycan forming a new band below 50 kDa that removed the polydisperse 55-260 kDa band. Decorin formed a duplet by migrating around 40-50 kDa by removing the band above 50 kDa. However, some high molecular weight decorin remained incompletely digested.

DISCUSSION

SLRPS play an important role in regulation of the ECM including fibrillogenesis, fibrosis and connective tissue remodelling. Western blot analysis identified SLRPS in the canine CCL. The heterogeneous distribution of both decorin and biglycan may indicates that there are GAG substitutions and other carbohydrates present and the dense band on fibromodulin and lumican may illustrate that they are keratan sulphate substituted. The multiple bands shown on osteoglycin and keratocan may be due to nonspecific binding of the antibody. Pre-digestion of decorin and biglycan with chondroitinase ABC showed that the molecular mass of decorin and biglycan were reduced under 50 kDa. Migration of both decorin and biglycan under 50 kDa suggests that their heterogeneous bands represent chondroitin/dermatan sulphate substitutes. Further investigation includes purifying glycosaminoglycans with the use of specific polysaccharide-degrading enzymes (e.g. keratanase). Finally, we will use densitometry to semi quantify proteoglycans in each region of CCL.

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MRI DEPICTION OF TRIPLE-BUNDLE STRUCTURE IN THE NATIVE HUMAN ACL

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INTRODUCTION

We reported that the anterior cruciate ligament (ACL) could macroscopically be divided into not only two but also three fiber bundles: the anteromedial bundle-medial part (AMM), anteromedial bundle-lateral part (AML) and posterolateral (PL) bundles[1]. However, the three fiber bundles have never been depicted with any imaging techniques. If these three bundles could be assessed individually with MRI, partial tear of ACL could be diagnosed more easily. Thus we tried to depict the three ACL bundles with 3-T ultra-high-field strength MRI.

MATERIALS AND METHODS

Twenty-four normal volunteers were examined using 3D isotropic sagittal MRI with the sequence of Coherent Oscillatory State acquisition for the Manipulation of Image Contrast (COSMIC) with fat suppression. Isotropic data from 3D-COSMIC allows for reformations in arbitrary planes, making multiple 2D acquisitions unnecessary. All images were evaluated on the GE MR workstation. The viewing plane was decided to maximally visualize the three bundles. One experienced musculoskeletal radiologist and one orthopaedic surgeon analyzed all images, and findings were reported by consensus of agreement. A 3D color-coded shell map was obtained to depict each fiber bundle.

RESULTS

ACL bundle anatomy was best depicted in the oblique axial plane at the center of the ACL in all knees (Fig.1). The three bundles were clearly observed in 22 of 24 knees (92%)(Fig.1, Fig.2). While the AMM and AML bundles were clearly depicted in all knees, the PL bundle was visualized only in 22 of 24 knees (92%).

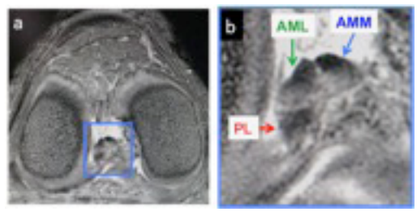

Fig. 1: a, The oblique axial plane of the right knee; **B**, AMM, AML and PL bundles of the ACL

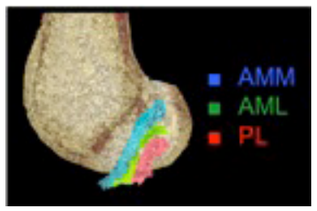

Fig. 2: 3D color-coded shell map

DISCUSSION

The current study is the first one to depict the three ACL bundles with MRI. The triple-bundle structure in the native ACL was distinguishably visualized with 3D-COSMIC isotropic imaging of 3-T MRI. With this depiction technique, the precise diagnosis of partial tear of ACL could be made. Thus selective reconstruction for the ruptured portion of the ligament with the intact portion preserved could be planned more consistently.

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SURGICAL TREATMENT OF COMBINED POSTERO LATERAL CORNER AND ACL INJURY

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INTRODUCTION

Injuries to the posterolateral corner are often associated with lesions of the central pivot; for this reason their clinical diagnosis is difficult and can easily be misunderstood, resulting in a residual instability that can threaten the survival of the reconstruction of the cruciate ligaments. The aim of this study was to evaluate retrospectively 30 patients who underwent ACL reconstruction combined PLC and to determine the quality of the clinical outcome of at least 5 years folllow up.

