International Symposium on Ligaments and Tendons-X

The Chinese University of Hong Kong

HONG KONG

February 5-6, 2010

Edited by: **Kai Ming Chan, MD**
Savio L-Y. Woo, PhD, Dsc, DEng
Pauline P.Y. Lui, PhD
Chih Hwa Chen, MD

Volume 10

Table of Contents

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Symposium Sponsors

We gratefully acknowledge our kind sponsors. They contribute to the greatness of the meeting.

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Welcoming Note by Prof. Savio L-Y. Woo

Welcome!

It is my pleasure and honor to join Professor K.M. Chan to welcome you to Hong Kong for the Tenth International Symposium on Ligaments and Tendons (ISL&T-X)!

The first ISL&T meeting took place over a decade ago. Our mission remains to bring together researchers with diverse backgrounds to present and discuss new developments and important topics related to ligaments and tendons. This annual symposium is a place where graduate students as well as junior and senior level biologists, engineers and clinicians can exchange ideas freely, learn from one another, and establish collaborations.

As ISL&T is maturing, it is crucial for us to move onto next stage and bring the meeting outside of the U.S. and on the international stage for all to enjoy and participate. I'd like to thank Prof. Chan for taking on the challenge and bringing the first ever ISL&T to Asia. His able organizing committee has also done a wonderful job in keeping with the traditions of ISL&T. Further, they have added their own unique mark to the meeting by including an extra half-day for an education session, research and professional development, plus a special session on tendinopathy. As the ISL&T is so dear to my heart, I am truly delighted that we can have a wonderful one and a half days to be amongst good friends and colleagues.

Last year, we formed the International Advisory Committee (IAC) for ISL&T. This is a body of experts of ligaments and tendons, which has been charged to govern this organization. The IAC will develop new strategies to move the field forward, including the formation of a society as well as creating an association to a high quality international journal.

I trust that you will all enjoy the beautiful scientific program that was put together by our Program Co-Chairs, Dr. Pauline Lui and Prof. Chih-Hwa Chen. Please participate by actively sharing your ideas and new data, as well as asking a lot of questions. I also wish to congratulate all our younger colleagues who are finalists for the various awards as you are representing the best of the best! A special acknowledgement must also be given to Dr. and Mrs. Al Banes of Flexcell International for their generous support of the ISL&T awards over the years!

I'm also happy to announce that the next ISL&T meeting will take place in Long Beach, California on January 12, 2011, a day before the ORS, and our host will be Dr. Thay Lee of the University of California, Irvine. Please mark your calendar and plan to attend!

Finally, we are all very thankful for the most generous hospitality of Prof. Chan and his great team, as well as to our sponsors for their support of this meeting.

Please enjoy the day!

Savio L-Y. Woo, Ph.D., D.Sc. (Hon.), D.Eng. (Hon.)

Welcoming Note by Prof. Kai Ming Chan

Dear Friends and Colleagues,

Welcome to Hong Kong - our vibrant and exciting city!

We take great pride to host the ISL&T-X in Hong Kong, the very first one outside the US. This is an extraordinary honor and privilege.

I would like to thank the entire Planning Committee and International Program Committee, particularly the guidance of Prof. Savio L-Y. Woo and the able leadership of the Program Chairs, Prof. Chih Hwa Chen and Prof. Pauline Lui. We are expecting more than 120 participants from 15 countries/ regions. This is a truly international meeting with a galaxy of expertise.

The program highlights the following:

1. Tendinopathy-Pathogenesis and Treatment
2. Tendon Development and Tendon Cell Differentiation
3. Translational Research for Tendons and Ligaments
4. Functional Tissue Engineering and Repair of Ligaments and Tendons
5. Tissue Mechanics
6. ACL Reconstruction-Biology of Healing and In Vivo Knee Kinematics

Following the tradition of ISL&T, we will encourage interaction amongst the podium and poster presenters and the audience in a lively atmosphere that we hope will generate new ideas, innovations and continuing networking. We do hope that the ISL&T-X in Hong Kong will showcase a model how this initiative from the US would be radiated to the rest of the world with additional vigor. We are most excited to maintain this momentum to bring ligament and tendon research into the limelight of attention in the clinical and the research arena.

The Bone and Joint Decade 2000-2010 will be coming to a concluding chapter soon. There are intense discussions to extend the Bone and Joint Decade to a new era with different emphases and formats. Certainly with the deeper understanding of the burden of disease, the socio-economic impact, the clinical and research potential, tendon and ligament will be high on the agenda. ISL&T-X will be a major impetus in bringing it to international prominence.

With the backdrop of the coming Chinese New Year of the Tiger, we hope you will take time to enjoy the superb hospitality and the glamorous festivity around. I wish you a most enjoyable stay in Hong Kong.

Prof. KM Chan
Chair, Planning Committee, ISL&T-X

General Information of ISL&T-X

AIMS OF THE SYMPOSIUM

The International Symposium on Ligaments & Tendons provides a forum to discuss state-of-the-art ligament and tendon research. By bringing together leaders as well as budding investigators in our field, we hope to address challenging problems in clinical management of ligament and tendon injuries, and set new directions in biomechanical and biological research that hold great potential for the future.

● PLANNING COMMITTEE

Kai Ming Chan, MD, Chair
Pauline P. Y. Lui, PhD
Ling Qin, PhD
Daniel Fong, PhD
Francis Chan, PhD
Savio L-Y. Woo, PhD, DSc
Leung Kim Hung, MD
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Rui Liang, MD
Gabriel Ng, PhD
Chih Hwa Chen, MD
Gang Li, PhD
Diann Decenzo, MS
Matthew B. Fisher, BS
Patrick S.H. Yung, MD

● INTERNATIONAL PROGRAM COMMITTEE

Chin Hwa Chen, MD, Co-chair

Pauline P.Y. Lui, PhD, Co-chair

Committee Members

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Hiromichi Fujie, PhD (Asia)
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Ryosuke Kuroda, MD (Asia)
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Masahiro Kurosaka, MD (Asia)
Gang Li, PhD (Asia)
Zong Ming Li, PhD (North America)
Gabriel Ng, PhD (Asia)
Hongwei Ouyang, MD (Asia)
Graham P. Riley, PhD (Europe)
Hirotaka Sano, MD (Asia)
Louis Soslowsky, PhD (North America)
Ting Ting Tang, PhD (Asia)
Gail M. Thornton, PhD (North America)
Michael Torry, PhD (North America)
Vincent Wang, PhD (North America)
Shinichi Yoshiya, MD (Asia)
Ming Hao Zheng, PhD (Asia)

- **INTERNATIONAL ADVISORY COMMITTEE**

Instructions to Presenters

I. Podium Presenters

To encourage discussion, the presentation time is limited. The presentation formats are listed below. Your moderators have been asked to adhere to the time allotted for your presentation as well as the number of slides.

Important: Pleae report to our IT panel 15 minutes before your session. You have to double check your presentation with our technicians. And please be seated at the designated areas at least 10 minutes before your presentation.

Presentation Requirements

For 15 minute time slots

◊ 10 min. presentations each immediately followed by a 5 min. discussion.

◊ Maximum **10 PowerPoint slides** for presentation**.**

For 5 minute time slots

◊ 5 min. presentations followed by a 5 min. group discussion of 2-3 papers.

◊ Maximum **5 PowerPoint slides** for presentation.

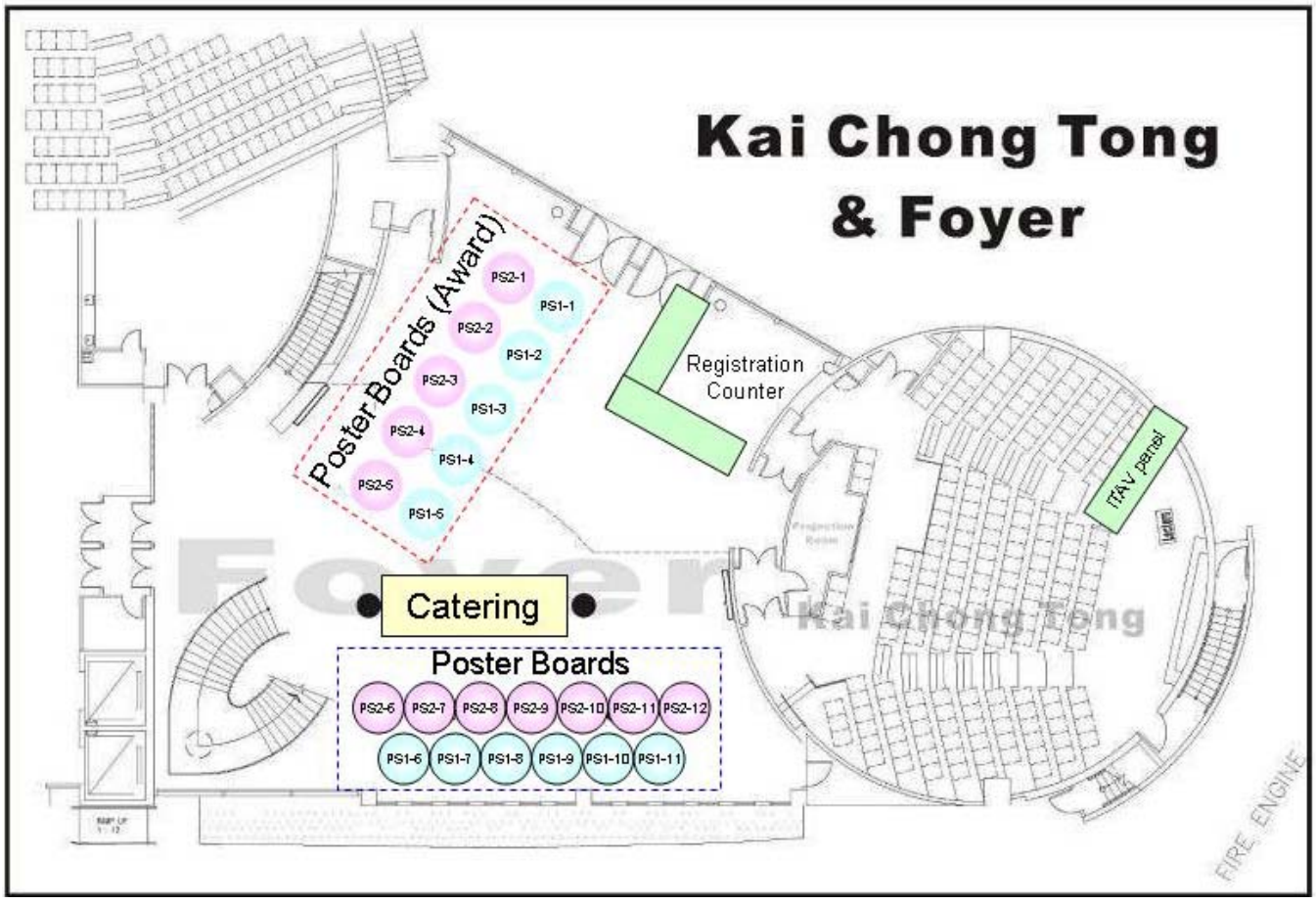
Note: In view of time and the large number of talks, there will be no opportunity to use your personal computer or load your PowerPoint file during the symposium.

Instructions to Presenters

II. Poster Presenters

All posters should be no larger than 155cm (Height) x 114cm (Width). Poster boards will be available in the lobby. Your poster space is indicated by the corresponding code in the program book. Please set up your poster between 8:00 - 8:30am and take it down between 5:45 – 6:15pm.

Note: Please stand next to your poster in case other participants would like to ask questions about your poster. Candidates of Best Poster Award are given 2 minutes of oral presentation at poster sessions. The poster moderators will coordinate the sessions. Please attend the session at the assigned time.



ISL&T-X Awards

It is always our honor to have great papers presented at ISL&T-X. We established awards to stimulate high quality scientific research by investigators in the study of ligaments and tendons. The awardees are selected by members of the program committee based on the quality of the abstract and presentation as well as the overall merit of the study.
To acknowledge the work done by students, fellows, and young faculties, we have the following awards:

Best Student Paper Award (up to 2)
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 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.
- **Selection Criteria** Applicant's abstract submitted for the ISL&T-X 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. Result announcement will be made at the banquet.
- **Acknowledgements** Sponsored by Flexcell International Corporation and Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong.

Best Research Fellow Paper Award
Award: USD$200 and Certificate

- **Ligibility** Open to clinical fellows or post-doctoral fellows. Applicant must be the first author of the abstract and be present at the ISL&T meeting 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 the award.
- **Selection Criteria** Applicant's abstract submitted for the ISL&T-X 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. Result announcement will be made at the banquet.
- **Acknowledgements** Sponsored by Flexcell International Corporation.

Best Poster Award
Award: USD$200 and Certificate

- **Eligibility** Open to all participants of poster presentation. Applicant must be the first author of the abstract and be present at the ISL&T meeting 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 the award.
- **Selection Criteria** Applicant's abstract submitted for the ISL&T-X 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. Result announcement will be made at the banquet.
- **Acknowledgements** Sponsored by Flexcell International Corporation.

Young Faculty Paper Award (NEW!)
Award: USD$200 and Certificate

- **Eligibility** Open to faculty aged under 40. Applicant must be the first author of the abstract and be present at the ISL&T meeting 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 the award.
- **Selection Criteria** Applicant's abstract submitted for the ISL&T-X 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. Result announcement will be made at the banquet.

Acknowledgements Sponsored by Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong

Savio Woo Young Researcher Award (up to 4) (NEW!)
Award: up to USD$1000 and Certificate

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 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-X 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 meeting 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 alternative

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

Selection Process

1. A number of highly meritorious abstracts that have a high impact with a clear motivation and relevance as well as quality experimental methods and scientific reasoning in the field of ligament and tendon research are invited by the award committee to submit a 3-page extended abstract.
2. The award committee will conduct a thorough review and select the winners.

Award Committee

Albert Banes, PhD – Chair, Award Committee
Kai-Ming Chan, MD – Chair, ISL&T-X
Jennifer Wayne, PhD - Biomechanics
Steven Arnoczky, DVM - Biology
Nicola Maffulli, MD - Clinical

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

Program Schedules

Main Program

February 5, 2010, Postgraduate Education Centre

Starts	Ends	Min	Code	Topics	Speakers
8:00am	8:30am	30	-	Registration and Light Breakfast	
8:30am	**8:35am**	**5**	OR1	**Opening Remark**	**Savio L-Y. Woo**
8:35am	**8:40am**	**5**	OR2	**Opening Remark**	**Kai Ming Chan**
Session 1: Tendinopathy-Pathogenesis and Treatment					**Moderators: Christer G. Rolf Roger K.W. Smith**
8:40am	8:50am	10	S1-1	Keynote Lecture: B to B in Tendinopathy, Where is the Missing Gap? (p.33)	Kai Ming Chan
8:50am	9:00am	10	S1-2	Keynote Lecture: Update of Clinical Trial on Autologous Tenocyte Therapy for Tendon Degeneration (p.34)	Paul Anderson
9:00am	9:05am	5	-	Discussion	
9:05am	9:15am	10	S1-3	Tendinopathies: Is the Ageing Cell Responsible for Reduced Tendon Matrix Turnover in Older Aged Individuals? (p.35)	Helen L. Birch
9:15am	9:20am	5	-	Discussion	
9:20am	9:30am	10	S1-4	Tenocyte Hypercellularity and Vascular Proliferation in a Rabbit Model of Tendinopathy – Contralateral Effects Suggest the Involvement of Central Neuronal Mechanisms (p.36)	Gustav Andersson *Student paper award list
9:30am	9:35am	5	-	Discussion	
9:35am	9:40am	5	S1-5	Mechanical Stretching Increased the Expression of BMP-2 which Promoted Osteogenic/Chondrogenic and Inhibited Tenogenic Differentiation of Tendon-Derived Stem Cells (TDSCS) *In Vitro* (p.37)	Yun Feng Rui *Student paper award list
9:40am	9:45am	5	S1-6	Eccentric & Concentric Calf Muscle Loading: An *In Vivo* Study of Force & EMG (p.27-32)	Saira Chaudhry *Savio Woo Young Reseracher Award winner *Student paper award list
9:45am	9:50am	5	S1-7	Efficacy of Bone-Marrow Derived Mesenchymal Progenitor Cells for Naturally-Occurring Tendinopathy in the Horse (p.38)	Roger K.W. Smith
9:50am	9:55am	5	-	Discussion	
9:55am	10:25am	30	-	**Break and Poster Session 1**	**Moderators: Masataka Deie Hirotaka Sano**

Starts	Ends	Min	Code	Topics	Speakers
Session 2: Tendon Development and Tendon Cell Differentiation					**Moderators: Catherine Kuo Pauline P.Y. Lui**
10:25am	10:40am	15	S2-1	Keynote Lecture: Overview on Current Tendon Development and Differentiation Researches (p.39)	Hong Wei Ouyang
10:40am	10:50am	10	S2-2	Tendon-lineage Differentiation of Human Embryonic Stem Cells Induced Tendon-lineage Differentiation by Overexpression of Scleraxis and Dynamic Mechanical Stress (p.24-26)	Xiao Chen *Savio Woo Young Reseracher Award winner
10:50am	10:55am	5	-	Discussion	
10:55am	11:00am	5	S2-3	Effect of GDF-6 on the Tenogenic Differentiation of Rat Bone Marrow-Derived Mesenchymal Stem Cells *In Vitro* (p.40)	Ming Ni *Student paper award list
11:00am	11:05am	5	S2-4	Tendon Stem Cells Exhibit Differential Properties from Tenocytes (p.41)	James H.C. Wang
11:05am	11:10am	5	-	Discussion	
11:10am	11:15am	5	S2-5	Control of Tendon Stem Cell Differentiation by Nano-Topography through Integrin and Myosin II Pathway (p.42)	Zi Yin
11:15am	11:20am	5	S2-6	Isolation and Characterization of Human ACL-Derived Vascular Stem Cells (p.43)	Tomoyuki Matsumoto
11:20am	11:25am	5	-	Discussion	
Session 3: Translational Research for Tendons and Ligaments					**Moderator: Gang Li Wei Hsiu Hsu**
11:25am	11:40am	15	S3-1	Keynote Lecture: Regulation of Adult Stem Cells Fate and Function in Tissue Engineering and Regeneration (p.44)	Rocky S. Tuan
11:40am	11:50am	10	S3-2	Propagation of Full-Thickness Rotator Cuff Tears - A Three-Dimensional Finite Element Analysis (p.45)	Hirotaka Sano
11:50am	11:55am	5	-	Discussion	
11:55am	12:05pm	10	S3-3	A Novel Extracellular Matrix Bioscaffold can Enhance ACL Healing (p.46)	Matthew B. Fisher
12:05pm	12:10pm	5	-	Discussion	
12:10pm	12:15pm	5	S3-4	*In Vivo* Osteogenesis of Ligamentum Flavum Cells in Photo-Responsive Hydrogel Encapsulated Bone Morphogenetic Protein-2: a Nude Mice Model (p.47)	Shu Wen Whu *Y. Faculty award & *Rs. fellow award list
12:15pm	12:20pm	5	S3-5	Does a Biomechanically Ideal Ovine Anterior Cruciate Ligament Autograft Present Early Signs of Degradation? (p.48)	May A. Chung
12:20pm	12:25pm	5	S3-6	Effect of Enamel Matrix Derivative on Tendon–Bone Interface in Anterior Cruciate Ligament Reconstruction in Rats (p.49)	Masataka Deie
12:25pm	12:30pm	5	-	Discussion	
12:30pm	**1:30pm**	**60**	**Lunch**		