MATERIALS AND METHODS

We evaluated 30 patients who underwent combined ACL reconstruction and PLC between 2000 and 2005 at our hospital. In all patients, ACL reconstruction was performed using a single tunnel technique with semitendinosus and gracilis tendons or posterior tibial tendon from cadaver. The reconstruction of the PLC technique was performed by Larson modified gracilis tendon from a cadaver. In 12 patients underwent a meniscal suture of the meniscus with all-inside sutures. All patients were subjected to clinical evaluation in 1,3,6,12 and 24 months after surgery and then re-evaluated at a distance for the execution of this study with a mean follow-up of 7.2 years. The average age of the patients was 34.7 years at the time of surgery.

RESULTS

24 of 30 patients examined at follow up at a distance were clinically stable, did not report any subjective instability and were satisfied. 4 patients had laxity of I-II grade at Lachman test and a positive dial test (grade I). However, no reported subjective instability and were satisfied intervention. In 3 patients the reconstruction failed and was necessitated surgical revision of the ACL. 1 patient was excluded from the study because it incurred a complex fracture of the proximal tibia to 8 years after reconstruction.

DISCUSSION

The results of this study encourage the ligament reconstructions are present when combined posterolateral instability associated with lesions of the central pivot. We believe that the standardization of the diagnosis and treatments of these kind of injuries is very important. The choice of using a non-anatomical but isometric technique's is the great chance to have a more reproducible surgery technique with less complications and better clinical outcomes.

CONCLUSIONS

The diagnosis and surgery of combined instability of the knee are not easy thing. It is important to have a methodical approach from the clinical to the treatment aspects to recognize and repair these injury getting an optimal recovery of joint function.

BIOMECHANICAL COMPARISON BETWEEN ANATOMICAL RECTANGULAR TUNNEL AND ISOMETRIC ROUND TUNNEL ACL RECONSTRUCTIONS WITH A BONE-PATELLA TENDON BONE GRAFT

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INTRODUCTION

The objective of this study was to evaluate the effectiveness of the two ACL reconstruction procedures of with a bone-patellar tendon-bone (BTB) graft: the anatomical rectangular tunnel (ART) and the isometric round tunnel (IRT) ones. We hypothesized that the ART reconstruction technique was more biomechanically efficient than the conventional IRT reconstruction.

METHODS

Five fresh-frozen human cadaveric knees were mounted to the robotic knee joint simulator with the universal force-moment sensor, and then were passively flexed and extended under 6 DOF, while their 3-dimensional paths were recorded. The ART reconstruction was performed with the initial graft tension of 10N at 20°, while the knees underwent the same movement as before. The kinematics of the knees with an intact and a reconstructed ACL, as well as the *in situ* force in the intact ACL, were determined in response to external load. Then, the IRT reconstruction was performed to the same knee with the initial graft tension of 40N at 20°, while the knees underwent the same movement as before. The kinematics and the *in situ* force of the reconstructed knees were determined.

RESULTS

The both ART and IRT ACL reconstructions were successful in limiting anterior tibial translation under anterior tibial loads, while the initial tension to restore the normal A-P laxity (Laxity Match Pretension :LMP) of 40N in IRT reconstruction was greater and that of 10N in ART reconstruction. However, the increased initial tension in the IRT reconstruction lead to proximal, posterior, and lateral displacement of the tibia along with tibial external and valgus rotation.

DISCUSSION

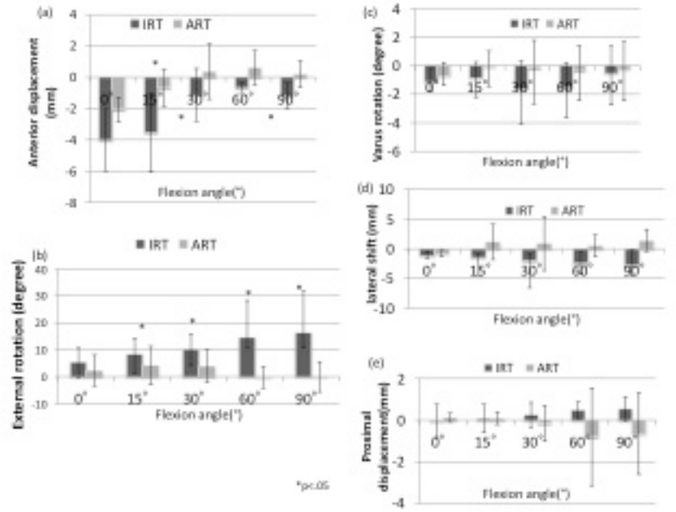
Taking it into account that the *in situ* force of the native ACL 20° at LMP is around 0N, and that the LMP in the ART procedure was much smaller, the reconstructed graft by ART reconstruction is much closer to the normal ACL.