Starts	Ends	Min	Code	Topics	Speakers
Session 4: Functional Tissue Engineering and Repair of Ligaments and Tendons					**Moderators:** **Wei Liu** **Yin Chih Fu**
1:30pm	1:45pm	15	S4-1	Keynote Lecture: Research of Tissue Engineered Tendon and Its Clinical Trial in Repairing of Ligaments and Tendons (p.50)	Hui Qi Xie
1:45pm	1:55pm	10	S4-2	Lubricin Reduces Adhesion Formation and Impairs the Healing of Repaired Flexor Tendon (p.51)	Yu Long Sun *Rs. fellow award list
1:55pm	2:00pm	5	-	Discussion	
2:00pm	2:10pm	10	S4-3	Cellular Response and Extracellular Matrix Breakdown in Rotator Cuff Tendon Rupture (p.52)	Bing Wu
2:10pm	2:15pm	5	-	Discussion	
2:15pm	2:20pm	5	S4-4	Monitoring Neovascularization in Tendon Healing by Three-Dimensional Doppler Ultrasound Imaging (p.53)	Bruma S.C. Fu
2:20pm	2:25pm	5	S4-5	Spatial and Temporal Changes of Collagens and Proteolycans in a Tendon Window Injury Model (p.54)	Pauline P.Y. Lui
2:25pm	2:30pm	5	S4-6	A Comparative Study on the Histological and Mechanical Properties of Bone-to-Bone, Bone-to-Tendon and Tendon-to-Tendon Healing – A Goat Achilles Tendon – Calcaneus Model (p.55)	Peng Zhang *Rs. fellow award list
2:30pm	2:35pm	5	-	Discussion	
Session 5: Tissue Mechanics					**Moderators:** **Ling Qin** **Edmond Y.S. Chao**
2:35pm	2:50pm	15	S5-1	Keynote Lecture: Mechanics Rules Cell Biology (p.56)	James H.C. Wang
2:50pm	3:00pm	10	S5-2	Specimen Dimensions Influence the Material Properties of Tendon Fascicles: Insights into Structure-Function Relationships (p.57)	Kirsten Legerlotz
3:00pm	3:05pm	5	-	Discussion	
3:05pm	3:10pm	5	S5-3	Four Loading Episodes during Early Tendon Healing Improve Tissue Quality (p.58)	Pernilla Eliasson *Student paper award list
3:10pm	3:15pm	5	S5-4	Anisotropic Properties of Stem Cell-Based Self-Assembled Tissues Cultured on a Micro Pattern-Processed Glass Plate (p.59)	Hiroki Sudama *Student paper award list
3:15pm	3:20pm	5	S5-5	Mechanical Stimulation Enhances Engineered Tendon Formation *In Vitro* and *In Vivo* (p.60)	Wei Liu *Rs. fellow award list
3:20pm	3:25pm	5	-	Discussion	
3:25pm	3:30pm	5	S5-6	*In Vivo* Length Changes of Ligaments Stabilizing the Trapezium and Trapeziometacarpal Joint during Thumb Movement (p.61)	Jing Xu *Y. faculty award list

Starts	Ends	Min	Code	Topics	Speakers
3:30pm	3:35pm	5	S5-7	Effects of Cyclic Tensioning Culture on a Stem Cell-Based Self Assembled Tissue (scSAT) Derived from Synovium (p.62)	Saito Kei *Student paper award list
3:35pm	3:40pm	5	-	Discussion	
3:40pm	4:10pm	30	-	**Break and Poster Session 2**	**Moderators:** **Masataka Deie** **Masahiro Kurosaka** **Hirotaka Sano**
Session 6: ACL Reconstruction-Biology of Healing and *In Vivo* Knee Kinematics					**Moderators:** **Harukazu Tohyama** **Shinichi Yoshiya**
4:10pm	4:25pm	15	S6-1	Keynote Lecture: The Graft Fixation Sequence Affects their Force Distributions in Double Bundle Anterior Cruciate Ligament Reconstruction (p.63)	Guoan Li
4:25pm	4:40pm	15	S6-2	Keynote Lecture: Technique and Problems of Anatomic Double Bundle ACL Reconstruction (p.64)	Masahiro Kurosaka
4:40pm	4:50pm	10	S6-3	Periosteum-Like Cell Sheets Enhanced Tendon-Bone Healing in an Anterior Cruciate Ligament Reconstruction (p.65)	Chih Hsiang Chang *Rs. fellow award list
4:50pm	4:55pm	5	-	Discussion	
4:55pm	5:05pm	10	S6-4	Immunohistochemical and Gene Expression Analysis in the Ruptured Human Anterior Cruciate Ligament ~Expression of Activated Stat3~ (p.66)	Takuya Naraoka
5:05pm	5:10pm	5	-	Discussion	
5:10pm	5:15pm	5	S6-5	Biomechanical Function of Anterior Cruciate Ligament Remnants: Effects of Remnant Pattern and Duration between Injury and Surgery on Knee Stability Evaluated with a Navigation System (p.67)	Atsuo Nakamae *Y. faculty award list
5:15pm	5:20pm	5	S6-6	*In-Situ* Force in the Three Bundles of the Human Anterior Cruciate Ligament (p.68)	Hitoshi Yagi
5:20pm	5:25pm	5	-	Discussion	
5:25pm	5:30pm	5	S6-7	Transforming Growth Factor-β1 Gene Transfer Therapy Improves Achilles Tendon Healing by Promoting Collagen Formation (p.69)	Yu Hou
5:30pm	5:35pm	5	S6-8	Effects of Low-Intensity Resistance Training with Restricted Muscle Blood Flow on Tendon and Ligament Maturation-Study of after ACL Reconstruction (p.70)	Rieko Kuramochi
5:35pm	5:40pm	5	-	Discussion	
5:40pm	**5:45pm**	**5**	**-**	**Closing Remark**	**Chih Hwa Chen**
6:45pm	9:45pm			Maxim Palace, New Town Plaza, Shatin	

Poster Sessions

Starts	Ends	Code	Topics	Speakers
9:55am	10:25am	**Poster Session 1**		**Moderators: Masataka Deie Hirotaka Sano**
9:55am	9:58am	PS1-1	Local Administration of TGFB1/VEGF165 Gene Transduced Mesenchymal Stem Cells on Properties of Achilles Allograft Replacement of ACL in Rabbits (p.71) *(Best Poster Award competition presentation time: 2mins)*	Xue Lei Wei
9:58am	10:01am	PS1-2	The Assembly of hESC-MSC and Knitted Silk Scaffold Combined with Collagen Matrix Develop to Engineered Tendon under Mechanical Stress (p.72) *(Best Poster Award competition presentation time: 2mins)*	Zi Yin
10:01am	10:04am	PS1-3	An observational study to identify the presence of Achilles Tendinopathy and ultrasound detected changes in elite footballers and gender-matched controls (p.73) *(Best Poster Award competition presentation time: 2mins)*	Mark Perry
10:04am	10:07am	PS1-4	Neovascularization is an Essential Factor in Medial Collateral Ligament (MCL) Healing. (p.74) *(Best Poster Award competition presentation time: 2mins)*	Katsumasa Tei
10:07am	10:10am	PS1-5	Stress Changes of Lateral Collateral Ligament under Various Motions (p.75) *(Best Poster Award competition presentation time: 2mins)*	You Wang
		PS1-6	Histological Evaluation of Patellar Tendon and Its Enthesis in Trained, Untrained and Detrained Rats: Experimental Study, Preliminary Results (p.76)	Antonio Frizziero
		PS1-7	Serum Concentrations of the Neurotrophin BDNF and Those of TNF-Receptor1 Arecorrelated in Individuals with Achilles Tendinosis but Not in Healthy Controls (p.77)	Johan Bagge
		PS1-9	Will Multiple Freeze/Thaw Cycles Change the Tensile Properties of Human Patellar Tendons? (p.78)	Ho Joong Jung
		PS1-10	Arbitrary Starting Point of Separation Affect the Morphology of the Two Bundles of Anterior Cruciate Ligament at Insertion Sites (p.79)	Jin Zhong Zhao
		PS1-11	Effect of CTGF on the Tenogenic Differentiation of Rat Bone Marrow-Derived Mesenchymal Stem Cells *In Vitro* (p.80)	Chao Song
		PS1-12	Measurement of normal tibialis anterior muscle architecture by ultrasound in elite athletes and controls-A cross-sectional study (p.81)	Matt Wilson

Starts	Ends	Code	Topics	Speakers
3:40pm	4:10pm	**Poster Session 2**		**Moderators: Masataka Deie Masahiro Kurosaka Hirotaka Sano**
3:40pm	3:43pm	PS2-1	An Experiment Of Human Acellular Dermal Matrix on the Rotator Cuff Repair in a Canine Model (p.82) *(Best Poster Award competition presentation time: 2mins)*	Da Mi Choi
3:43pm	3:46pm	PS2-2	Effect of Cyclic Stretching on the Tenogenic Differentiation of Rat Bone Marrow-Derived Mesenchymal Stem Cells *In Vitro* (p.83) *(Best Poster Award competition presentation time: 2mins)*	Ming Ni
3:46pm	3:49pm	PS2-3	Dynamic Strain-Mediated Tendinogenic Differentiation of Bone Marrow Stromal Cells on Small Intestinal Submucosa Membrane (p.84) *(Best Poster Award competition presentation time: 2mins)*	Ting Wu Qin
3:49pm	3:52pm	PS2-4	Regulatory Effect of Collagen V on the Fibrillogenesis of Tenocytes in a Tissue Engineering Model (p.85) *(Best Poster Award competition presentation time: 2mins)*	Ping Lu
3:52pm	3:55pm	PS2-5	Stromal Cell-Derived Factor 1 Enhances the Regeneration of Tendon Using a Knitted Silk Scaffold Combined with Collagen Matrix (p.86) *(Best Poster Award competition presentation time: 2mins)*	Wei Liang Shen
		PS2-6	Effect of GDF-7 on the Tenogenic Differentiation of Rat Bone Marrow-Derived Mesenchymal Stem Cells *In Vitro* (p.87)	Chao Song
		PS2-7	Metabolism of Extracllular Matrix in Flexor Tendon with Stress Deprivation (p.88)	Yu Long Sun
		PS2-8	Carpal Tunnel Size and Shape Alteration Induced by Transverse Carpal Arch Deformation (p.89)	Hong Pan
		PS2-9	Induced Pluripotent Stem Cells as a Model to Treat and Study Human Osteoarticular Diseases (p.90)	Miguel Esteban
		PS2-10	High Efficiency Differentiation of Neuronal Cells from Mouse Embryonic Stem Cells by Gradual Medium Replacement (p.91)	Xi Ning Pang
		PS2-11	Does the site of maximum neovascularisation correlate with the site of pain in recalcitrant mid-tendon Achilles Tendinopathy? A prospective observational study (p.92)	Kiran Divani
		PS2-12	The Effect of Alendronate on the Mechanical Strength of the Tendon Graft to Bone Tunnel Complex after ACL Reconstruction (p.93)	Pauline P.Y. Lui

Associative Program

Feb 6, 2010, 4/F Li Ka Shing Medical Sciences Building

This is a brand-new element of ISL&T-X.

Mentor Group will give you useful hints on paper writing skills and conducting translational research for clinical and industry needs. And you will also listen to and discuss with your experts on controversial ligaments and tendons research issues.

9:15-10:15am **Mentor Group** **Rm 407**	***Translational Research for clinical and industry needs*** **Cheng Kung Cheng**
	Skills on writing papers **Savio L-Y. Woo**
10:15am-12:15pm	**Option A: Lab Tour** **Rm 406**
10:15am-1:15pm	**Option B: Research Forum** **Rm 407**

Option A: Lab tour (Rm 406)

It will give you a live demonstration of the advanced lab equipment and advanced research techniques in the hosting University. Participants will be divided in small groups and guided by our teams to tour around laboratories to see demonstration on

- micro-CT imaging technique for the imaging of calcification in tendons and tunnel healing in ACL reconstruction;
- High resolution animal ultrasound imaging for monitoring tendon structure and vascularization after injury;
- *In vivo* imaging system for cell tracing
- Cell migration system
- GMP laboratory for tissue transplantation in human
- Gait laboratory for studying human motions

Option B: Research Forum (Rm407)

- Discussion on controversial research issues related to ligaments and tendons.
- Moderators will introduce the topic and raise specific questions to experts in the field for comments. Then, moderators will pick 2-3 questions from the audience.
- Each topic will last for 30 – 40 mins.

Tentative questions are listed as follows:

A) Tendon cell differentiation

Moderator: Gang Li
Experts: James H.C. Wang & Wei Liu

1. How to define tendon stem cells?
2. How to define tenocyte?
3. What are the key factors for tenogenic differentiation?
4. Is mechanical factor essential for tendon cell differentiation?
5. Can we make tendon tissues in vitro?

B) Roles of inflammation in tendinopathy

Moderator: Christer G. Rolf
Experts: James H.C. Wang, Wen Chung Tsai & Roger K.W. Smith

1. What type and function do you describe for "the tendon cells" which is found in the hypercellular areas of tendinopathy?
2. What is the role of the corresponding hypervascularity?
3. Was there ever an acute inflammation in tendinopathy?
4. Will NSAID halt the healing process in tendinopathy?
5. Is there any evidence based rationale for treatments such as "sclerotic injections' and low frequency ultrasound for the management of tendinopathy?
6. What are the future lines of research for possible cure of 1) symptoms and 2) structural and biological dysfunction of tendinopathy?

C) Animal model of tendinopathy

Moderator: Pauline P.Y. Lui
Experts: Nicola Maffulli, Roger K.W. Smith, Christer G. Rolf

1. Criteria and basis for assessment of animal models
2. Re-assessment of current animal models based on the criteria
3. Suggestions for the improvement of existing animal models

D) Innovations in ACL reconstruction

Moderator: Chih Hwa Chen

Experts: Yung Bok Jung, Harukazu Tohyama, Wei Liu, Patrick S.H. Yung

1. Anatomical or isometric reconstruction
2. Double bundle vs single bundle
3. New fixation devices for ACL graft fixation
4. Graft healing in the tunnel
5. Graft ligamentization in the joint
6. Graft choice in the future (Tissue engineering ligament, artificial ligament)
7. Ligament healing after ACL partial tear

SAVIO WOO YOUNG RESEARCHER AWARD WINNER

Biological Research

TENDON-LINEAGE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS BY OVEREXPRESSION OF SCLERAXIS AND DYNAMIC MECHANICAL STRESS

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[1] Center for Stem Cell and Tissue Engineering, School of Medicine, Zhejiang University, Hangzhou, China
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INTRODUCTION

Tendons are frequently damaged during sports and other strenuous activities [1, 2] and have quite poor self-repair capacity. Their healing occurs via the formation of a fibrotic scar and results in significant dysfunction and disability.

Currently, stem cells and tissue engineering techniques show great potential for tendon regeneration. Adult tendons lack regeneration capability; however, fetal tendons have great regeneration potential. More interestingly, this capability is intrinsic to the fetal tendon itself [6]. Thus, fetal cells seem to play a crucial role in fetal tendon regeneration. Embryonic stem cells (ESCs), which are primary embryonic cells and hold tremendous potential for cell-based therapies, may have the potential for tendon regeneration. As shown in our previous work, ESCs can be used for tendon regeneration by stepwise induction. However, the maturation of tendon-like tissues and regeneration of repaired tendon are yet to be achieved.

No optimal method to induce ESC into tenocyte differentiation for tendon regeneration has been found yet. The signals that are specific to tendon development may be used to induce tendon-lineage differentiation. In embryonic development, TGF and FGF signals, ectoderm signals and mechanical stress [3, 4] are critical for tendon development and associated with tenocyte recruitment and differentiation. Scleraxis, a bHLH transcription factor, is a highly specific marker for tendons, and scleraxis knockout causes severe defects in force-transmitting tendon [5]. This suggests that mechanical stress and scleraxis may play a synergic role in the tendon development. However, ectopic expression of scleraxis is not enough to induce ectopic tendon formation. Dynamic mechanical stress could also induce tendon-lineage differentiation of bMSC. The signaling mechanisms that mediate force-induced tenocyte differentiation and collagen expression are currently not defined. In this study, we tested the hypothesis that mechanical stress interacts with the transfer growth factor-beta (TGF-beta) pathway and scleraxis transcription factor to stimulate tendon-lineage differentiation and tendon regeneration.

METHODS

Cell Culture Human ESCs were first induced to differentiate into mesenchymal stem cells (MSCs) as previously MSCs (hESC-MSCs) was analyzed by flow cytometry. described [6]. The immuno-phenotype of hESC-derived

Scleraxis Transfection and Mechanical Stress Scleraxis-hESC-MSCs were obtained by transfection with scleraxis-lentivirus followed by selection with blasticin (2 μg/ml). LacZ or GFP genes were used for control. To fabricate engineered tendons, the cells were then embedded in collagen gel at 10^7 cells/ml, seeded on knitted silk scaffolds, and cultured for 2days. The engineered tendon then was subjected to a dynamic mechanical stress of 1HZ for 2h/day to evaluate the combination effect of scleraxis and dynamic mechanical stress.

Fabrication of scaffold-free Engineered Tendon hESC-MSCs formed cell sheets after 14 days of culture, and engineered tendons were formed in vitro [6]. The engineered

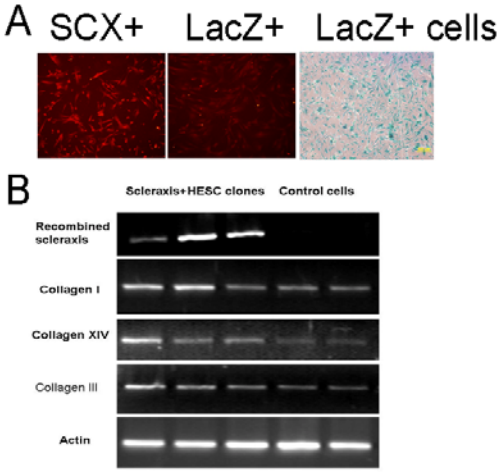


Fig.1 Scleraxis overexpression induce tendon ECM expression. A. Immunofluorescence of scleraxis and virus transfection efficiency. B. scleraxis positive clones showed high scleraxis expression and higher expression of collagen I, III and XIV

tendons were subjected to a dynamic mechanical stress of 1HZ at 10% strain for 2h/day for 7 days. Then the regeneration potential of the engineered tendon tissues was evaluated in an in-situ rat patellar tendon window repair model.

Cell labeling To identify genetically engineered hESC-MSCs within the site of implantation in vivo, the cells were infected either with DiI [6].

RESULTS

hESC differentiation into MSCs hESC-MSCs had the potential to differentiate into the three mesenchymal lineages, including osteogenesis, adipogenesis and chondrogenesis, and were positive for MSC surface markers (data not shown).

Scleraxis initiate mesodermal differentiation After selection with blasticin, 100 percent of cells were transfected by the recombinant genes. Scleraxis expression in the transfected cells was significantly higher than the control (Fig 1A). Scleraxis overexpression increased the collagen I, III, XIV expression in clones, reduced collagen II promoter activation (Fig 2A) and bmp induced smad activation(Fig 2B). However, ALP activity and alizarin red staining showed scleraxis also increased the bone induction.

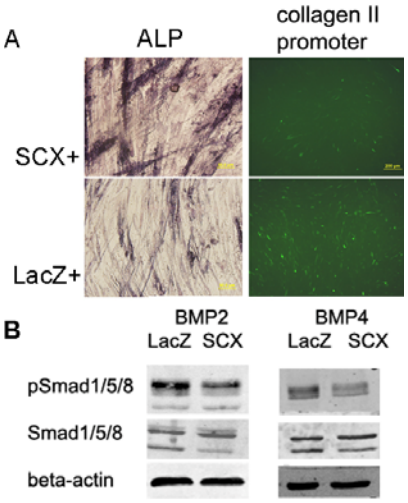


Fig.2 Scleraxis affect osteogenesis and chondrogenesis. A scleraxis increase ALP activity and reduce collagen II promoter activation. B scleraxis reduce bmp2 and bmp4 induced smad activation

Scleraxis and mechanical stress synergistically induce tendon differentiation The expression of tendon-specific genes, such as collagen I α1, collagen I α2, collagen XIV was higher and osteo-/chrondro- specific genes, such as osteocalcin, PTC, aggrecan were lower in scleraxis-hESC-MSCs. However, osteo-specific gene runx2 was higher and several tendon-specific genes, such as tenomodulin, nafatc4 were lower in scleraxis-hESC-MSCs. In the in-vitro tissue engineering tendon model, 4 hour cyclic mechanical stress significantly reduced runx2 and increased tenomodulin and nafatc4 expression in scleraxis-hESC-MSCs, while mechanical stimulus had no such effect in control group. (Fig.3). The findings implicated that scleraxis initiate both teno- and osteo-lineage early differentitiation, while mechanical stress synergistically promote the teno-differentiation effect of scleraxis and inhibits its osteo-differentiation effect.

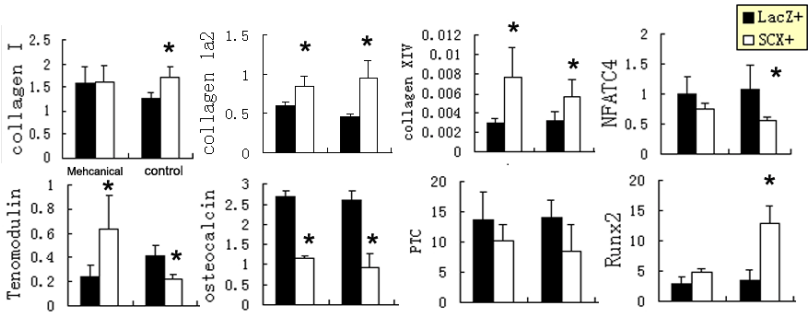


Fig.3 scleraxis and mechanical stress effect tendon-/osteo- specific gene expression
*P<0.05 compared to LacZ+ cells

Scleraxis and mechanical stress promote tendon differentiation and regeneration in vivo In the in-vivo ectopic implantation models, mechanical stress induce larger collagen fibers and scleraxis showed synergetic effect (Fig.4). In the in-situ tendon repair model, the engineered tendon treated with scleraxis-hESC-MSCs and mechanical stress had significantly better mechanical properties than those of the control group (Fig.5). Furthermore, hESC-MSCs remained viable at the tendon wound site for at least 4 week. Moreover, no teratoma was found in any samples.

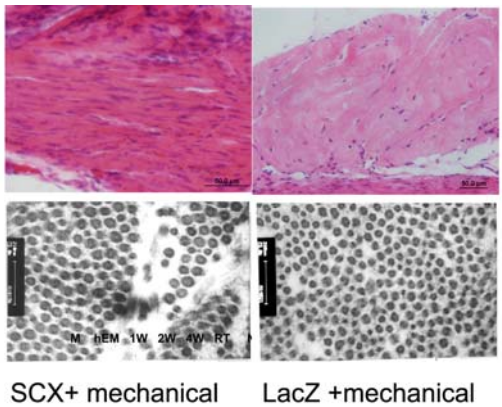
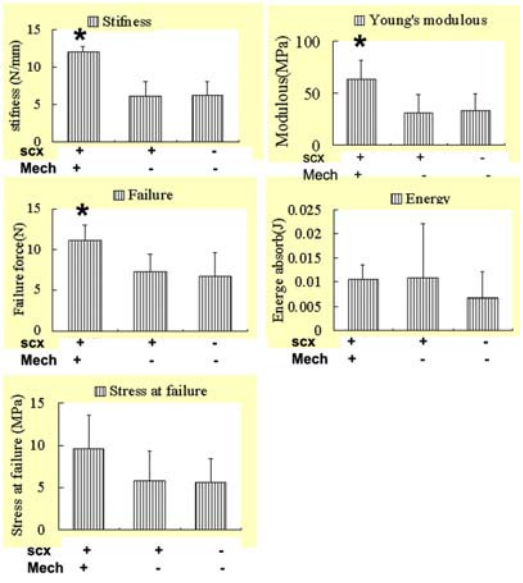


Fig.4 scleraxis and mechanical stress increase maturation of tendon-like tissues in vivo

DISSCUSION

The present study demonstrates that mechanical stress and scleraxis have a synergetic function on tendon

differentiation of hESC-MSC as well as tendon regeneration. Previous research indicated that scleraxis is also upstream genes of bone and cartilage specific genes, such as collagen I, collagen II and aggrecan, and it is involved in bone and cartilage differentiation and development [7-10]. Scleraxis alone is not enough to induce tendon lineage differentiation both in-vitro and in-vivo [7, 11]. As shown in the present study, which is consistent with previous work, scleraxis may not only be involved in tendon differentiation, but also in bone and cartilage differentiation. Scleraxis increases tendon specific ECM, such as collagen I α1, I α2 and collagen XIV. It also increases runx2 expression in hESC-MSC engineered tendon. The role of scleraxis on tendon differentiation is partially to change the activation of BMP-smad pathway.



Dynamic mechanical stress could also induce tendon-lineage differentiation of bMSCs. However, the mechanism involved and the effect on maturation of collagen fibers are not mentioned [12, 13]. This study showed that mechanical stress have synergetic function on inducing teno-lineage differentiation by reducing runx2 and increasing tenomodulin and nafatc4 expression in scleraxis-hESC-MSC engineered tendon. These results were confirmed by the in-vivo study that mechanical stress with scleraxis induces larger collagen fibers. Patellar tendons treated with the mechanical-scleraxis- hESC-MSCs engineered tendon had much better mechanical properties than did controls.

These findings may have considerable importance in understanding the roles of mechanical stress and scleraxis on tendon differentiation as well as developing therapeutics for tendon regeneration.

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**Finalist of the Best Student Paper Award*

SAVIO WOO YOUNG RESEARCHER AWARD WINNER

Clinical Research

ECCENTRIC & CONCENTRIC CALF MUSCLE LOADING:

AN IN VIVO STUDY OF FORCE & EMG

S. Chaudhry [1, 2], H.R.C. Screen[1], R.C. Woledge [2], D.Bader[1], D. Morrissey[2]

Queen Mary University of London, [1]School of Engineering and Material Science, [2]Centre for Sports and Exercise Medicine, London, UK

INTRODUCTION

Achilles tendinopathy is a painful condition occurring in and around the Achilles tendon (AT), thought to be a failed healing response. It is often characterized by disruption of collagen fibres, an increase in non-collagenous matrix and random proliferation of tenocytes as shown by tendon thickening, disordered tendon fibrils and neo-vascularisation on ultrasound imaging. Excessive repetitive overloading of the Achilles is one of the main stimuli leading to tendinopathy [1], hence it is common in athletes with 11-24% of runners suffering with this disease [2]. However, it also occurs in non-athletes, with around 33% of tendinopathic presentations being in sedentary individuals [3].

Achilles tendinopathy is prone to recurrence, as current treatments are only partially successful. The main conservative treatment is heavy load eccentric training (ET) of triceps surae, shown to be effective in various controlled clinical trials and systemic reviews, with a success rate of 60-82% [4, 5]. The ET protocol was proposed by Stanish et al back in 1986 [6], and the first controlled trial of this method carried out by Alfredson in 1998 [4] confirmed the superior outcomes of EC training over concentric training (CT). Since this time, a number of studies have evaluated the efficacy of ET providing a sufficient level of evidence to support this treatment method [4, 5, 7, 8].

Eccentric training involves the lengthening of the muscle-tendon unit during the application of load. This is the opposite to concentric loading where the muscle-tendon unit gets shorter and differs from isometric exercises, in which the muscle tendon unit length remains unchanged. ET has been shown to result in positive clinical outcomes for Achilles and other tendinopathies, improving pain, patient satisfaction and function when compared with concentric exercise. It has also been shown to increase the total strength and mass of the muscle as compared to CT [9]. However, it is unclear why ET results in better outcomes in Achilles Tendinopathy than CT and the mechanism behind the superior muscle strength and mass gain during EC loading remains unclear. Based on the specificity principle of strength training, it has been postulated that eccentric and concentric actions provide different stimuli to the triceps surae, and therefore produce different muscle and tendon adaptations [10]. It is likely that the whole triceps surae unit will be involved in mechanical remodelling and not just the Achilles tendon, hence the aim of our study was to compare eccentric and concentric loading modalities to investigate differences in muscle activity and tendon force (TF) across the muscles of the triceps surae. Thus, this study hypothesized that CT and ET of the triceps surea would lead to different force production and muscle activation patterns.

METHODS

Twelve healthy volunteers (6 male and 6 female, mean age = 27.8 ± 1.9 years (SD)) performed eccentric and concentric loading exercises for the triceps surae. For ET, subjects were asked to stand on the toes of one foot with the heel raised, before lowering the heel in a controlled manner. The exercise was performed off the edge of a step to allow full dorsi-flexion to be reached (Fig.1A). For CT, subjects started with the heel below the toes and raised the heel during the exercise in the same controlled manner (Fig.1B). In order to keep speed consistent, subjects were taught to complete the heel raise or heel drop during a count of four beats on a metronome. After completing either exercise, the subjects used the other leg to assist in returning to the starting position, before repeating the exercise. A single data collection consisted of 2 cycles of either CT or ET performed consecutively. Three sets of such data were recorded for each loading paradigm for each subject, in a randomized order. Tendon force and extension were measured during each exercise.

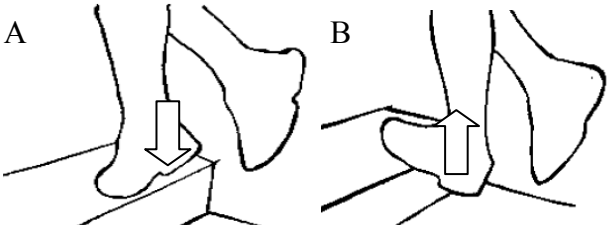

Fig.1 A) Eccentric loading B) Concentric loading

An active infra red motion analysis system (CODA, CX1, Charnwood Dynamics, Rothley, UK) was used to determine gross movements of the leg, with simultaneous ultrasound tracking of the medial gastrocnemius muscle-tendon junction (MTJ). The ultrasound probe was placed on the leg such that the medial gastrocnemius muscle–tendon junction (MTJ) could be imaged (Voluson e, GE Healthcare, UK), and three motion tracking markers placed on the probe, so its location could be embedded into the laboratory coordinate system in order to spatially synchronise the data. In addition, electromyography (EMG) recordings were used to determine muscle activity using dual electrodes with a 20mm inter-electrode distance placed on the belly of the soleus, lateral gastrocnemius, medial gastrocnemius and tibialis anterior muscles following the EU guidelines [11]. EMG data was then rectified and smoothed using MATLAB in order to compare the activation patterns during ET and CT.

RESULTS

While some inter-subject variability was apparent, CT and ET resulted in distinctly different loading patterns and muscle activation patterns across all subjects. ET resulted in greater values for both the rate of change of tendon force, and the maximal force (1604±89.5N) compared with CT (1410±79.7N) $p < 0.01$. In contrast, when looking at EMG data both the rate of change of muscle activation and the maximal activation values were significantly higher ($p < 0.01$) in CT than ET. Pairing eccentric and concentric EMG data for each subject highlighted that, whilst the mean CT muscle activity levels were higher, the ratio of mean concentric to mean eccentric activity for each subject varied between muscles, with values of 1.08-2.00 for soleus, 1.25-3.03 for lateral gastrocnemius, 1.08-1.48 for medial gastrocnemius and 0.09-1.83 for tibialis anterior. Figure 2 shows the variation of the muscles activity and force with respect to heel height (HHt). As the heel position varies between its highest and lowest positions, there is high rate of change of force during ET while high rate of change of EMG activity across four muscles during CT.

Figure 3 shows the variation of force with tendon length during ET for one subject. Mean data for all subjects will be available before the February meeting. The significant drop in force is explained by the bilateral weight bearing during the return phase of ET; however, a typical hysteresis curve is demonstrated.

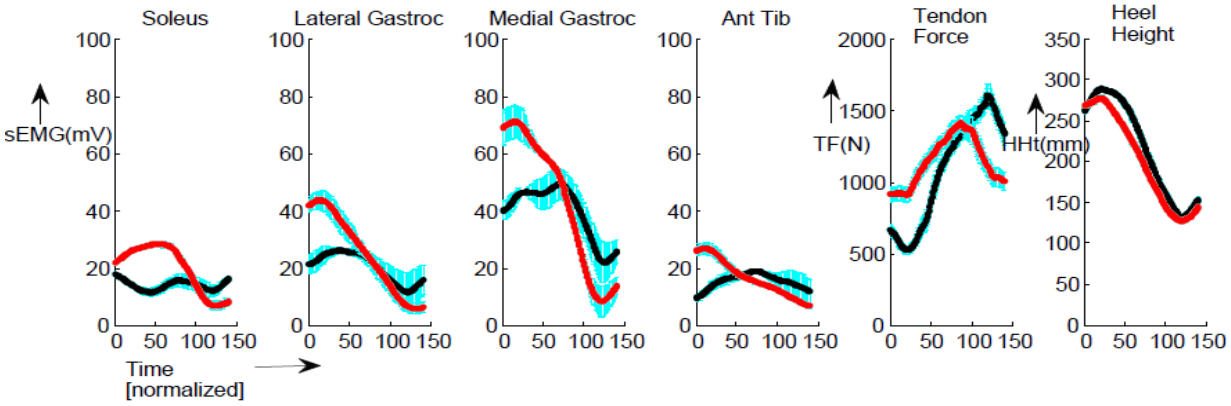

Fig.2 Graphs of mean (SEM in blue) soleus / lateral gastrocnemius / medial gastrocnemius / anterior tibialis EMG and tendon force are shown against normalised time. In addition heel height is shown in order that the previous graphs can be interpreted with respect to position. In all graphs the eccentric action is in black, and the concentric in red.

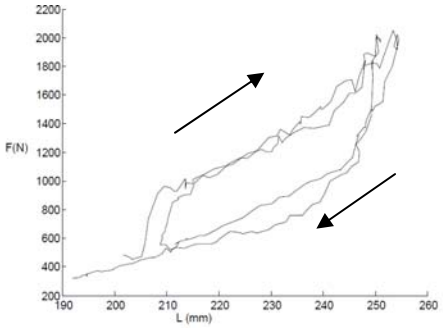

Fig.3 Achilles tendon length and force measurements against time for two consecutive ET cycles in one subject. Arrows indicate the general trend for rise and fall of the force against length.

An active infra red motion analysis system (CODA, CX1, Charnwood Dynamics, Rothley, UK) was used to determine gross movements of the leg, with simultaneous ultrasound tracking of the medial gastrocnemius muscle-tendon junction (MTJ). The ultrasound probe was placed on the leg such that the medial gastrocnemius muscle–tendon junction (MTJ) could be imaged (Voluson e, GE Healthcare, UK), and three motion tracking markers placed on the probe, so its location could be embedded into the laboratory coordinate system in order to spatially synchronise the data. In addition, electromyography (EMG) recordings were used to determine muscle activity using dual electrodes with a 20mm inter-electrode distance placed on the belly of the soleus, lateral gastrocnemius, medial gastrocnemius and tibialis anterior muscles following the EU guidelines [11]. EMG data was then rectified and smoothed using MATLAB in order to compare the activation patterns during ET and CT.

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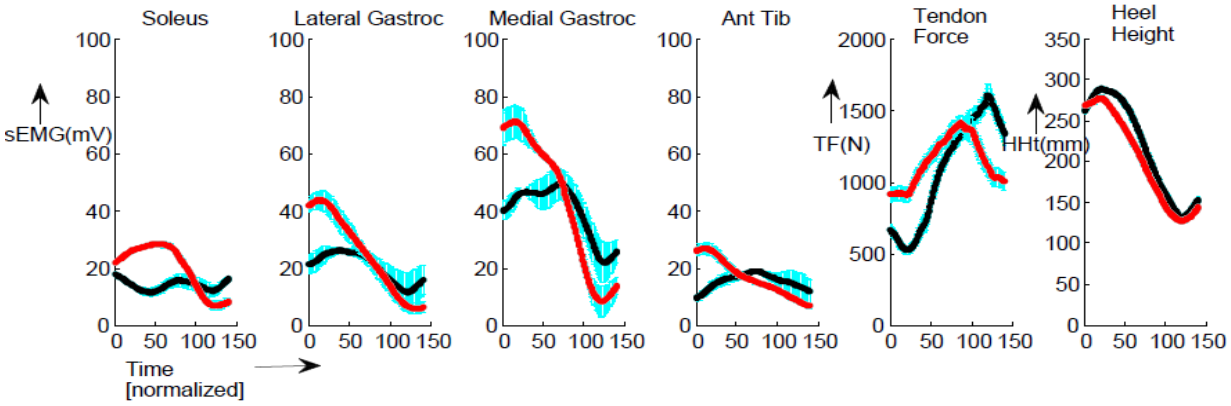

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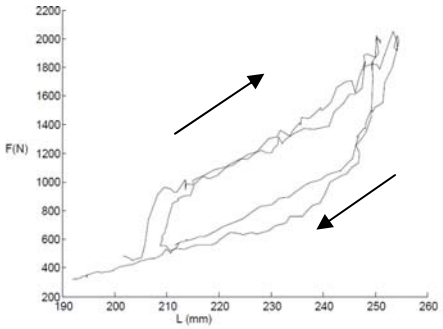

Fig.3 Achilles tendon length and force measurements against time for two consecutive ET cycles in one subject. Arrows indicate the general trend for rise and fall of the force against length.