CONCLUSION

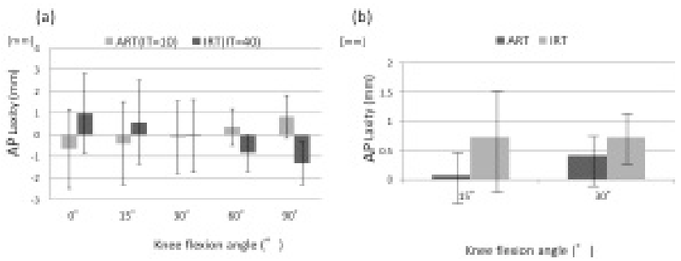
The anatomical rectangular tunnel (ART) ACL reconstruction is more efficacious to control anterior instability than the isometric round tunnel (IRT) one.

FIGURE

The position of the tibia in the ACL-R knee compared with that in the normal knee during flexion-extension test (a)-(e)



Anterior laxity under±100N of anterior tibial force (a), under combined rotatory load (b)



ACHILLES TENDON TEARS TREATED WITH L.A.R.S.

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The achilles tendon tears represent a debating problem between fautors of surgical and non surgical treatment. Whatever the treatment of choice, until now, the recovery need about 10-12 weeks, if we consider sport may need 6-12 month.
The L.A.R.S. (ligament advanced reinforcement system) made of polyester tereftalato, consist of a proximal part that must be fixed at the proximal miotenineus junction , a central third of free fibbers that must leaved into the lesion and a cylindrical distal part that must be fixed with an interference screw (30 mm length and 6 mm I diameter) into the calcaneal half tunnel. Between April 2004 and December 2008 we have treated surgically 40 achilles tendon lesions with an open end to end suture and lars augmentation.
Thank to the artificial augmentation , that give a strong stability, we let the patient weight bearing with a heel raising of 2,5 mm from the second week and total weight bearing from the fifth week. At a mean follow up of 6 years (min 5 max 9) we revised and evaluated all the patients with the ATTRS (Achilles tendon total rupture score). The results was excellent and good in all cases. We report a delayed healing of a wound treated for two weeks only with medication and we need to remove a screw for pain in 1 Patient. Ours results are similar to those of literature and we consider the augmentation with LARS a surgical option in the treatment of such lesion particular in case of high degenerated tendon, high demand patient when you need an augmentation.

EFFECT OF THE MATRIX ON MECHANICAL REGULATION IN HUMAN TENOCYTES

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INTRODUCTION

Tendinopathies are a range of diseases characterised by pain and insidious degeneration. Although poorly understood, onset is often associated with physical activity. We have previously investigated the regulation by mechanical strain of metalloproteinase gene expression in human tenocyte in a 3D collagen matrix. Integrins are important in cellular interaction with the ECM and are reported to mediate mechanotransduction in various non-tendon tissues. We have reported that TGFβ activation is a key player in the regulation of metalloproteinases in response to mechanical load, which may be mediated by integrins. This project aims to investigate the effect of cyclic loading and TGFβ stimulation on integrin expression by human tenocytes, in collagen and fibrin matrices.

METHODS

Human tenocytes were seeded at $1.5x10^6$ cells/ml into collagen (rat tail type I, 1mg/ml) or fibrin (fibrinogen 6mg/ml, Thrombin 0.2u/ml) gels and stretched using a sinusoidal waveform of 0-5% at 1Hz using the Flexcell FX4000T™ system. Cultures were treated with or without 1ng/ml TGFβ1 and load for 0-48 hours. Taqman Low density Array was used to asses a range of integrin, including ITGA1-6, ITGA10 and ITGA11 as well as ITGB1-5 (n=3). A cell based luciferase assay was used to measure active and total TGFβ.