DISCUSSION

This study demonstrates that eccentric calf muscle loading produces higher forces within the tendon than concentric loading with lower muscle activation levels. Results from our study show that at the start of concentric movement the calf muscles are activated, the AT force increases and the subject accelerates upwards. The peak AT force occurs in the middle of the movement, possibly as the moment arm of the triceps surae is furthest from the ankle joint axis for sagittal plane movement during this phase. In the eccentric movement, the subject starts to drop 'under control'. The movement is controlled (resisted) by lengthening of the activated calf muscle and by rate–dependent stretching of the AT (figure 3). The maximal derived AT force occurs at the end of the eccentric movement when maximum force is required to decelerate the subject against gravity. It was observed that there was no significant differences in the forces midway between the two loading cycles which is consistent with the previous findings reported by Rees et al. [12] where they compared similar ET and CT loading approaches. Our study has a greater subject number and additionally shows significantly greater forces occurring at the start and end of loading. If the mechanism of action of ET is via higher force fluctuation, then these differences may have therapeutic relevance [6].

The higher progressive force on tendon during eccentric loading may partially explain the difference in therapeutic effect, as tendon cells respond to mechanical forces by adapting their production of neo-matrix. These higher tendon forces may lead to improved matrix anabolism, thus improving the structural and mechanical properties of the tendon. The differences in the peak load and the rate of change of tendon force are consistent with previous findings in other muscle groups. For example, Westing and Serge found significantly higher mean eccentric compared to concentric torques in quadriceps and hamstring muscles [13].

One of the aims of this study was to observe calf muscle activity during the two loading protocols. The relatively low EMG activity during eccentric action was expected. The combination of the EMG and force data indicates that eccentric contractions required lower levels of voluntary activation by the nervous system to achieve a given muscle force, applied to the tendon. The reduced EMG observed during eccentric loading suggests an incomplete activation of the motoneurons that innervate the muscle, which may take the form of a lower level of activation distributed across the entire population of motoneurons or be the activation of only a subset of the entire population. It may be that the associated energy preservation during ET, via ATP sparing, allows a greater volume of exercise to be carried out under eccentric conditions than concentric.

In conclusion, significant differences in EMG and tendon force were demonstrated between eccentric and concentric loading protocols, which may underlie some of the differences in therapeutic effect. Future, imminent, work will clarify group stress – strain relationships.

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B TO B IN TENDINOPATHY, WHERE IS THE MISSING GAP?

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Recent investigations on tendinopathy have revealed more and more clues for the pathogenesis, but effective treatments for tendinopathy are still unavailable. The missing gap between the bench work and the bedside's needs may reside on the translational research, which have not received sufficient emphasis. Based on our current understanding on tendinopathy, it is possible that the pivot of the pathological development is the erroneous differentiation of tendon stem cells. As a result, how to safeguard tenogenic differentiation in tendon healing would be a turnkey solution for treatment of tendinopathy. In order to perform pre-clinical tests, establishment of representative animal models of tendinopathy is a pre-requisite. We found that the spontaneous failed healing of collagenase-induced tendon injuries in a rat model resembled most key characteristics of tendinopathy in humans, including histopathological changes and activity-related pain. Erroneous acquisition of chondrogenic and/or osteogenic phenotypes by the tendon cells in this animal model was also observed. Investigations to modulate tendogenic differentiation in vitro and in vivo may reveal the possibilities to rectify erroneous tendon cell differentiation in tendinopathy. As the use of tenogenic mesenchymal stem cells (MSC) may have great potentials for cure of tendinopathy, technical hurdles for clinical application of MSC must be overcome. For examples, the source of autologous MSCs, the in vitro manipulation of MSCs and the mechanisms of cell delivery will be key topics for translation research with respect to stem cell therapy for tendinopathy.

AUTOLOGOUS TENOCYTES THERAPY FOR TENNIS ELBOW: THE PERTH CLINICAL TRIAL UPDATE

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Tennis elbow is a general discretion for a painful elbow lesion, which is also known as epicondylitis for an acute condition or epicondylosis for the chronic condition. It has been proven that the pathogenesis of tennis elbow is not a inflammatory condition but a result of degenerative changes including disruption of collagen matrix, calcification, vascularisation and adipogenesis. As current treatments are based largely on empirical experience and did not address the fundamental pathogenesis of cellular deficiency. We reasoned that autologous tenocyte therapy may be able to restore the tissue architecture of tendon and thus eliminate pain through the treatments regenerative capacity of the tendon. Subsequently, it results in a very high recurrent rate. We have conducted a first phase I clinical trial on 30 patients in 2008 under approval of Australia Therapeutic Good Adminstration (TGA). The major objective of the study is to validate the safety, tolerability and early phase treatment efficacy of autologous tenocytes therapy in patients with tennis elbow. All of patients enrolled in the trial have history of tennis elbow longer than 6 months and were resistant to current available non-surgical treatment. Tenocytes, the major cell type that maintain the metabolism of tendon were obtained from patella tendon biopsy of the patients. These cells were cultured and expanded under the TGA approved GMP lab. Cells were then injected into the elbow lesion under ultrasound by radiologists. MRI and ultrasound imaging evaluation and comprehensive scoring regimes including Extreme Functional Score; Quick Disabilities of the Arm, Shoulder and Hand Score and Upper American Shoulder and Elbow Surgeon Scale were used to evaluate patient outcome over 12 months period. To date there are 21 patients received autologus tenocyte therapy. No major adverse events were reported in all these patients. Preliminary assessment of image evaluation and functional assessment showed improvement of patient outcome but final assessment of efficacy is not yet established. The conclusion established for the trial at present is that introduction of autologous tenocyte therapy poses minimal risk in patient safety.

TENDINOPATHIES: IS THE AGEING CELL RESPONSIBLE FOR REDUCED TENDON MATRIX TURNOVER IN OLDER AGED INDIVIDUALS?

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INTRODUCTION

The prevalence of tendon pathology and incidence of tendon injury are higher in older age in both human and equine subjects. Tendinopathies are recognised as degenerative conditions and although the aetiology is unknown the accumulation of micro-damage in structural proteins is often suggested as a possible factor. In addition, the extracellular matrix macromolecules are susceptible to irreversible chemical modifications which can disrupt function and contribute to age related decline. Our previous work has shown a significant positive correlation between the advanced glycation end product (AGE) pentosidine and age in equine superficial digital flexor tendon (SDFT) tissue. The time dependant racemization of aspartic acid also showed a significant increase with increasing horse age in SDFT tissue, as expected. Further analysis of our racemization data showed that collagen half life increased with age, indicating that the turnover of this relatively long lived protein decreases even further with advancing age. A slowing of the turnover rate with increasing age suggests that the ability of the tendon to repair any micro-damage may be impaired in older individuals. We hypothesise that cells in the equine SDFT have a reduced ability to synthesise and degrade components of the extracellular matrix as age increases. The objectives of this study were to assess transcription levels for a panel of genes associated with the structural components of the matrix and their degradation, and mRNA translation by measuring degradative enzyme activity in a group of horses selected with a wide age range.

METHODS

The mid-metacarpal region of the SDFT was harvested from 32 horses aged 4 to 30 years euthanased for reasons other than tendon injury. All tendons were normal on gross pathological examination. Cell activity was assessed by measuring the expression of genes coding for matrix macromolecules and degradative enzymes using real-time reverse transcriptase polymerase chain reaction (RT-PCR). Collagen degradation was assessed in tendon extracts at the protein level using a commercially available RIA for the cross-linked C-terminal peptide of Type I collagen (ICTP) and a commercially available ELISA that recognises the C-terminal of the ¾ peptide (C1,2C) generated when triple helical collagen is cleaved by collagenases. Protein levels of collagenase (MMP-13) were assessed using a fluorogenic assay. Statistical significance was assessed using Pearson Product Moment Correlation and was set at $p<0.05$.

RESULTS

Expression of genes coding for collagens in tendon (Col1A1, Col3A1, Col5A1) and non-collagenous matrix proteins (aggrecan, biglycan, decorin, fibromodulin, lumican, COMP) did not decrease significantly with increasing horse age. Expression of collagen degrading enzymes (MMP-1, 2, 9, 13) did not decrease significantly with increasing horse age and MMP13 activity levels were not significantly different between horses of different ages. However levels of the MMP generated neoepitope, C1,2C in tendon tissue were significantly ($p=0.008$) higher in older horses while the amount of ICTP extracted from the tendon tissue decreased significantly with increasing horse age ($p<0.001$). Expression of proteoglycan degrading enzymes (MMP-3 & 10) increased significantly with horse age ($p\leq0.043$).

DISCUSSION

The results indicate that the increased half life of matrix proteins does not result from an age related decline in the ability of cells to synthesise structural proteins. Levels of collagen degrading enzyme expression also appear to remain stable as the horse ages, suggesting there is no change in the cells' ability to degrade the matrix. However an increase in MMP generated neo-epitopes in collagen suggests degraded collagen may be retained in the matrix while the reduced levels of ICTP extracted from the tissue may be a reflection of changes to the matrix making proteins less soluble. These results suggest that ageing of tendon and reduced collagen turnover may be due to changes to the extracellular matrix rather than a decline in cell activity. Accumulation of damaged and/or partially degraded collagen within the tendon has implications for tendon mechanical integrity.

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**Finalist of the Best Student Paper Award*

TENOCYTE HYPERCELLULARITY AND VASCULAR PROLIFERATION IN A RABBIT MODEL OF TENDINOPATHY-CONTRALATERAL EFFECTS SUGGEST THE INVOLVEMENT OF CENTRAL NEURONAL MECHANISMS

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INTRODUCTION

There is a need for validated experimental models of tendinopathy in order to understand the dynamic processes that cannot be captured in cross-sectional human studies. These models can also be used to evaluate potential interventions. Many animal models exercise one limb while using the non-exercised limb as a control. Clinically we have observed that patients with unilateral tendinopathy symptoms often display bilateral tissue changes on ultrasound, disputing the use of the contralateral limb as an appropriate control. Therefore, the aims of this study were (1) to validate a rabbit Achilles tendinopathy model by objectively measuring tenocyte number and vascularity in tendon tissue, and (2) to determine whether the contralateral (non-exercised) limb is an appropriate control.

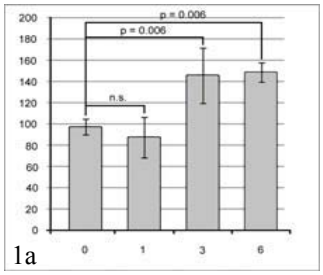
METHODS

Four groups of New Zealand adult white rabbits (6 per group) were used. Animals were subjected to low frequency electrical stimulation and passive flexion-extension of the right triceps surae muscle for 1, 3 or 6 weeks. The animals of one (control) group were not subjected to exercise/stimulation. The Achilles tendon of both limbs was collected and examined. Tenocyte number was counted in standardised areas, and vascular density was graded using the vascular subsection of the Bonar scale. In addition, morphological evaluations were performed as well as in-situ hybridisation for vascular endothelial growth factor (VEGF)-mRNA.

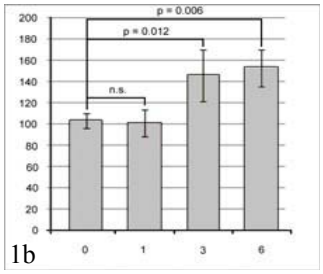
RESULTS

Fig 1a: The mean number of tenocytes in the right leg (exercised leg in test groups) was significantly higher in those animals exercised for 3 weeks and 6 weeks as compared to control animals (0). There was no difference in tenocyte numbers between animals exercised for 1 week and the controls.

Fig 1b: Notably, the same findings were seen in the left (non-exercised) leg, as were detected in the exercised leg (cf. Fig. 1a).



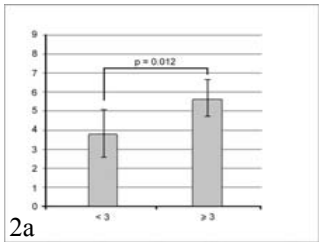
1a



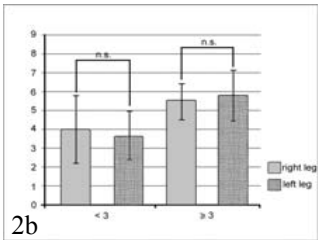
1b

Fig 2a: The vascularity (Bonar scale) was increased in the animals exercised for 3 weeks or more compared to those exercised for less than 3 weeks (including control group). Data from right and left leg were combined.

Fig 2b: In the animals exercised for less than 3 weeks there was no difference in vascularity between the right leg and left leg. The same was seen in the animals exercised for 3 weeks or more.

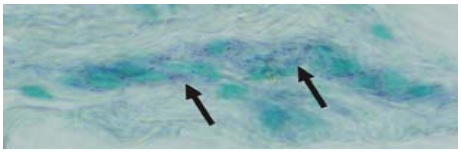


2a



2b

Fig 3: Blood vessels within the tendon tissue displayed VEGF mRNA (arrows) via in-situ hybridisation (AP-detection). These reactions were more pronounced in the rabbits exercised for 6 weeks as compared to control animals.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DISCUSSION

There were objective tissue changes characteristic of tendinopathy in the Achilles tendons of rabbits exercised for 3 weeks or more. One week of exercise was not sufficient to induce tendon changes. Notably, the same changes were observed in the non-exercised limb. These bilateral changes suggest that central neuronal mechanisms may be involved. Our findings indicate that the contralateral side is not an appropriate control in animal models.

**Finalist of the Best Student Paper Award*

MECHANICAL STRETCHING INCREASED THE EXPRESSION OF BMP-2 WHICH PROMOTED OSTEOGENIC/CHONDROGENIC AND INHIBITED TENOGENIC DIFFERENTIATION OF TENDON-DERIVED STEM CELLS (TDSCs) *IN VITRO*

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INTRODUCTION:

This study aimed to investigate the effect of repetitive tensile load on the expression of BMP-2 and the effect of BMP-2 on the osteogenic, chondrogenic and tenogenic differentiation of TDSCs in vitro.

METHODS:

Repetitive stretching was applied to TDSCs isolated from rat patellar tendon at 0, 4 and 8%, 0.5Hz. The expression of BMP-2 was detected by Western blotting and RT-PCR. BMP-2 was added to the TDSC monolayer for the detection of ALP activity at day 3, calcium nodule formation by alizarin red staining at day 10 and mRNA expression of osteogenic (RunX2, osteopontin), chondrogenic (Sox9 and aggrecan) and tenogenic (tenomodulin, tenascin C) markers at day 0, 1, 3, 7 and 10 by real-time RT-PCR.

RESULTS:

TDSCs adhered, proliferated and aligned along the direction of externally-applied tensile force while they were randomly oriented in the control group. Western blotting showed increased expression of BMP-2 in 4% and 8% stretching groups but not in the control group. Upregulation of BMP-2 mRNA was also observed in the 4% stretching group. BMP-2 increased the osteogenic differentiation of TDSCs as indicated by higher ALP cytochemical staining, ALP activity and calcium nodule formation. There was upregulation of mRNA expression of osteogenic and chondrogenic markers as well as down regulation of tenogenic markers upon treatment with BMP-2.

DISCUSSION:

Repetitive tensile load increased the expression of BMP-2 which enhanced osteogenic and chondrogenic while decreased tenogenic differentiation of TDSCs. Activation of BMP-2 expression in TDSCs during tendon overuse might account for ectopic chondrogenesis/calcification and failed healing in calcifying tendinopathy.

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This work was supported by equipment / resources donated by the Hong Kong Jockey Club Charities Trust, the Restructuring and Collaboration Fund from University Grant Council and the CUHK Direct Grant.

EFFICACY OF BONE-MARROW DERIVED MESENCHYMAL PROGENITOR CELLS FOR NATURALLY-OCCURRING TENDINOPATHY IN THE HORSE

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INTRODUCTION:

Exercise-induced tendinopathy is common in human and equine athletes. Healing results in the formation of scar tissue within the tendon characterised by increased structural stiffness, disorganised matrix and accumulated gycoaminoglycans and is associated with reduced performance and risk of re-injury. Mesenchymal progenitor cells (MPC's) offer the potential for tendon regeneration and improved functional outcome and have been used clinically in injured equine superficial digital flexor tendons[1] (SDFT). An evaluation of efficacy in clinical disease cases at the tissue level has not yet been reported.

Hypothesis: Autologous MPC implantation induces greater normalization of the tendon matrix and reduces re-injury rates compared to conventionally managed horses.

METHODS:

Experimental study: 8 horses with naturally occurring SDFT injury were used. Autologous bone marrow derived MPCs were expanded *in vitro,* suspended in citrated bone marrow supernatant and $1x10^7$ implanted in the damaged SDFT of 4 horses under ultrasound guidance. Saline was injected into 4 controls. Horses received controlled exercise and were euthanased after 6 months. Non-destructive mechanical testing assessed structural stiffness of the SDFT. Morphological and compositional analysis was performed. *Clinical study:* MPCs were implanted into 25 National Hunt racehorses with naturally occurring SDFT injury. Re-injury rate over a period of 2-years after a return to full work was compared with published conventionally treated National Hunt racehorses using the same selection and follow-up criteria in 17 horses[2].

RESULTS:

Experimental study: MPC-treated SDFT had greater elasticity than saline-treated SDFT ($p<0.05$). Cross-sectional area of MCP-treated tendons was lower than saline-treated tendons ($p<.0.05$). Histologically, MPC-treated tendons had reduced cellularity and improved organisational scores at the injured site and were comparable to uninjured sites of the treated horses. The lower cellularity was supported by lower DNA content and cell count ($p<0.05$). Collagen content was unaltered between groups, while GAG content was lower in MPC-treated SDFT although statistically significant ($p=0.06$). *Clinical study:* Re-injury rates for MPC-treated horses was lower (24%) than in conventionally managed horses (56%; $p<0.05$).

DISCUSSION & CONCLUSIONS:

Markers of regeneration have not been identified in tendon but a normalisation of biomechanical (reduced stiffness), histological (lower scores) and compositional parameters (lower GAG content) towards those levels in normal (or less injured) tendon could be considered surrogate markers of regeneration. The evidence of optimised healing seen experimentally is supported clinically where a reduction in re-injury rate was found. MPC implantation results in a tissue more like normal matrix rather than fibrous scar tissue formed after natural repair. These findings support the potential use of MPC's in human tendon and ligament injuries which share many similarities with equine tendinopathy.

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TENDON DEVELOPMENT AND TENDON CELL DIFFERENTIATION

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Tendon is a quite specific fibrous tissue that connects muscle to bone with poor self-repair capability. Currently, novel tendon tissue-engineering techniques combine the application of biodegradable biomaterials, cell, growth factors and gene transfer have been explored. However, there are no optimal methods for tendon tissue engineering. The lack of optimal induction for tenocytes differentiation hinders the process of tendon tissue engineering. Until now, little is known about the regulatory signals involved in tendon formation and differentiation and this lack of understanding has hindered the therapies for tendon repair. It is not clear whether tendon embryogenesis process resurrected during stem cells differentiation and regeneration.

Recent studies of embryonic tendon development identified scleraxis (Scx), a bHLH transcription factor gene, as a highly specific marker of tendons. The roles of Scx on vertebrate tendon development, induction and differentiation during embryogenesis has been investigated [1, 2]. Scx knockout led to the severe force-transmitting tendon defects while other tendons remains almost normal. Also TGF-smad, FGF-Pea3 signal and ectoderm signal is critical for tendon development and associate with tenocytes differentiation. Anyone of these signal defect cause the lost of tendon.

In vitro studies of tendon differentiation were much less when compare to other skeletal tissues, such as muscles, bone and cartilage. This is due to the lack of knowledge on tendon specific markers and differentiation factors. The mechanical stress has been mostly investigated on teno-lineage differentiation as mechanical stimulation seems to be of great significance in tendon healing and differentiation. Some other growth factors and transcription factors, including TGF-beta, BMP2, GDF 5, 6 ,7 and smad8 have been reported to be the possible inducer of the teno-lineage differentiation. Moreover, Tgf-betas shifted the differentiation outcome of the cultures from chondrogenesis to tendon-fibrogenesis [3]. However, although the tendon-like tissues were found in those studies, the native tendon tissues were not yet formed.

Here, the process of embryonic tendon development and current induction researches of tendon differentiation, as well as the tendon specific parameters for evaluation were reviewed. Also further directions of tendon development and tendon differentiation researches were discussed.

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**Finalist of the Best Student Paper Award*

EFFECT OF GDF-6 ON THE TENOGENIC DIFFERENTIATION OF RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN VITRO

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INTRODUCTION

Bone marrow-derived mesenchymal stem cells (BMSCs) isolated from adult bone marrow have been shown to have multi-differentiation potential. The application of BMSCs for bone and cartilage repair has been studied for many years and has been used as a new treatment in clinic. Recent investigations have tried to use BMSCs for tendon repair, but ectopic calcification was reported in some cases (Seib FP et al., 2009). In order to promote tendon repair with BMSCs, the in vitro differentiation of BMSCs to tenocytes before transplantation is suggested. Growth and Differentiation Factor-6 (GDF-6) was reported to promote tendon healing and increase tensile strength of tendon regenerate (Aspenberg et al., 1999; Vishal Mehta et al., 2005). In this study, we investigated the effect of GDF-6 on the tenogenic differentiation of BMSCs in vitro.

METHODS

BMSCs were isolated from bone marrow tissue of GFP (Green Fluorescent Protein) rats by density gradient centrifugation method. Chondrogenic, osteogenic and adipogenic differentiation assays were used to demonstrate the multi-differentiation potential of the isolated BMSCs. BMSCs at passage 3 were cultured with different concentrations of GDF-6 (0ng/ml, 5ng/ml, 10ng/ml, and 20ng/ml) for 2 weeks (5 wells / group). Afterwards, mRNA expression of scleraxis, tendomodulin, tenascin C and type I collagen (COL1A1) from 4 wells in each group was examined by qRT-PCR. The remaining sample (n=1) from each group was used for immunocytochemistry staining of tenomodulin. The protein expression of tendomodulin in cells treated with GDF-6 (10ng/ml) for two weeks was also examined by Western blotting. Kruskal-Wallis test was used to compare the difference in relative mRNA expression among different groups. If there was a difference, Mann-Whitney U test was used for 2-group comparison. All the data analysis was done using SPSS (SPSS Inc, Chicago, IL, version 16.0)，$P<0.05$ was regarded as statistically significant.

RESULT

Multi-differentiation assays have confirmed that BMSCs have osteogenic, chondrogenic and adipogenic differentiation potentials. There was increase in the expression of tendomodulin and scleraxis after treatment with GDF-6 for 2 weeks. The mRNA expression of tendomodulin significantly increased by 5.61 and 2.57 folds for the high and middle dose respectively compared to that in the control group ($P<0.05$) while the expression of scleraxis significantly increased by 4.18, 2.96 and 1.62 fold for the high dose, middle dose and low dose respectively compared to that in the control group ($P<0.05$). The mRNA expression of tenascin C and COL1A1 had no significant difference among 4 groups. Immunocytochemistry staining showed that tenomodulin expression was detected in the three treatment groups but not in control group. This was further confirmed by Western blotting which showed higher protein expression of tenomodulin in the medium dose (10ng/ml) group compared to that in the control group.

CONCLUSION

GDF-6 increased scleraxis and tenomodulin mRNA expression of rat BMSCs in dose-dependent manner in vitro.

DISCUSSION

GDF-6 may be used to promote in vitro tenogenic differentiation of rat BMSCs before transplantation for tendon repair.

ACKNOWLEDGMENT

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TENDON STEM CELLS EXHIBIT DIFFERENTIAL PROPERTIES FROM TENOCYTES

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INTRODUCTION

Tendons are subjected to large mechanical loads in vivo. As a result, they are prone to injury. Injured tendons heal slowly and often result in the formation of inferior scar tissue, which increases the risk of re-injury at the repair site. Tendons are also susceptible to loading-induced tendinopathy. Despite its high prevalence, current treatments of tendinopathy are largely palliative. Thus, the development of new effective cell therapies for the restoration of normal tendon structure and function is highly desirable, but progress has been hindered by a lack of characterization of tendon cells. Recently, remarkable progress has been made in that human and mouse tendons were shown to contain tendon stem cells (TSCs) [1] in addition to tenocytes, the known resident fibroblast-like cells in tendons. However, whether TSCs share common properties with tenocytes is not known. This study aims to define the differential properties of TSCs and tenocytes.

MATERIALS AND METHODS

TSCs and tenocytes were isolated from patellar and Achilles tendons of young rabbits accoring to a published protocol [2]. Morphology, colony formation, and proliferation of both TSCs and tenocytes were examined. In addition, the differentiation potential and cell marker expression of the two types of cells were determined using histochemical, immunohistochemical, and qRT-PCR analysis as well as in vivo implantation.

RESULTS

Morphologically, TSCs possessed smaller cell bodies and larger nuclei than ordinary tenocytes and had cobblestone-like morphology in confluent culture whereas tenocytes were highly elongated (Fig. 1). TSCs also proliferated more quickly than tenocytes in culture. Moreover, TSCs were able to differentiate into adipocytes, chondrocytes, and osteocytes in vitro, and form tendon-like, cartilage-like, and bone-like tissues in vivo. In contrast, tenocytes had little such differentiation potential. Finally, TSCs expressed the stem cell markers Oct-4, SSEA-4, and nucleostemin, whereas tenocytes expressed none of these markers.

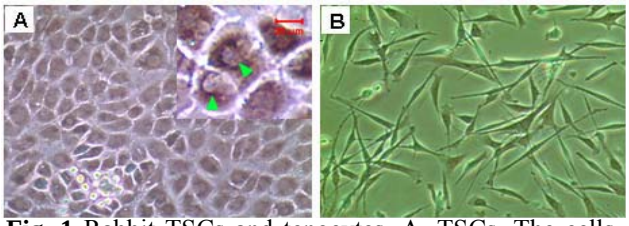

Fig. 1 Rabbit TSCs and tenocytes. A. TSCs. The cells were cobblestone-like under confluent conditions and had large nuclei (inset, arrows). B. Tenocytes. The cells exhibited a highly elongated shape, a typical fibroblast phenotype. Besides the differences in cell morphology, TSCs differed from tenocytes in their cell marker expression, proliferative, and differentiation potential (see text).

DISCUSSION

This study showed that TSCs differ from tenocytes in morphology in culture, proliferative potential, and expression of stem cell markers (Oct-4, SSEA-4, and nucleostemin). Additionally, unlike tenocytes, TSCs were shown to possess multi-differentiation potential. Future research should determine whether TSCs can be used for more effective repair or possibly for regeneration of injured tendons. In addition, considering that tendons are constantly subjected to mechanical loading, future studies should look into the mechanobiology of TSCs and the interactions of TSCs with tenocytes, so that tendon physiology and pathology (e.g. tendinopathy) can be better understood.

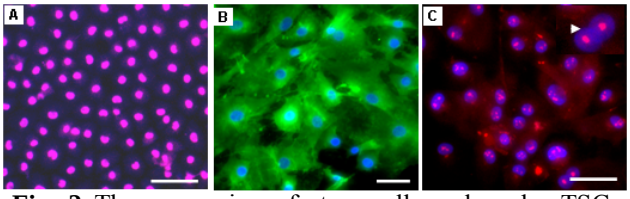

Fig. 2 The expression of stem cell markers by TSCs derived from rabbit patellar tendons. A. Oct-4; B. SSEA-4; and C. nucleostemin (arrow points to nucleostemin in pink). Similar findings were obtained on TSCs derived from Achilles tendons. However, expression of these markers was not detected on tenocytes (data not shown).
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ACKNOWLEDGEMENT

This work was supported in part by NIH AR049921 and AR049921S2 (JHW).

CONTROL OF TENDON STEM CELL DIFFERENTIATION BY NANO-TOPOGRAPHY THROUGH INTEGRIN AND MYOSIN II PATHWAY

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INTRODUCTION

Tendon is a specific connective tissue composed of parallel nano-grade collagen fibers. The effect of this tissue-specific matrix orientation on stem cell differentiation has not been investigated. This study aimed to determine the effects of nano-topography on the differentiation of human tendon stem/progenitor cells (hTSPCs) [1] and develop a biomimetic scaffold for tendon tissue engineering.

METHODS

Aligned and randomly oriented poly (l-lactic acid) nanofibrous membranes were fabricated by electrospining. The immuno-phenotype of fetal hTSPCs was identified by flow cytometry. Scanning electron micrographs was used to examine cell morphology on the nanofibers. Gene expression was detected by real-time PCR. Histological analysis was conducted to evaluate *in vivo* results.

RESULTS

The multipotency of hTSPCs toward osteogenesis, adipogenesis and chondrogenesis was confirmed. SEM micrographs showed that hTSPCs were spindle-shaped and well orientated on the aligned nanofibers. The expression of tendon-specific genes was significantly higher in hTSPCs growing on aligned nanofibers than those on randomly oriented nanofibers in both normal and osteogenic media. And the osteogenic markers were expressed more in the hTSPCs growing on random group than aligned ones. In addition, ALP activity and alizarin red staining showed that the randomly oriented nano-scaffold induced osteogenesis, while the aligned scaffold hindered the process. Moreover, aligned cells expressed higher levels of integrin α1, α5 and β1 subunits and myosin II B ($p < 0.05$). In *in vivo* experiments, the aligned nanofibers induced the formation of spindle-shaped cells and tendon-like tissue.

DISCUSSION

The present study is the first demonstration that an aligned nanofibrous scaffold induces teno-lineage differentiation of human tendon stem cells. More importantly, this matrix-specified lineage resisted the power of osteogenic induction medium. Furthermore, the aligned nanofibrous scaffold influenced the differentiation of hTSPCs through an integrin-mediated mechanotransduction pathway. In conclusion, the aligned electrospun nanofiber structure provides an instructive microenvironment for hTSPC differentiation and could serve as an optimal scaffold for tendon regeneration.

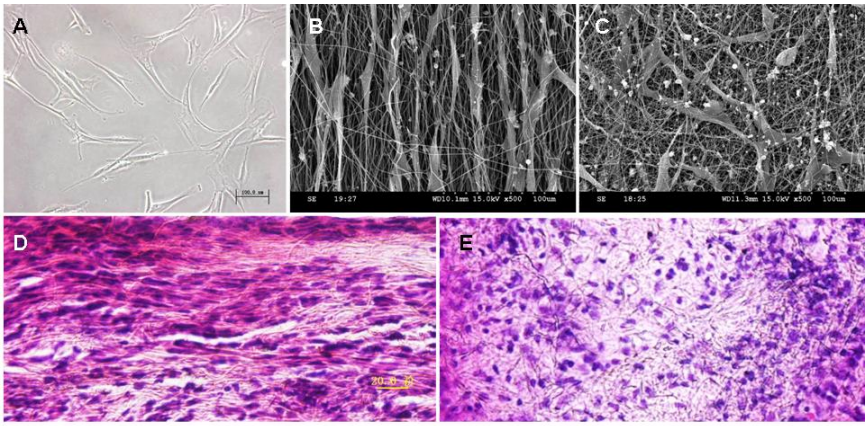

Fig. (A) Morphology of human tendon stem/progenitor cells at P2. Morphological changes of hTSPCs growing on the topographical nano-scaffolds. (B) and (C) show hTSPCs cultured on the aligned and randomly-oriented nanofibrous scaffold respectively. Histology of hTSPCs cultured on nano-scaffolds implanted subcutaneously in nude mice 1 week after surgery. (D) and (E) Typical hematoxylin and eosin staining of aligned and randomly-oriented constructs, respectively.

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ISOLATION AND CHARACTERIZATION OF HUMAN ACL-DERIVED VASCULAR STEM CELLS

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INTRODUCTION

Anterior cruciate ligament (ACL) usually fails to heal after rupture mainly due to the inability of the cells and blood vessels within the ACL tissue to build an adequate healing process, in which cases graft reconstructions are essential. However, some reports have shown healing potential of ACL with primary suture repair. Whereas some reports show the existence of mesenchymal stem cell-like cells in human ACL tissues as a potential explanation for healing, their origin and characteristics still remain unclear. Recently, blood vessels have been reported to be a richer supply of stem/progenitor cells with a characteristic of expression of CD34 and CD146 surface maker [1-4]. Therefore, we attempted to validate the hypothesis that there exist CD34 and CD146-expressing vascular cells in the ACL tissues which have a potential for multi-lineage differentiation and are recruited to the ruptured site of ACL for intrinsic healing.

METHODS

Human adult ACL ruptured tissues were harvested from subjects undergoing ACL reconstruction (22.5±3.8 years old, 5.1±1.9 post-injury, n=8).

RESULTS

Immunohistochemistry and flow cytometry analysis demonstrated that human ACL tissue contained significantly more CD34 (stem cell marker) and CD146 (pericyte marker) positive cells in the rupture site compared to the mid-substance ($p<0.05$). Isolated CD34+CD45- cells from the ruptured site, which converted into mesenchymal stem cell-like cells after cultivation, showed significantly higher expansion potential compared to CD146+CD45- and CD34-CD146-CD45- cells ($p<0.05$). Cultured CD34+CD45- cells have a high multilineage differentiation potential including chondrogenesis especially for the osteogenic, adipogenic, and endothelial differentiation compared to the other cell populations ($p<0.05$).

DISCUSSION

Our study showed CD34 and CD146+ cells, which were found around and wall of blood vessels more in the ruptured site than in the mid-substance, exhibited potential for multilineage differentiation including osteogenesis, chondrogenesis, adipogenesis, and endotheliogenesis, suggesting that these populations exhibit vascular stem cell characteristics. Especially, CD34+ cells in the ruptured site, which have higher proliferation ability and differentiation potential of osteogenesis and endotheliogeneis, could be a therapeutic strategy for tendon-bone healing and regeneration following ACL reconstruction.

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REGULATION OF ADULT STEM CELL FATE AND FUNCTION IN TISSUE ENGINEERING AND REGENERATION

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Multipotent adult mesenchymal stem cells (MSCs) are considered a promising cell type for the engineering and regeneration of diseased and injured tissues. Key functions of MSCs that are critical to their regenerative activities are: (1) the ability to undergo guided or induced differentiation into specific cell and tissue lineage; (2) extensive proliferative ability to produce sufficient cell number for clinical applications; and (3) recently discovered trophic activities that allow MSCs to regulate and interact with host cells to enhance intrinsic tissue reparative and regenerative potential. Successful application of MSCs in tissue engineering and regeneration is critically dependent on understanding the underlying mechanisms responsible for these characteristics of MSCs. We have undertaken a high-throughput screening for candidate genes that act to regulate MSC cell fate and differentiation, and have begun to characterize the mechanism of action of these genes. The concept of niche in regulating MSC fate is an important concept in stem cell biology, and refers to the biological actions of microenvironmental factors that include secreted signaling molecules, metabolites, extracellular matrix, cell- and cell-matrix interactions, bioactive gases, and mechanical influences. MSCs also participate, in both autocrine and paracrine fashion, in the action of the niche. The trophic action of MSCs includes anti-inflammatory, pro-survival, and pro-regenerative activities, and is likely to feature prominently in successful regenerative strategies. Finally, delivery of MSCs to target tissues must be optimized, and requires the development and application of biomimetic scaffolds that provide a functional haven for cell proliferation, differentiation, and interactions. Stem cell-based tissue engineering and regeneration serves as an exciting platform for collaborative interactions among life scientists, engineers, and clinicians.

PROPAGATION OF FULL-THICKNESS ROTATOR CUFF TEARS – A THREE-DIMENSIONAL FINITE ELEMENT ANALYSIS

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OBJECTIVE:

The propagation pattern of rotator cuff tearing has not been fully clarified yet. The purpose of this study was to investigate the pathomechanism of the propagation of the full-thickness rotator cuff tears using 3-dimensional (3D) finite element method.

METHODS:

A normal cadaveric humeral head (69-year-old male) was used for the current study. All soft tissues except rotator cuff tendons were removed, which was then examined with CT scan. The DICOM data of the specimen were imported to the commercial software for the finite element analysis, Mechanical Finder (extended edition, version 6.0, RCCM, Japan). A 3D model of the rotator cuff tendons attached to the humeral head was developed (Fig. 1).

To recreate the contact condition, GAP elements were inserted between the articular cartilage of humeral head and the deep surface of rotator cuff tendons. A semicircular cuff defect (diameter: 1, 2, 3 cm) was created on the supraspinatus tendon close to its insertion. The material properties of bone were calculated using its CT number based on the data proposed by Keyak, et al. The Young's modulus and the Poisson's ratio of tendon were determined as 305.5 MPa and 0.497, respectively. To simulate the abduction of the shoulder, tensile loads (supraspinatus: 50N, subscapularis: 23N, infraspinatus: 63N) were applied to the proximal end of cuff tendons based on Kronberg's report. Then, the distribution of Dracker-Prager's equivalent stress was calculated.

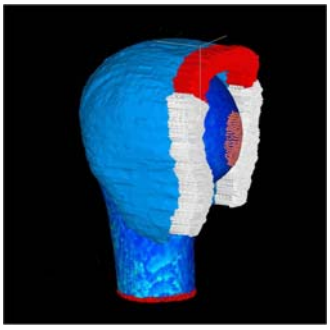

Fig. 1: 3D model of the humeral head with rotator cuff tendons.

RESULTS:

In all three models, the highest stress concentration was seen at the lateral margin in the torn tendon stump, which extended both anteriorly and posteriorly (Fig.2). The highest value of the equivalent stress increased with the size of tears (1cm: 1.6MPa、2cm: 2.2MPa、3cm: 2.7MPa).

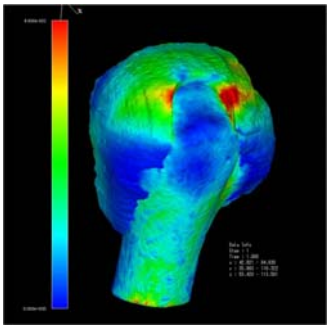

Fig. 2: Stress distribution. High stress is seen at the lateral margin in the torn tendon stump.

DISCUSSION AND CONCLUSION:

It was known that the complete tear of the rotator cuff tendon gradually propagated with time. The results of the current study demonstrated that the full-thickness tear of the rotator cuff tendon propagated both anterior and posterior directions with the zipper phenomenon.