RESULTS

In collagen cultures all integrins assayed were detectable (Ct < 35). ITGB1 was increased 2 fold with 48 hours of cyclic strain (p=0.006). ITGA6 and ITGA10 were decreased 1.4 and 2 fold with TGFβ treatment after 24 hours (p=0.019, p=0.006). ITGA3 and ITGB3 were significantly decreased 7.6 and 8.3 fold with TGFβ treatment after 48 hours (p=0.012, p=0.023). ITGA5 and ITGB1 showed similar responses with strain and TGFβ, i.e. an increased trend. However, the other integrins showed a dissimilar response to strain and TGFβ. Tenocytes seeded in collagen matrices showed an increase in active TGFβ in strained compared to non-strained conditions. However, tenocytes seeded into fibrin gels did not produce the same response.

DISCUSSION

We have shown that integrin mRNA expression is regulated by the application of mechanical load. This indicates that mechanical loading may modify cell sensitivity to perceive further load through increased interaction with the ECM. Any differences in the cellular response to load in collagen and fibrin cultures, indicates that cellular interaction with the ECM is an important factor in the detection of load.

Due to the differential regulation of some of the integrins with strain and TGFβ, it appears that TGFβ may not be responsible for the regulation of all integrins with strain. However this remains unconfirmed and may be explained by a temporal difference. Further analysis of how integrins are regulated in response to mechanical load and how this expression is translated to the protein level is required.

Due to the absence of the activation of TGFβ in response to strain in fibrin cultures it appears that TGFβ activation requires interaction with type I collagen in order to trigger activation. This could be a consequence of direct cellular interaction with the collagen matrix resulting in downstream activation of TGFβ.

EFFECT PF THE MATRIX ON THE FOOT BIOMECHANICS IN THE AETIOLOGY OF ACHILLES TENDINOPATHY

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INTRODUCTION

In recent years many studies have focused on Achilles tendinopathy. Repetitive and excessive stretching of the Achilles tendon may be considered the primary stimulus of tendinosis.
We asked whether the overstretching of the Achilles tendon was related to foot structure specifically to arch height.
Our aim was to investigate the foot structure in patients with Achilles tendinopathy and to explain how a specific foot structure can lead to Achilles tendinopathy.

METHODS

From 2007 to 2012 we have analyzed 380 patients with Achilles tendinopathy over 50 years old.
For the diagnosis of this disease, we performed for each patient a physical examination and an ultrasound exam of the bilateral tendons measuring proximal and distal tendon diameters and assessing for the presence of hypoechoic change, intratendon defects, calcification and neovascularity.
We performed a baropodometric and X ray exam of the feet to identify the type of anatomical structure.

RESULTS

Ultrasound abnormalities were common among patients, both male and female, and in bilateral Achilles tendons of each patient. Disabling tendon-related symptoms developed in 80 patients. The moderate or severe hypoechoic defects were showed in 12% of asymptomatic tendons. All patients had cavus feet and we found that the feet with Achilles tendinopathy were higher arch structure than normal feet.

DISCUSSION

In this study, the ultrasound abnormalities were common in Achilles tendinopathy.
Also, the asymptomatic tendons showed the tendinosis defects. We found a significant relationship between Achilles tendinosis and the foot structure. The cavus foot is an anatomical factor that may be related to greater risk of Achilles tendinosis. Likely, there is the relationship between the overstretching of Achilles tendon, cavus foot and tendinosis.
We believe that the repeated and/or prolonged tendon strain can lead to degenerative changes that exceeds the reparative ability of the tendon. Also, we believe that rebalancing the cavus foot biomechanics, the tendinopathy can heal.

FIXATION OF ACUTE DISTAL BICEPS TENDON RUPTURES USING MITEK ANCHORS A RETROSPECTIVE STUDY

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INTRODUCTION

Ruptures of the distal biceps tendon are relatively uncommon with an incidence of 1.2 per 100000 persons. These ruptures are usually seen in the dominant arm of men between 40 and 60 years of age who underwent a traumatic event in which an unexpected extension force is applied to an elbow in flexion.