A NOVEL EXTRACELLULAR MATRIX BIOSCAFFOLD CAN ENHANCE ACL HEALING

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Swanson School of Engineering, University of Pittsburgh, Pittsburgh, PA

INTRODUCTION:

Recently, there has been a renewed clinical interest in healing the anterior cruciate ligament (ACL) by means of biological augmentation [1]. Laboratory studies has also shown that ACL healing could take place using tissue engineering; however, the neo-tissue is hypertrophic while its biomechanical properties are abnormal. Previously, we have success in utilizing extracellular matrix bioscaffolds, namely the porcine small intestinal submucosa (ECM-SIS), as a biological agent to improve the biomechanical properties of healing ligaments and tendons [2]. Thus, we wished to investigate how ECM-SIS in combination with its hydrogel could enhance healing of a fully transected ACL following primary repair. We hypothesize that the bioactive agents in the SIS hydrogel[3] can accelerate the formation of healing tissue, while a layer of ECM-SIS wrapped around the injury site can guide neo-tissue formation with limited hypertrophy [2]; and as a result, the two could work in synergy to heal an injured ACL with sufficient biomechanical properties that could contribute positively to knee stability.

METHODS:

Fourteen skeletally mature female goats were used. Seven animals had the ACL of their right stifle joint transected and repaired with sutures so that the degree of lack of healing could be established. In the remaining seven animals, the ACL was transected and repaired, and an ECM-SIS sheet was wrapped around the injured ACL. The SIS hydrogel was then injected directly into the injury site. The ECM-SIS bioscaffolds used in this study were obtained from genetically-modified pigs, in which the galactosyl-α(1,3)galactose (αGal) epitope is eliminated, in order to limit the immunogenicity porcine tissues in potential applications in humans [4]. The left stifle joint was sham-operated and served as a control. After 12 weeks, all animals were euthanized and assessed in terms of gross morphology. Two animals in each group were assigned forhistological studies. For the αGal(-) SIS-treated group, five specimens were used for biomechanical testing. The knee kinematics and in-situ force of the ACL in response to a 67N anterior-posterior (A-P) load were measured using a robotic/universal force-moment sensor testing system[5]. After, the cross-sectional area (CSA) of the ACL was measured [6]. The femur-ACL-tibia complexes (FATCs) were subjected to uniaxial tensile testing and its stiffness and ultimate load were obtained.

RESULTS:

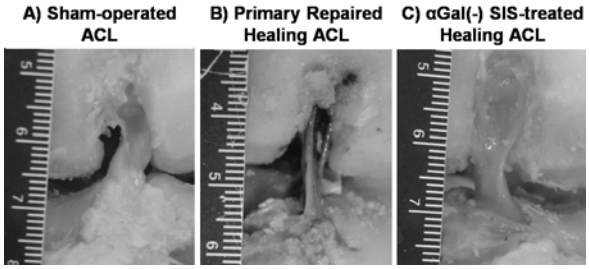

Figure 1. Gross morphology of ACL after 12 weeks. (A) sham-operated, (B) following primary repair only, and

Indeed, gross observation of the primary repair only group showed significant ACL resorption (Fig. 1B). Out of seven stifle joints, one ACL had completely resorbed in the synovial environment, three had only a small amount of tissue, while the remaining three still had some tissue concentrated around the sutures. Histological evaluation revealed only loose connective tissue without normal collagen orientation. On the other hand, the healing ACL following αGal(-) SIS-treatment showed continuous neo tissue formation (Fig. 1C), and histological observation revealed aligned collagen fibers and spindle-shaped cells. However, the fibers were less dense than the sham-operated control. Also, a synovium covering over the surface of the neo tissue was clearly visible. Functionally, the A-P tibial translation of the knee was less than one half of the ACL-deficient knee ($p<0.05$). Values for in-situ force in the healing ACL were 98% and 93% of those for the sham-operated ACL at 30° and 60° of knee flexion. The cross sectional area of the healed ACL was also similar to the sham-operated ACL (21.4±9.1mm² vs. 22.2±4.5mm², respectively, $p>0.05$). In terms of stiffness, the FATCs following αGal(-) SIS-treatment reached 42% of those for the sham-operated controls (48 ± 20 N/mm vs. 114 ±19 N/mm, respectively, $p<0.05$). The ultimate load of the αGal(-) ECM treated group reached 186 ± 86 N. Both the stiffness and ultimate load were comparable to those for ACL reconstruction in goats at twelve weeks post surgery[7].

DISCUSSION:

This study showed that the application of αGal(-) SIS bioscaffolds in combination with αGal(-) SIS hydrogel together with primary repair had positively contributed to ACL healing with tissue formation but without tissue hypertrophy. The healing ACL could function to reduce knee instability, supporting our hypothesis. Thus, this study offers the future possibility for further development and improvement of biological augmentation, e.g. combination of bioscaffolds and hydrogels as well as other biopolymers with the controlled and sustained release of key growth factors, to promote the healing of ACL.

ACKNOWLEDGEMENTS:

Financial support from Commonwealth of Pennsylvania and NSF Engineering Research Center Grant (#0812348). REFERENCES: 1. Murray et al. JOR 2007. 2. Liang et al. JOR 2006. 3. Freytes et al. Biomaterials 2008. 4. Phelps et al. Science 2003. 5. Livesay et al. ABME 1995. 6. Woo SL-Y et al. JBME 1990. 7. Ng et al. JOR 1995.

**Finalist of the Young Faculty Paper Award and the Best Research Fellow Paper Award*

IN VIVO OSTEOGENESIS OF LIGAMENTUM FLAVUM CELLS IN PHOTO-RESPONSIVE HYDROGEL ENCAPSULATED BONE MORPHOGENETIC PROTEIN-2: A NUDE MICE MODEL

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INTRODUCTION:

Osteogenesis of the ligamentum flavum (LF) is a widely recognized pathophysiologic factor in spinal stenosis and in other pathologic ossifications of the spinal ligament. Bone morphogenetic protein-2 (BMP-2) and the BMP-2 receptor have been reported to be present in the ossification of LF, which suggests a mechanism of osteogenic differentiation of degenerated LF. [1] The potential for biomimetic constructs with co-immobilized adhesion and rhBMP-2 to induce osteoinduction and osteogenesis was demonstrated in our recent study.[2] Ligamentum flavum excised during the decompressive spinal procedure will be sent for histological study or discard. We expect the novel rhBMP-2 tether photopolymerized gel will modified the encapsulated ligamentum flavum cells to be osteogenic and induce osteogenesis in vivo. This preliminary study will define the role of ligamentum flavum cells in the application of tissue engineering for osteogenesis.

METHODS:

The Ligamentum flavum (LF) tissue was obtained from spinal stenosis patients during surgical spinal decompressive procedures (Figure 1-A). LF tissue specimens were digested in Neuman-Tytell serumless medium (Gibco BRL) containing 250 U/mL type 1A collagenase (Worthington Biochemical Corp) at 37 C in 5% CO2 for cell isolation (Figure 1-B). RhBMP-2 (250 ng/ml) was conjugated to polyethylene glycol (PEG) by reacted with an equimolar amount of acrylate-PEG-N-hydroxysuccinimide .3 Three 2¡Ñ105 cells/construct groups were prepared, included LF cells encapsulated in no BMP-2 (Control group), in soluble 250 ng/ml BMP-2 (BMP group), and in PEG-tethered 250 ng/ml BMP-2 (PEG-BMP group). A 50-£gl aliquot of cell.polymer. photoinitiator solution was subsequently loaded into disk-shaped molds with a 4-mm internal diameter, followed by photopolymerization with 365 nm UV light to gelate the cell.polymer constructs (Figure 1-C). The constructs were washed with sterile PBS containing 1% penicillin-streptomycin, and incubated in a bioreactor under 20 rpm for 7 days before surgery. Nude mice (BALB/c nu/nu) were anesthetized, aseptic preparation, and draping over the dorsum of the mouse. Constructs were implanted into the back of nude mice (Figure 1-D), respectively. After operation for 6 weeks, constructs were taken out. Alkaline phosphatase activity, amount of DNA, histological and immunofluorescence assays will be examined for the evidence of osteogenesis.

RESULTS AND DISCUSSION:

The significant opaque constructs in PEG-BMP group were obtained after 6 weeks (Figure-2-A). Clear constructs were maintained in control group (Figure-2-C). Amount of DNA was the same among three group. The tendency of alkaline phosphatase (ALP) expression in construct was PEG-BMP> BMP> Control (Figure-3). The higher expression of collagen type I and alkaline phosphates activity of LF cells in PEG-BMP group than in BMP and control group at 6 weeks was found (Figure-4). LF cells performed low osteogenesis without BMP-2. Free BMP-2 was lost easily from PEGDA. The LF cells maintained osteogenesis in PEG-BMP group longer due to chemical bond between PEG and BMP-2. This result in vivo was the same as our previous studies in vitro. Ligamentum flavum cells in PEG-BMP group had shown potential in the application of tissue engineering for osteogenesis.

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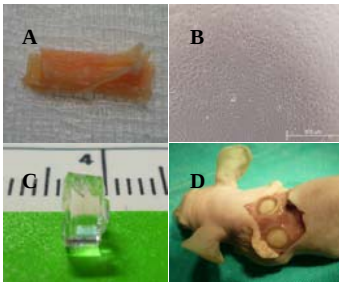

Figure 1. A: human LF tissue, B: LF cells, C: LF cells constructs, D: LF cells constructs implanted into a nude mice for 6 weeks.

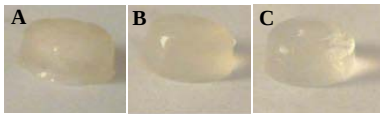

Figure 2. LF constructs in nude mice for 6 weeks. A: PEG-BMP, B: BMP, C: Control.

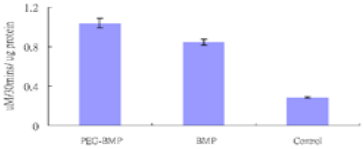

Figure 3. Alkaline phosphatase activity in nude mice at 6 weeks.

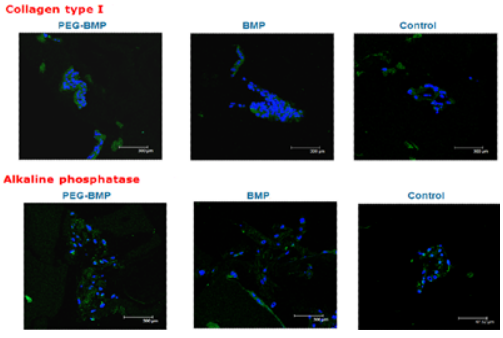

Figure 4. Collagen type I and alkaline phosphatase expression in nude mice at 6 weeks.

DOES A BIOMECHANICALLY IDEAL OVINE ANTERIOR CRUCIATE LIGAMENT AUTOGRAFT PRESENT EARLY SIGNS OF DEGRADATION?

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PURPOSE

The accurate position of the graft is believed to be vital for the long term functioning and success for any reconstructed ACL (Brophy and Pearle 2009). Currently, there are intense investigations into optimizing the biomechanics of ACL replacements (Brophy and Pearle 2009, Zantop et al. 2006) using tendon autografts based on the concept that if initial mechanics were perfectly reproduced, graft biology of each autograft will be optimized and graft degradation prevented. The theory is that if grafts don't break down and normal joint stability is maintained over time, then potential meniscal damage and onset of osteoarthritis (OA) may also be prevented. The aim of this study was to test this hypothesis in an ovine model of a biomechanically ideal ACL autograft (using the intact normal ACL itself as a "graft") looking for early signs of degradative inflammation within this structure during its early healing processes.

METHODS

Eighteen skeletally mature 3-4 year old female Suffolk-cross sheep were allocated into 3 groups: surgical, surgical sham, and control. The surgical group was composed of 9 animals that had ACL autograft surgery via arthrotomy. The surgical sham group consisted of 3 animals, 6 animals were allocated to the control group. At 2 weeks post surgery, each animal was sacrificed and both the treated and contralateral limbs were harvested. The ACL was dissected out of the joint and the anterior medial (AM) and posterior lateral (PL) bands were examined via real time RT-PCR for mRNA levels of inflammatory and degradative markers, IL1-beta and MMP 13 respectively. Two tailed Student's T-tests for unequal variance was used to verify the potential differences between groups. Changes between surgical and the corresponding contralateral, surgical and sham surgical, and finally surgical and normal control were compared. Differences were considered significant when $p<0.05$.

RESULTS

The AM band of the ACL exhibited significant elevations in IL1-beta mRNA levels in the surgical group when compared to the contralateral un-operated limbs, surgical shams, and control groups. Additionally, similar elevations in IL-1beta levels were observed in the PL band of the experimental group when compared to surgical shams and controls. The PL bands also displayed a significant IL1-beta elevation when the surgically operated limb was compared to its contralateral un-operated limbs. MMP 13 levels were elevated in the ACL, interestingly MMP-13 mRNA levels are significantly increased in the PL band when compared to the AM band.

DISCUSSION

In this ovine model, the ideal ACL graft undergoes a significant inflammatory and degradative response, despite being anatomically placed. In other words anatomic placement did not protect the graft from the expected inflammatory remodelling effects, implying that other biologic strategies are likely required to protect any graft (and particularly less anatomically placed tendon grafts) from these biological effects.

The strengths of this ovine model are realized in the physical size of the animal, it is large enough to ensure that normal anatomy is truly being recreated and it provides a considerable amount of tissue for evaluation. It must be noted that we are studying a quadruped model with potential load sharing differences from those of bipeds. This short term study concentrated only on a focused number of degradative/ inflammatory markers making it difficult to form predictions on the longevity of the graft. Therefore, we will have to be expanded this study to later time points in order to asses the physical impact on the graft material, as well as the health of the joint as a whole, to determine if degradative effects do, in fact, dominate in this model.

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EFFECT OF ENAMEL MATRIX DERIVATIVE ON TENDON–BONE INTERFACE IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION IN RATS

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INTRODUCTION:

Anterior cruciate ligament (ACL) reconstruction with a graft of semitendinosus or/and gracilis tendon is common and well-established treatment for ligament rupture. However, the weak link during the early healing process in the attachment site between the tendon and bone and the rate of healing and strength of this attachment could be one of the critical factors for successful ACL reconstruction. To improve this limitation, recent studies have shown that various growth factors have potential for tendon-bone integration. In this study, we focused on enamel matrix derivative (EMD) for tendon-bone integration. This EMD has a potential for stimulation of mesenchymal stem cells following up-regulation of Sharpey's fiber formation and has already been applied to dental field for treatment of tooth root. Inspired these backgrounds, we hypothesized that EMD applied to tendon-bone junction during ACL reconstruction could accelerate tendon-bone healing with up-regulation of Sharpey's fiber formation. The purpose of this study is to evaluate the histological and mechanical effect of EMD on tendon-bone interface in ACL reconstruction.

METHODS:

Surgical Procedure

Female Sprague-Dawley rats were used in this study. The both knees of each animal were underwent ACL reconstruction using the flexor digitorum tendon. Using ø2mm of the dental drill, bone tunnels were made in the proximal tibia and the distal femur. Then the graft was passed through the bone tunnels. After fixation of proximal side on femur, the distal side of graft was secured on the proximal tibia under pretension 100g. Rats were allowed to bear full weight with no limitation of range of motion.

Material preparation

We used commercially available EMD (EMDOGAIN®, Seikagaku Corporation, Osaka, Japan). In left knee joint, around the tendon-bone interface on tibia side was filled in 40µl of EMD with propylene glycol alginate (PGA) as a carrier of proteins (EMD). In right knee joint, around the tendon-bone interface on tibia side was curetted and filled in only PGA (control). All animals were evaluated at 4, 8, 12 and 16 weeks with histological and biomechanical analysis (n = 6 in each time point).

Histological Evaluation

At 4,8 and 12 weeks, the knee joints were harvested and fixed in 10% buffered formalin. After decalcification, samples were embedded in paraffin and cut into 5-µm thick longitudinal sections to the bony tunnels in the femur and tibia. Haematoxylin- eosin (H-E) and Azan staining were carried out using these slides.

Mechanical Evaluation

The mechanical properties of the graft were measured using the both knee specimens of each rat in a conventional tensile tester. After removal of all extraneous soft tissues from the femur-graft-tibia units, the femur and tibia were set and measured the ultimate load to failure at a cross-head speed of 1 mm/min in line with the long axis of the ACL.

RESULTS:

Histological analysis

We examined six samples at 4,8,and 12 weeks after treatment in both groups. In the EMD group, significant increase of collagen fibers and progressive maturation in the interface was observed, although tendon-bone interface was composed of cellular and vascular fibrous tissues in the control group at 8 weeks after surgery.

Mechanical analysis

The average load to failure in the treated specimens was significantly greater than in the control specimens for the entire study population as well as at the individual 8-, 12-, and 16-week time points. At 8- and 12-week time points, specimens treated with EMD significantly stronger than control specimens.

DISCUSSION:

In this study, it was demonstrated that EMD has a potential for improvement of tendon-bone infiltration after ACL reconstruction histologically and mechanically especially in early period. In the clinical setting, ACL reconstruction procedures require healing of tendon grafts in the surgically created bone tunnel. Firm attachment of the tendon graft to the bone is a critical factor allowing earlier and more aggressive rehabilitation and earlier return to sports and work. However, more precise investigations would be required for applying to clinical situations, this clinically available material could be an attractive option supporting successful ACL reconstruction in the near future.

RESEARCH OF TISSUE ENGINEERED TENDON AND ITS CLINICAL TRIAL IN REPAIRING OF LIGAMENTS AND TENDONS

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AIMS

Tendon injury is one of the most common problems in clinical surgery. Because of the special anatomic characters of the tendon, it is easily adhered with the surrounding tissue. At least 60% of repaired tendons need supplement surgery of tendolysis in China. On the basis of our previous research, the flexor digitorum in tendon sheath in *Macaca mulatto* was repaired with the tissue engineering technique. Based on our in-depth basic research for 20 years, 5 volunteers with old defect of Achilles tendon, 11 with coracoclavicular ligament injury were repaired by tissue engineered tendon.

METHODS

Animal experiment——After amplification culture, the tendon cells were seeded into the bio-derived tendon scaffold material for co-culture. The 3 flexor digitorum profundus of fingers of each hand in 15 *Macaca mulattos* were resected and 25mm defects in the tendon sheath were made as experimental model. They were divided into three groups according to the repair methods. Group A: tissue engineered tendon which was composited of co-culture of allogeneic tendon cells and bio-derived material; Group B: simple bio-derived material; Group C: autograft (autogeneic tendon transplantation). In the different stages (1^{st}, 2nd, 3rd, 6th, 12th week), their morphological, histological and ultrastructural characters were observed. The content of ammonia terminal peptide of type Ⅰ procollagen (PINP) were measured by radio immunoassay. After 12 weeks, DNA was extracted from implanted micro-tissue and performed PCR to show the electrophoresis typing of two sensitive short tandem repeat locus (CSF1PO and PLA2A).

RESULTS

Animal experiment——All the animals were survived. The wound were healed with first intention. There were no ruptured in the anastomotic site between the implanted tendon and host tendon. In group A, the new tendon was white and sheeny, the surface was smooth and no obvious adhesion with surrounding tissue, and it can glide in the sheath. The histological examination showed that PGA was almost completely degraded, the tendon cells were spindle-like or slight strip- like, no obvious lymphocytes infiltration was observed and the tissue fibers arranged uniformly and regularly with capillary hyperplasia. Scanning electron microscope observation revealed that the tendon cells aligned regularly and evenly among the materials; the collagen fibers formed network, whose main direction was aligned with the longitudinal axis of the materials. The analysis of PINP content showed that the collagen synthesis of group A and C was higher than that of group B ($P<0.05$), and no statistical significant difference between group A and C ($P< 0.05$). The labeled cells were positive by IHC methods in group A before 6 weeks of implantation, and it was weak positive at the 12th week. There were no positive labelled cells could be observed in group B and group C. The electrophoresis typing of short tandem repeat loci showed that there was non-autogeneic allele in the implanted tissue in group A. It proved the tissue engineered tendon was survived in vivo. Clinical trial——The patient were followed up over 35 months. The clinical results were satisfactory. The patients could lift heel and carry weight. The shoulder function of the patient was recovered well after operation, and no local or systemic immunological rejection were occurred. MRI examination showed similar signal between repaired side and healthy side.