Several surgical techniques were described for the repair of the distal biceps brachi tendon and, to date, there is no consensus as to which surgical technique should be used best.

Seven consecutive patients between July 2005 and November 2011 underwent a distal biceps tendon repair using Mitek suture anchors.

METHODS

Functional results were assessed using range of motion measurements and the Disabilities of the Arm, Schoulder and Hand (DASH) questionnaire and range of motion measurement. The DASH questionnaire is a self-administered region-specific outcome instrument developed as a measure of self rated upper extremity disability and symptoms. The DASH consists of a 30-item disability/ symptom scale, scored 0 (no disability) to 100 (severe disability).

Detailed anatomical illustrations are made for the visual understanding of the operative technique.

OPERATIVE TECHNIQUE

The patients were operated in a supine position under general anaesthesia and a tourniquet of the upper limb. A lazy ''S" shaped single incision over the antecubital fossa is made and the torn biceps tendon is identified. Preparation is continued along the natural tunnell of the tendon to the radial tuberosity. Four unicortical holes are made at the site of the footprint followed by creating a hatch in the first bone cortex between the four holes using an osteotome. Drillholes are made in the opposite cortex sing the Mitek drill bit and the Mitek anchors are placed through the holes in the opposite cortex. Finally the tendon is fixed in the hole by knotting the sutures according to Bunell's technique and the skin is closed in layers.

Post operatively, the elbow was immobilised in a plaster in 90 degrees flexion for 5 weeks. Hereafter active, low demand exercises were started. After 3 months, loading the tendon-bone complex was gradually increased. 6 months post-operatively full loading of the elbow was permitted.

RESULTS

The average duration of surgery was 65 minutes (median 63, range 51-80). Five patients have been discharged on the same day of surgery. The two remaining patients were discharged one or two days following the day of surgery respectively.

Six patients have fully completed and returned the DASH questionnaire. Three patients had a DASH score of 0, one patient a DASH score of 5.8 and 2 patients a DASH score of 10. An MRI of the elbow of 4 out of 4 patients showed full integration of the tendon in the radial tuberosity with no heterotophic ossification.

CONCLUSIONS

It is a successful method for integrating a tendon into bone, as has been shown in ACL reconstruction surgery. Functional results are uniformly good or excellent, with all patients achieving return of strength.

Limitations of this study include the small sample size due to the low incidence of distal biceps tendon ruptures, preventing solid statistical conclusions.

INFLUENCE OF INTRAMUSCULAR INJECTION OF PLATELET-RICH PLASMA IN SERUM CONCENTRATIONS OF IGF-I AND CRP CONCENTRATIONS IN DOGS

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INTRODUCTION

The clinical use of PRP intramuscular application was prohibited by WADA due to the suspicion that these factors can produce systemic effects in Insulin-like growth factor I (IGF-I) concentrations. In addition, it is not known if the administration of PRGF can have any influence on the inflammatory state of the organism.

Studies suggest that the anabolic effect of IGF-I is not present in preparations of platelet-rich plasma because the isoform IGF-IE_a (produced in the liver) and present in this preparation, has no anabolic effect, unlike the IGF-IE_c isoform derived from muscle which is not present in this preparation (Matheny y Nindl, 2011).

The purpose of this study was to evaluate the influence of local PRGF® injection in healthy muscle on serum concentrations of IGF-1 and CRP (as a marker of inflammation) in the canine species.

METHODS

*A p*rospective crossover study design was performed. Eight Beagles were injected with 3 different preparations in healthy left lumbar muscles:

A) Treatment 1, Placebo, (PCB): 1 ml of saline (SS) with 0.05 ml Calcium Chloride (Ca_2Cl).

B) Treatment 2, Plasma Rich in Growth Factors (PRGF): Regular doses (1ml) of Plasma Rich in Growth Factors (PRGF®) with 0.05 ml Ca_2Cl.

C) Treatment 3 High Doses Plasma Rich in Growth Factors (HPRGF): (3 ml) of PRGF® (high dose) with 0.15 ml Ca_2Cl.

In each of the treatments, blood samples were obtained by jugular venipuncture at baseline and 1', 15', 30', 1h, 6h, 12h, 24h, 3, 7, 14, 21, 28, 35, 42, 49, and 56 days post injection. The treatments were applied in the following order: first a, then b and last c, with a month break between them. IGF-I was analyzed by automated immunoassay system and CRP was determined through an immunoturbidimetric assay, both validated in dogs. To assess the muscle dimensions and the response to PRGF infiltration, ultrasound and TC studies were performed. The ultrasounds were done at baseline (before injection) and at 1', 7, 14, 21, 28, 35, 42, 49 and 56 days after inoculation and after obtaining the blood samples. In all the data, normality was assessed and log transformed if necessary. For each treatment comparison of means of CRP and IGF-I over time was analyzed with an ANOVA and *post hoc Tukey test*. A Kendall's Tau-b correlation test was performed. To assess the PRGF quality, the concentration of IGF-I was studied and then compared to blood and PRGF with an ANOVA repeated measures.

RESULTS AND DISCUSSION

When IGF-I and CRP were compared throughout the procedure, there were no differences between times in the three protocols (Figure 1 and 2), and there was a significant negative correlation between them.

Comparing the muscle area between the infiltrated and non- infiltrated sides of the three groups in the days of study, there were no significant changes in muscle size on both sides, after intramuscular injection. When compared the time evolution of the muscle area, in each of the sides (right/not infiltrated and left/infiltrated) and between the study groups over time, there were no statistically significant differences. We can conclude that IGF-I and CRP concentrations do not increase at a systemic level after intramuscular injection of PRGF, and the muscular area keeps constant, without increasing in size. There is no anabolic effect with the use of this product intramuscularly.

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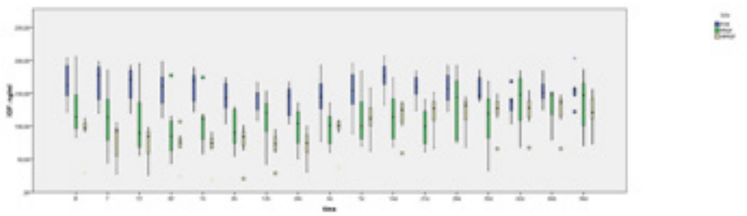

Figure 1: Evolution on time of IGF-I serum levels in the three groups.

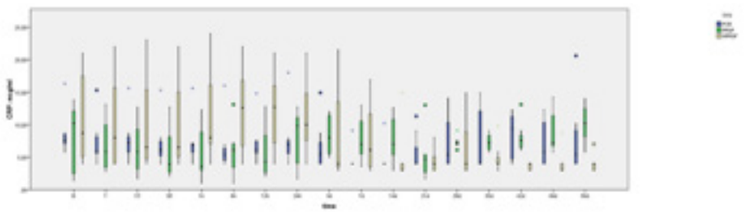

Figure 2: Evolution on time of CRP serum levels in the three groups

SCAFFOLD AND GRWOTH FACTORS IN THE TREATMENT OF THE ACUTE LESION OF ANTERIOR TALO-FIBULAR LIGAMENT

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INTRODUCTION

The incidence of ankle sprains has been estimated at 1 per 10.000 persons per day and it is considered one of the most common musculoskeletal injuries, comprising over 25% of all sports related trauma .85% of this lesions involve the lateral ligaments .Although most patients fully recover with nonsurgical treatment and physical rehabilitation, symptomatic chronic instability (C.A.I. Chronic Ankle Instability)is found in 20% to 40% of patients. C.A.I. is defined as repetitive bouts of lateral ankle instability resulting in numerous ankle sprains **;** 72% of people with C.A.I. is unable to return to their previous level of activity because a decreasing the level of exercise, change in type of sport, and withdrawing from occupational activity . In addition activity limitation is not confined to the injured limb; problems have been reported in the controlateral ankle of 85% of people who develop C.A.I. after unilateral sprain. Which are the reasons of C.A.I. or we can better say : which mechanisms are changed as far as to produce C.A.I. ?The mechanisms changed are the ankle movements , the decreasing of the reaction speed of peroneal muscles to a inversion stress and the variation of the proprioception due to ligament lesion. To explane why these altereted mechanisms may lead to a CAI we may imagine an example:

1. Ankle sprain with a lesion of the talo-fibular ligament
2. Alteration of the faster proprioception derived from the ligament
3. Istantaneous instability
4. Changement of the ankle movement
5. Low recovery of proprioception thanks to extra ligament receptors
6. The changement of the talus movementis so big that the extra ligaments remain not- calibrated and they are not successful to balance the lack of ligaments receptors ! So we can say that the distorsion of the refined system (proprioception) would be balanced by extra ligament receptors but the distorsion of the rough system (movement alteration) may cause the distorsion of the balance system.