DISCUSSIONS

Autogeneic healthy tendon transplantation is the common method to repair the tendon damage in clinic situation, which increases the patient's pain, complication and cost, and still most of repaired tendons need supplement surgery of tendolysis, especially in the area of tendon sheath. The characters of anatomic configuration and structure of hand in monkey are mostly similar to that of human being. The results reveal that tissue engineering technique may be a probable novel method to solve those problems. The clinical trial showed that the repair of Achilles tendon and coracoclavicular ligament injuries by tissue engineered tendon is a feasible method.

**Finalist of the Best Research Fellow Paper Award*

LUBRICIN REDUCES ADHESION FORMATION AND IMPAIRS THE HEALING OF REPAIRED FLEXOR TENDON

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INTRODUCTION

Restoration of flexor tendon function after flexor tendon laceration especially in zones 1 and 2 has been recognized as a difficult surgical problem. Adhesion formation continues to be a common problem after flexor tendon repair[1]. It was found the gliding resistance between flexor tendon and pulley significantly increased after flexor tendons and pulley were repaired[2]. The high friction suture techniques caused more adhesion formation than the lower friction suture techniques[3]. In order to improve tendon gliding and digit function, the low frictional suture materials and methods have been developed[3,4]. However, the outcomes of repair are still far from satisfaction. New approaches are necessary for further development.

Lubricin, also known as superficial zone protein and proteoglycan 4, was first identified in synovial fluid[5]. It is well known that lubricin plays an important role in boundary lubrication. The surface modification of the repaired flexor tendon with lubricin reduced the gliding resistance and improved the gliding ability of a repaired flexor tendon in a canine model in vitro[6]. In addition, lubricin limits the incorporation of the separated cartilaginous tissues[7,8]. Therefore, it may further inhibit the adhesion formation of the flexor tendon. The purpose of this study was to investigate the effects of lubricin after flexor tendon repair in a canine model.

METHODS

Sixty dogs were randomly assigned to have the repaired tendons treated either with lubricin (n=30) or saline (control group, n=30). The 2nd and 5th FDP tendons in one front paw of each dog were fully lacerated, repaired in zone II, and treated with 260 μg/ml lubricin or saline. Passive digit and wrist motion rehabilitation was initiated at day 5 postoperatively and continued daily until sacrifice at 10 days (n=20), 21 days (n=20), and 42 days (n=20). The adhesion formation of the repaired tendons was evaluated with normalized work of flexion (nWOF). The healing of the tendon was assessed with histology, repair strength and incidence of gap formation and tendon rupture.

RESULTS

The nWOF of the repaired tendons treated with lubricin were significantly lower than those of the untreated repaired tendons at all time points ($p < 0.05$)(Fig. 1). However, the repair failure strength of the lubricin-treated group was also significantly lower than that of the control group at days 21 and 42 ($p < 0.05$) (Fig. 2). The total incidence of gap formation and rupture almost doubled with the lubricin treatment (50%) comparing to control (27%) (Table.1). The rates of large gap and rupture were higher in lubricin-treated tendons than that of control tendons, especially day-42. Thirteen of 60 repaired tendons in the lubricin group ruptured, but only two in the control group.

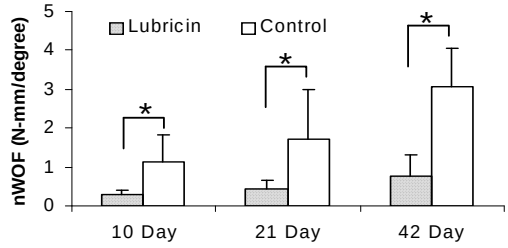

Fig. 1. The normalized work of flexion of the repaired

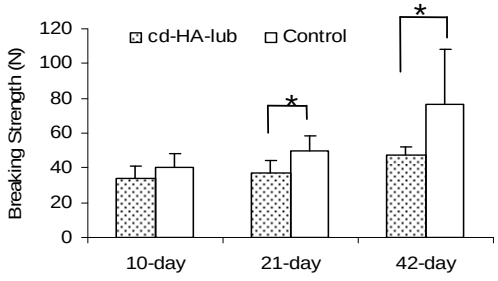

Fig. 2. Breaking strength of the repaired tendons

DISCUSSION

Lubricin effectively decreased the adhesion formation after flexor tendon repair. This could result from both physical and biological abilities of lubricin: lubrication and inhibition of tissue integration, which were identified in cartilaginous tissues[5,7,8]. Lubricin may biologically limit the integration of the repaired flexor tendon with the surrounding tissues. Consistent with adhesion reduction, we found that lubricin inhibited the healing of the repaired tendon. This study provides evidence that lubricin is an important factor in inhibiting the healing of flexor tendon. Lubricin may also be detrimental to the healing of other intrasynovial tissues, such as ACL and rotator cuff. A new strategy is needed to localize lubricin on the surface of the repaired flexor tendon combined with better protection of the lacerated tendon ends from lubricin penetration.

Table 1. Incidence of gap and rupture.

	Control/Lubricin			Total
Time/days	10	21	42	60/60
1-3 mm gap	5/5	5/2	1/2	11/9
>3mm gap	2/2	1/2	0/4	3/8
Rupture	1/3	1/3	0/7	2/13
Total	8/10	7/7	1/13	16/30

ACKNOWLEDGE

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CELLULAR RESPONSE AND EXTRACELLULAR MATRIX BREAKDOWN IN ROTATOR CUFF TENDON RUPTURE

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PURPOSE

Rotator cuff tendonopathy is considered to be a degenerative disorder characterized by the disruption of extracellular matrix (ECM). The aim of this study is to investigate the relationship between the disruption of ECM and cellular response including autophagic cell death, apoptosis and cell differentiation into myofibroblasts in the degenerative rotator cuff.

METHODS

Tendon samples were collected from 30 patients undergong surgery for rotator cuff tears. Autophagic cell death of the tendon cells in ruptured rotator cuff tendon was detected by immunohistochemical staining for ubiquitin. Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL assay) was performed to detect apoptosis. Myofibroblasts were identified immunohistochemically with anti-alpha-smooth muscle actin anti-body. The distribution of autophagic cell death, apoptosis, myofibroblasts and cell density were assessed correlated with the disruption of ECM which was graded 0-3 points using a customized scoring system.

RESULTS

Autophagic cell death, apoptosis and myofibroblasts were observed in all of the samples. The highest percentage of autophagic cell death (51.9±1.5%) was evidenced in grade 2 matrix, significantly different from that in matrix graded 0, 1 and 3 ($P_{2Vs0}<0.001$; $P_{2Vs1}<0.001$; $P_{2Vs3}=0.008$, respectively), while the highest apoptosis (34.8±1.6%) was found in grade 3 matrix($P_{3Vs0}<0.001$; $P_{3Vs1}<0.001$; $P_{3Vs2}=0.044$, respectively). The percentages of myofibroblasts significantly increased as the ECM degenerated, with the peaked percentage in grade 3 matrix (19.8±1.3%)($P_{3Vs0}<0.001$; $P_{3Vs1}<0.001$; $P_{3Vs2}=0.044$, respectively). The total cell density varied among the 4 ECM grade with significant maximal and minimal cell population found in graded 1 (674±27) and 3 (395±17) area respectively ($P_{1Vs3}<0.001$).

CONCLUSION

This study indicates that autophagic cell death, apoptosis and myofibroblast cell differentiation occur in ruptured rotator cuff tissue. These events are closely related to the extent of damage of ECM structure.

MONITORING NEOVASCULARIZATION IN TENDON HEALING BY THREE-DIMENSIONAL DOPPLER ULTRASOUND IMAGING

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INTRODUCTION

Neovascularization is thought to be an important determinant for tendon healing. Previous studies on neovascularization in tendon injuries mainly rely on histological examination with specific staining of endothelial cells, however, as neovessels may not evenly distributed in the healing tissues, there might be sampling bias with 2-D examination. As 3-Dimensional (3-D) Doppler ultrasound imaging has been used to quantify neovascularization in studies of tumour vasculature, we tried to explore and validate the use of 3-D Doppler ultrasound imaging to measure neovascularization in tendon healing.

MATERIALS AND METHODS

A well-established rat model of patellar tendon donor site injury was used. Sixteen rats were recruited in the present study and both knees of the rats were imaged by a 3-D Doppler ultrasound imaging system (Visualsonics) with a standardized positioning procedure before tendon injury was induced in the right knees. The rats were then imaged again at day 7, 14, 28 and 42 post-injury. Two rats were euthanized at each time point to obtain tendon samples for histological examination and immunostaining of alpha-smooth muscle actin (-SMA) and CD31. Microfil-perfusion computer tomography (Scanco) was performed on two rats for comparison of detected vasculature. Neovascularization was measured as increase in percentage volumetric vascular flow (% vascularity) within the patellar tendons, with 2 independent ultrasonographers to define the tendon volume of interest (VOI) in the ultrasound images with the Doppler signals turned off. Percentage vascularity was compared by Kruskal Wallis test and Wilcoxon's tests (between time points) at $p < 0.05$.

RESULTS

Matching of the results from Microfil-perfusion CT, 3-D Doppler ultrasound and histological images showed good consistency. Neovessels in the wound and the adjacent tendons were confirmed by immunostaining of -SMA and CD31. The intra-class coefficient between ultrasonographers for drawing VOI was 0.932. Vascular in-growth into the wound were associated with the vasculature from the above and the below of the patellar tendon and preferentially near the tibia side. Our results showed that % vascularity was significantly increased at day 7 post injury ($p=0.008$). Vascular regression was evident at day 14 post injury ($p = 0.004$), but an increase in % vascularity was detected at day 28 post-injury as compared to day 14 and day 42 post-injury ($p=0.016, 0.037$).

DISCUSSION AND CONCLUSION

We reported a useful method to measure neovascularization in healing tendons. We found that the re-vascularization process during tendon healing was complicated by a secondary surge in vascularity at the remodeling phase. It implies an intriguing regulation of neovascularization and vascular regression in tendon healing, which may contribute to the healing outcomes.

SPATIAL AND TEMPORAL CHANGES OF COLLAGENS AND PROTEOGLYCANS IN A TENDON WINDOW INJURY MODEL

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INTRODUCTION

The central one-third of patellar tendon is commonly used as graft for ACL reconstruction. Formation of scar tissues with reduced mechanical properties and development of tendinopathic-like changes have been reported clinically. We investigated the changes of major proteoglycans and collagens in regenerated tendon tissue in a tendon window injury model and correlated with chondro-osteogenesis observed in some samples in the same model.

METHODS

The central one-third of rat patellar tendon was removed while the contralateral tendon served as sham controls. Samples were harvested at various times for immunohistochemical analysis of collagen type I, type III, decorin, biglycan, fibromodulin and aggrecan.

RESULTS

There were transient increases in the expression of collagen type III and type I in tendon cells and matrix after injury. There was higher but insignificant expression of collagen type III and type III/type I in the calcified compared to the uncalcified region. Both the expression of decorin and fibromodulin decreased significantly at week 2. While the expression of fibromodulin returned to normal at weeks 4 and 12, the expression of decorin increased but remained significantly lower than controls. Sustained expression of biglycan was observed in the tendon matrix after injury with significantly higher expression in the calcified region compared to the uncalcified region at week 12. The expression of aggrecan decreased in the tendon matrix at week 2 and was significantly below control at week 12, with higher focal expression in calcified region.

DISCUSSION

There was sustained expression of biglycan and reduced expression of aggrecan and decorin in the tendon matrix as well as high expression of biglycan and aggrecan in the calcified region. Erroneous matrix deposition may account for poor mechanical property of the regenerated tissue. Higher expression of biglycan and aggrecan in the calcified region might be involved in ectopic chondro-osteogenesis after acute tendon injury.

ACKNOWLEDGEMENT

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A COMPARATIVE STUDY ON THE HISTOLOGICAL AND MECHANICAL PROPERTIES OF BONE-TO-BONE, BONE-TO-TENDON AND TENDON-TO-TENDON HEALING – A GOAT ACHILLES TENDON – CALCANEUS MODEL

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INTRODUCTION

This experimental study was performed to test the healing mode of Bone Tendon Junction (BTJ) at different injury sites using a goat Achilles tendon – Calcaneus (ATC) complex model of Bone-to-Bone (B-B), Bone-to-Tendon (B-T) or Tendon-to-Tendon (T-T) Healing.

METHODS

Forty-eight adolescent male Chinese goats were used for this experiment. ATC complex was surgically approached for osteotomy, separation at the BTJ, and tenotomy for study healing at the reattachment interface in B-B, B-T, and T-T groups. Serial histological studies involving conventional transmit light microscopy, polarized microscopy as well as immunohistochemistry were conducted to investigate the healing characteristics at different time points (6, 12, and 24 weeks after surgery). Tensile test was conducted to compare the mechanical properties of ATC healing complex among these three groups at the above mentioned time points. The intact ACT was treated as control.

RESULTS

Histology showed that all three types of repair progressed over time with the recovery of cell types and alignment of matrix collagen subjected to postoperative loading at the healing interface. However, when comparison was made among these three types at different time points, healing taking place in homogenous tissue, i.e. B-B or T-T groups, showed more better remodeling of the healing tissues towards normal intact structure than their B-T counterpart which displayed as poorly recovered fibrocartilage zone accompanying with a large amount of fibrous tissue. Mechanical testing showed that all mechanical variables, including load and stress at failure as well as energy required up to failure, improved with healing over time as compared among different time points after operation in these three groups. While the results of comparison among three groups at each postoperative time point indicated that the T-T group obtained higher stress and ultimate load than the B-B and B-T groups in the early period (6 and 12 weeks post surgery), which achieved the same level as the later two groups with tissue regeneration and remodeling over time (24 weeks post surgery), reaching 40-65% of the mechanical properties (maximal load, stress and energy) of the the intact control.

DISCUSSION

The mechanical properties of BTJ may increase to the same level at different injury sites (B-B,B-T,T-T), while the healing in homogeneous tissue (B-B, T-T) display better recovery than heterogeneous tissue (B-T) histologically, suggesting general better healing taking place within a homogeneous tissue.

ACKNOWLEDGEMENT

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MECHANICS RULES CELL BIOLOGY

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Humans live in an environment where various types of mechanical forces, including gravity and tensile and compressive forces, constantly act on human body. As a result, these forces influence tissue structure and function. For example, increased mechanical loads increase the mass and strength of the muscle. Another well known example is that mechanical loading changes the shape, density, and stiffness of bone. At the cellular level, many years of research have shown that mechanics in terms of mechanical forces, deformations, and even substrate stiffness, influences cell behavior and regulates cell structure and function.

In this presentation, I will highlight the key role of mechanics, mainly in the form of mechanical force and/or deformation, in regulation of various cell functions. Specifically, I will use examples from previous studies to show that

1. mechanical stretching modulates proliferation of human tendon fibroblasts and increases the cellular production of collagen type I, which is at least in part mediated by TGF-β1;
2. mechanical stretching induces the expression and production of COX-2 and PGE2 in a stretching magnitude-dependent manner;
3. mechanical stretching regulates self-renewal and differentiation of tendon stem cells in a stretching magnitude-dependent fashion;
4. mechanical forces generated by cells control cell proliferation and differentiation patterns.

Finally, the cell mechanics-based mechanotransduction mechanisms, by which cells sense and respond to mechanical forces in order to undergo a cascade of cellular and molecular events (e.g., ECM synthesis and cell differentiation), will also be discussed.

SPECIMEN DIMENSIONS INFLUENCE THE MATERIAL PROPERTIES OF TENDON FASCICLES: INSIGHTS INTO STRUCTURE-FUNCTION RELATIONSHIPS

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INTRODUCTION:

Based on the assumption that stress and strain describe the material properties of tendon tissue, and are thus not affected by specimen length or cross sectional area (CSA), it has been a struggle to explain the large strain values that are frequently described when analyzing the tendons of small mammals such as rats. However, there is evidence that the assumption of normalised material parameters should be challenged in non-homogenous soft-tissues[1,2]. The primary aim of our study was to characterize the influence of specimen length on stress, failure strain, and elastic modulus. Second, we wanted to explain the reason behind specimen length influencing mechanical parameters, hypothesising that this effect is largely influenced by the gripping of the specimen. Third, we wanted to investigate if the CSA of the fascicles has an effect on the mechanical properties and if this effect was correlated in any manner with varying specimen length.

METHODS

Tendon fascicles were dissected from five rat tails and five bovine foot extensors. Fascicle diameters were determined by a laser micrometer, the CSA calculated assuming a circular shape, and the fascicles then loaded to failure. Grip-to-grip length was varied, with 3-4 fascicles per animal for each sample length tested: rat tail fascicles were tested at lengths of 5, 10, 20, 40, 60, 80 and 100 mm (103 fascicles), bovine extensor fascicles were tested at 5, 10, 20 and 40 mm (67 fascicles). To determine differences in grip-section and mid-section strain, markers were placed every 5 mm along the bovine extensor fascicles (20 & 40 mm samples) and the tests to failure were filmed.

RESULTS

Strain to failure significantly ($p \leq 0.05$) decreased with increasing specimen length in both rat tail and bovine extensor fascicles, while the elastic modulus increased. Specimen length did not influence failure stress in rat tail fascicles, although in bovine fascicles it was significantly lower in the 40 mm specimens compared to the 5 & 10 mm specimens. The high failure strains in shorter specimens appeared to be the result of significantly higher strains in the grip section compared to the mid section, which has a disproportionately large effect on short specimens. Failure strain, stress and elastic modulus correlated significantly with CSA at certain specimen lengths. Only in short bovine extensor fascicles (of 5 and 10 mm length) was there a positive correlation with strain, while stress and linear elastic modulus were negatively correlated with CSA. In rat tail fascicles, failure strain correlated positively with CSA at specimen lengths of 5 & 20 mm, whilst stress and elastic modulus correlated negatively with CSA at 10-60 and 100 mm and 5-60 and 100 mm respectively. Furthermore, the effect of specimen dimensions differed between species/tendons as the shape of the stress-strain curve changed with specimen length in rat tail fascicles only and the effect of CSA on the mechanical properties was more pronounced in rat tail fascicles.