To prevent the occurrence of C.A.I. and to give a rapid recover to the patients with a talofibular lesion a new surgical procedure has been developed.

METHODS

A series of 18 consecutive patients (age 20-45) with 20 (2 bilateral) complete talo-fibular lesion were treated with a new surgical procedure. With minimally –invasive technique a biologic augmentation (ARTELON® Tissue Reinforcement) soaked with autologous PRP was fixed by two anchors to duplicate the talo-fibular ligament. All patients walk immediately with only a compressive and elastic bandage. The clinical follow up was made after 1-3-6 months with **the Foot & Ankle Disability Index (FADI) Score and, if possible , with the Foot & Ankle Disability Index (FADI) Score - Sports Module (12 patiens for 13 procedures). After 6 months a Nuclear Magnetic Resonance and a scan have been made. A rapid rehabilitation protocol began after 8 days from the procedure.**

RESULTS

Clinical evaluation showed improvement of FADI score from 86±4,9 to 93±5,3 after 1 months to 96,4±2,2 after 3 months 98±2 after 6 months; the FADI sport score raised from 71,2±12 to82,3±3 after 1 months, to 88,5±2,5 after 3 months and 97±2,3 after 6 months. The NMR and the scan after 6 months showed a good reconstruction of the ligament and the presence of the augmentation.

DISCUSSION

The high incidence of C.A.I. has drive us to search a procedure to control this problem. The new technique is mini- invasive, easy to do, and permit a rapid rehabilitation. In this first sequence we did not find any surgical complications, or recurrent sprain. All the patients restart sport after 30 days after surgery. Our study is associated with a number of limitations. First, this work is a non-controlled single cohort observational case report study of 18 patients with evaluation of the clinical efficacy and safety of this new technique. Cost-effectiveness was not evaluated. Second, the clinical data are limited to a 6 months clinical follow-up, thus lacking a long-term evaluation, since patients do not reach a longer follow-up so far. Durability of the repaired ligament and the potential to reduce subsequent CAI in a long a future perspective are unknown. However long-term, controlled studies and expanded methodology are needed to further evaluate this promising treatment method.

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ISL&T
International Symposium on LIGAMENTS AND TENDONS XIII

Friday, 18th October 2013
Italy, Tuscany, Arezzo

Arezzo Fiere e Congressi – Via L. Spallanzani, 23

07.00-08.00	Registration and Breakfast
08.00-08.15	Opening Ceremony ***S.L-Y. Woo, G. Cerulli***
08.15-09.09	**1st Session: Part I** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***S.L-Y. Woo, M.N. Doral***
09.09-10.09	**1st Session: Part II** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***G. Cerulli, C. Kuo***
10.09-10.39	**Coffee Break & Poster Session** Session Chairs: ***P. Renström, S. Zaffagnini***
10.39-11.48	**1st Session: Part III** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***K. Shino, F. Vercillo***
11.48-12.48	**1st Session: Part IV** **Biology and Biomechanics of Ligaments and Tendons** Session Chairs: ***R. Vanderby, G.M. Peretti***
12.48-13.50	*Light Lunch*
13.50-14.44	**2nd Session:** **Biological Augmentation (Stem Cells, PRP) for Tendon and Ligament Healing** Session Chairs: ***A. Banes, S. Bruè***
14.44-15.44	**3rd Session: Part I** **Tissue Engineering of Tendon and Ligament** Session Chairs: ***T. Wang, G. Zamarra***
15.44-16.23	**3rd Session: Part II** **Tissue Engineering of Tendon and Ligament** Session Chairs: ***A. Georgoulis, B. Innocenti***
16.23-16.53	*Coffee Break*
16.53-17.32	**3rd Session: Part III** **Tissue Engineering of Tendon and Ligament** Session Chairs: ***M. Handl, G. Riley***
17.47-18.02	Closing Remarks ***G. Cerulli, S.L-Y. Woo***
20.00	Gala Dinner & Award Ceremony at Arezzo Fiere e Congressi