DISCUSSION

We have demonstrated that specimen dimensions have a major influence on the mechanical parameters recorded when assessing the material properties of tendon. While the increase in strain with reduced specimen length can be explained by changes in the tissue structure due to stress concentrations at the gripping points, it is less obvious how CSA influences the measurement of mechanical properties. It could be assumed that collagen fibers in the centre of a larger fascicle experience less gripping pressure and are therefore more likely to slip, thereby increasing strain. Considering that tendon tissue is not homogenous, an increase in the relative contribution of connective sheaths in larger fascicles would result in a reduction of failure stress with increasing CSA. A different tendon composition and structure, as indicated by differing mechanical properties, resulting in a different adhesion of collagen fibers, could explain the differences between rat tail and bovine extensor fascicles regarding the correlation of CSA with mechanical properties at varying specimen length. Our findings have implications for the mechanical testing of tendon tissue: while it is not always possible to control for fascicle length and/or CSA, these parameters have to be taken into account when comparing samples of different dimensions. It seems advisable to use longer specimens whenever possible to reduce the variability within a given subgroup of a defined fascicle length.

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**Finalist of the Best Student Paper Award*

FOUR LOADING EPISODES DURING EARLY TENDON HEALING IMPROVE TISSUE QUALITY

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INTRODUCTION

Fifteen minutes of daily mechanical loading during the first 12 days after Achilles tendon rupture is sufficient to improve its healing in rats[1]. An additional 15 min loading episode 8 hours later each day does not further improve healing, which indicates that the response to a loading episode lasts for several hours[1]. We now investigated if even fewer daily loading episodes could improve healing. We also compared loading during a very early phase of healing with a later phase, to see if also early healing is sensitive to loading. We used tail suspension in rats to allow short and controlled periods of loading of the Achilles tendon. The suspended rats were either completely unloaded 24 hours a day, or allowed running periods of 30 min daily for 4 days.

MATERIALS AND METHODS

Forty female Sprague-Dawley rats ~200 g were habituated to a treadmill apparatus (12 m/min), before the experiment started. Thereafter, the Achilles tendon was transected and left to heal[1]. The day after surgery, all 40 rats were subjected to hindlimb unloading by tail suspension and divided into 4 groups: 2 groups were unloaded without exercise for 8 or 14 days (early control and late control group), and two groups were subjected to 30 min of daily exercise. The exercise groups were let down from suspension and ran on the treadmill for 30 minutes (9 m/min, slightly uphill) day 2-5 (early exercise group) or 8-11 (late exercise group). The animals were completely unloaded for 72 hours after the last exercise before they were killed together with the unloaded control animals on day 8 or 14. The tendons were harvested and tested mechanically. Results were analyzed with two-way Anova. Significant differences were further analyzed with Students t-test to evaluate the effect of loading at each time-point. The experiment was approved by the regional animals ethics committee. All evaluation was performed while blinded.

RESULTS

A 2-way Anova of all groups revealed that loading increased the peak force, stiffness ($p<0.0001$ for both), peak stress ($p=0.006$) and energy uptake ($p=0.002$). All these variables were also influenced by time ($p\leq0.002$ for all). Also elastic modulus was affected by time ($p=0.04$).

After 8 days of healing, the peak force was 60% higher in the early exercise group compared to the early unloaded controls ($p=0.01$, figure 1). The stiffness was also 30% higher in that group ($p=0.05$). Also the material properties were improved: there was a 40% higher peak stress in the early exercise group ($p=0.03$).

After 14 days of healing, the pattern was similar, with a 50% higher peak force ($p=0.006$), 30% higher stiffness ($p=0.01$) and a 40% higher peak stress ($p=0.05$) compared to the late unloaded control group.

One rat in each of the completely unloaded groups (early and late control groups) and two rats in the early exercise group were excluded because the suspension device on one occasion loosened from the tail, resulting in unwanted loading.

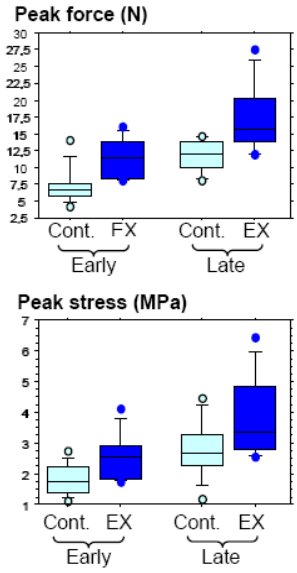

Figure 1. Peak force and Peak stress 8 and 14 days after tendon transection with or without loading. UL control is completely unloaded tendons where time-point for sacrifice corresponds to the early (day 8) or late (day 14) exercise groups. Exercise means 30 minutes of daily exercise on a treadmill for 4 days during early or late healing.

DISCUSSION

Single loading episodes days 2-5 improved the quality of the healing tissue at day 8. This was unexpected, considering that the mechanical stimulation was applied during the inflammatory phase of healing. However, this result is consistent with our previous finding that the expression of inflammation-related genes is reduced by mechanical loading in the early phase of healing[2].

The other important finding is that tissue quality (i e peak stress) was improved. Although this might seem uncontroversial, all our previous experiments in this model only showed increased force at failure due to an increased cross-sectional area[1]. The explanation is probably that we now for the first time waited for 3 days after the final loading episode, before killing the rats. Thus, it would seem that the early response to loading is mainly proliferative, whereas increased remodeling comes later, and requires that loading is discontinued.

The observation that only 4 episodes are necessary to create a strong response, suggests that we even might use just one loading episode, which would enable more detailed studies of how the response to loading develops over time.

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**Finalist of the Best Student Paper Award*

ANISOTROPIC PROPERTIES OF STEM CELL-BASED SELF-ASSEMBLED TISSUES CULTURED ON A MICRO PATTERN-PROCESSED GLASS PLATE

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INTRODUCTION

Ligaments and tendons are well-organized fibrous connective tissues. They are mainly composed of parallel aligned collagen fibers with fibroblasts. In case of the damage or rupture in the fibrous tissues, it is one of potential options to use cell-based therapies. We have been developing a novel tissue-engineering technique for the repair of ligaments and tendons which involve stem cell-based self-assembled tissues (scSAT) derived from synovium[1]. As the scSAT is composed of cells with their native extracellular matrix, it is free from concern regarding long-term immunological effects. For biological reconstruction of the microstructure of the ligaments and tendons, it is required for the scSAT to have anisotropic properties. Therefore, we developed a new cell culture method using a micro pattern-processed glass plate to meet the requirement.

MATERIALS AND METHODS

Micro pattern-processed glass plate having an array of parallel grooves (100 μm in width and 30 μm in depth) was developed through lithographic and isotropic wet etching. Mesenchymal stem cells were obtained from the synovial membranes of human knee joint by means of collagenase treatment. After subculture of seven times, the cells were plated on the 6 well-plates (control group) or micro pattern-processed glass plates (micro groove group) at a density of 4.0 x 10^5 cells/cm^2 in DMEM (10% FBS, 1% P/S, 0.2 mM ascorbic acid 2-phosphate). After thirty-five days, synthesized matrices were carefully detached from those plates and allowed to undergo active contraction for 1 hour to develop scSATs. The scSAT was, then, subjected to tensile testing at a rate of 0.05 mm/s in PBS at 37 °C using a custom-made micro tensile tester developed in our laboratory[2]. The tensile load was applied parallel or perpendicular to the oriented direction. Histological observation was performed for the surface structure of the scSAT using a differential interference contrast microscope (IX71, OLYMPUS, Japan).

RESULTS AND DISCUSSION

Microscopic observation indicated that the no orientation was observed in the control group, while cells and tissue were oriented along the direction of the grooves in the micro groove group (Fig.1). Typical stress-strain relationships of the control and micro groove groups are shown in Fig.2 (a). The control group exhibited linear fashion, while the micro groove group indicated J-shaped curve. The tangent modulus in 5-10% of strain was increased in the micro groove group than in the control group with a significant difference observed between the two groups. The tensile strength was shown in Fig.2 (b). As compared with the control group, the strength was significantly higher in the micro groove group. We also found that the tensile strength was significantly higher in the parallel direction of the groove than in perpendicular direction. These results suggest that micro pattern-processed glass plate provides the scSAT with anisotropic structure and properties.

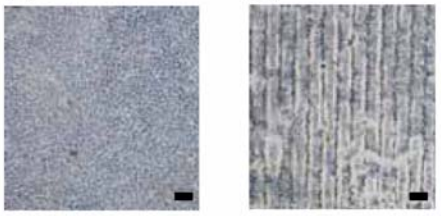

Fig.1 Differential interference contrast microscopic observation of the control (a) and the micro groove scSATs (b) (Bar: 100 μm).

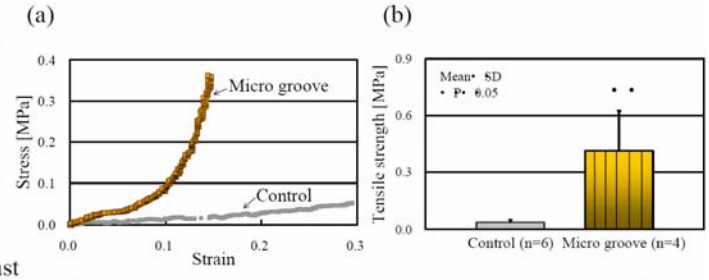

Fig.2 Typical stress-strain curves (a) and tensile strength (b) of the control and micro groove groups in tensile test

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ACKNOWLEDGMENT

The present study was financially supported in part by the Project-in-aid for the establishment of strategic research centers from the MEXT, Japan (BERC, Kogakuin University)

**Finalist of the Best Research Fellow Paper Award*

MECHANICAL STIMULATION ENHANCES ENGINEERED TENDON FORMATION IN VITRO AND IN VIVO

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INTRODUCTION

Tendon defect repair remains a major challenge in reconstructive surgery, because of lacking proper tissue graft source for tendon repair. Tissue engineering may become a promising approach for providing autologous tendon graft. Because tendon is subjected to constant mechanical loading in vivo naturally, mimicking this physiological niche would be an important consideration for engineering a functional tendon graft either in vitro or in vivo. This study examined the effect of mechanical loading on engineered tendon formation using in vitro and in vivo models.

METHODS

Human tenocytes or dermal fibroblasts were employed as the source of seed cells and polyglycolic acid (PGA) unwoven fibers were used as the scaffold. After the cell seeding onto the scaffold, the cell-scaffold constructs were loaded on a bioreactor with dynamic loading as an experimental group, or with a constant strain as a control for in vitro culture. After the culture for various time points, the in vitro engineered tendons were subjected to gross examination, histology, mechanical property analysis and electron microscope examination as well as proteomic study. In addition, the in vitro engineered tendons were also implanted in vivo in different animal models to observe the effect of mechanical loading on the tissue maturation and cell phenotype switch.

RESULTS

Either human tenocytes or dermal fibroblasts were able to form a neo-tendon tissue when cultured in vitro with a dynamic mechanical loading for 10-12 weeks. Grossly, a 3-4 cm long tendon-like tissue could be generated with a smooth surface and relatively good tissue elasticity and stiffness. Histologically, paralleled aligned collagen fibers as well elongated cells were observed. Transmission electron microscope examination also showed paralleled aligned and well-developed collagen fibrils with clear D-periodic structure. In addition, mechanical property analysis revealed a tensile strength of about 10 Newtons in these in vitro engineered tendon tissues. By contrast, the in vitro engineered tendon without dynamic loading showed poor tissue structure, weak mechanical property and poor collagen fibril development. Moreover, after in vivo implantation, the mechanical loading in vivo further enhanced the tissue maturation and mechanical strength of the loaded tendons compared to those implanted without mechanical loading. Proteomic analysis showed that tendon specific extracellular matrix molecules could be up-regulated by in vitro mechanical loading. Importantly, no significant difference was found between tenocyte and dermal fibroblast engineered tendons in gross view, histology, collagen fibril superstructure and mechanical property.

DISCUSSION

The results of this study demonstrated that it is feasible to engineer a tendon tissue by in vitro culture under dynamic mechanical loading, and mechanical stimulation plays an essential role in tendon tissue formation and maturation, mechanical strength enhancement and up-regulated expression of tendon specific extracellular matrix molecules. In addition, dermal fibroblasts might serve an alternative cell source for in vitro tendon engineering and tendon graft development.

**Finalist of the Young Faculty Paper Award*

IN VIVO LENGTH CHANGES OF LIAGAMENTS STABILIZING THE TRAPEZIUM AND TRAPEZIOMETACARPAL JOINT DURING THUMUB MOVEMENT

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INTRODUCTION

The thumb motion is chiefly dependent upon CMC joint motion. Our understanding of kinematics of this joint is limited; currently we do not know the length change of ligaments stabilizing the CMC joint during thumb movement in vivo. This study was to investigate changes in the lengths of selected stabilizing ligaments of the trapezium and trapeziometacarpal joint during thumb movement *in vivo*.

METHODS

We studied *in vivo* changes in the lengths of fibers of 5 stabilizing ligaments of the CMC joint during thumb movement in 6 hands of healthy volunteers using a noninvasive approach. Using serial computed tomography scans and volume registration techniques, the ligament lengths were examined at 4 positions: thumb in neutral position, maximal flexion, maximal abduction and opposition position. The 3-dimensional structures of the CMC joint were reconstructed using customized software. We modeled the paths of fibers of the 5 selected ligaments: dorsoradial ligament (DRL), posterior oblique ligament (POL), deep anterior oblique ligament (dAOL), intermetacarpal ligament (IML), dorsal intermetacarpal ligament (DIML). We analyzed changes in the lengths of these ligaments during thumb movement.

RESULTS

Majority of the ligaments showed statistically significant differences in the lengths during thumb movement compared with those at the neutral position. Compared with the neutral position, the DRL, POL, IML and DIML were elongated substantially at thumb flexion ($p < 0.01$ or $p < 0.001$); the elongation rate ranging from 20% to 50%. At thumb abduction, the DRL, POL, and DIML ligaments elongated statistically ($p < 0.05$ or $p < 0.01$). At thumb opposition, the changes in lengths of these ligaments were similar to those recorded at thumb flexion. At all 3 thumb movements, the lengths of the DAOL decreased compared with those at the neutral position.

CONCLUSION

Lengths of the ligaments stabilizing the thumb CMC joint change substantially during three principal thumb movements. Thumb abduction and opposition lead to similar changes in these ligaments. The changes in the lengths of these ligaments may indicate their different role in stablizing the joint during thumb motion, and may shed light on further defining the kinematic roles of these major CMC joint ligaments.

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EFFECTS OF CYCLIC TENSIONING CULTURE ON A STEM CELL-BASED SELF-ASSEMBLED TISSUE (SCSAT) DERIVED FROM SYNOVIUM

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INTRODUCTION

Tendons and ligaments functionally adapt to dynamic and static loads. Although a variety of biomechanical studies have been done so far to determine the mechanism of remodelling in fibrous tissues, it was difficult to obtain detailed information because of complicated constitution of the tissues. Meanwhile we have developed a stem cellbased self-assembled tissue (scSAT) for tissue engineering. Since the scSAT is consisted of synovium-derived mesenchymal stem cells (MSCs) and their native extracellular matrix, it is a good experimental model to determine the process of remodelling of fibrous tissues. Therefore, the effects of cyclic tensioning and its duration on the mechanical property of the scSAT were investigated in the present study.

MATERIALS AND METHODS

Stromal cells obtained from the synovial membranes of human knee joints were cultured in DMEM in monolayer. After the cell density reached to 6.0 × 105 cells/cm2, 0.2 mM of ascorbic acid 2-phosphate was added in the medium to promote the biosynthesis of extracellular matrix. After 28 days, synthesized matrices were carefully detached from culture plate and subsequently cultured for 1 hour to a develop scSAT (Time 0 group)1). Then the scSAT was subjected to cyclic tension for 3 or 6 days with the range of 4.0-8.0 mN controlled using a cyclic tensioning apparatus2) in an incubator at 37 ℃(L groups). In each day, the load was applied to the scSAT for 1 hour followed by an unloaded condition for 23 hours. In addition, other scSAT specimens were cultured with no load for 3 or 6 days in an incubator (N-L groups). The whole scSATs were then subjected to a tensile testing at a rate of 0.05 mm/s in PBS at 37 ℃ using a custom-made micro tensile tester2). The scSAT specimens of 3 day L group were tested in two different methods; the tensile load was applied parallel to the fiber orientation (load direction in the cyclic tensioning culture) in L group, while the tensile load was applied perpendicular to the fiber orientation in L-P group. Morphological observation was performed for the surface of the scSATs using a scanning electron microscope (VE-8800, KEYENCE). Using the image data of the fiber structure, anisotropic analysis was performed to determine the direction of principal fiber orientation (orientation angle) and the intensity of the orientation (orientation intensity) using the fiber orientation analysis Ver.7.02 3).

RESULTS and DISCUSSION

Scanning electron microscopic observation indicated that the 6 day loaded scSATs exhibited fibrous structures aligned with the loading direction in the cyclic tensioning culture (Fig.1). In the 6 day N-L group, the orientation angle and intensity were 42.4 degree and 1.18 respectively. However, in the 6 day L group, the fiber structure was strongly aligned parallel to the load direction in the tensioning culture along with a significant decrease of the orientation angle to 4.6 degree and a significant increase of the orientation intensity to 1.64. Tangent modulus between -10% and -5% from the breaking point of strain and tensile strength were indicated in Fig.2. In the 3 and 6 day L groups, the modulus and the strength became significantly higher as compared with the time 0 and corresponding N-L groups. In the 3 day and 6 day L group, the collagen-like fibrous structure was re-organized to align parallel to the load direction in the tensioning culture2). This may be a reason for the higher modulus and strength in both the groups as compared with those in the time 0 and N-L groups. The strength was increased in the 6 day L group than in the 3 day L group with no significant difference observed between the two groups. There may be a possibility of a production of new fibrous tissue in the 6 day L group. To verify this matter, we have to perform a quantitative and long-term analysis with regard to biological constituents of the scSAT specimens in future studies.

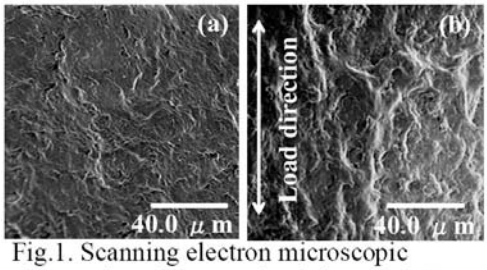

Fig.1. Scanning electron microscopic observation of the non-loaded control (a) and the 6 day loaded scSATs (b).

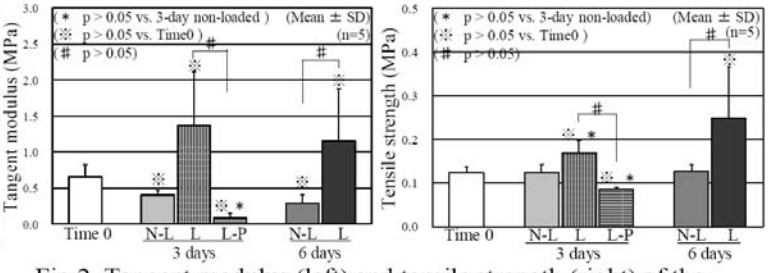

Fig.2. Tangent modulus (left) and tensile strength (right) of the loaded and control scSATs

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ACKNOWLEDGMENT

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THE GRAFT FIXATION SEQUENCE AFFECTS THEIR FORCE DISTRIBUTIONS IN DOUBLE BUNDLE ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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INTRODUCTION

Double bundle anterior cruciate ligament (ACL) reconstruction techniques are being advocated to reproduce the native anatomy of the ACL more closely and hence potentially provide better anterior-posterior and rotational stabilities. Owing to the distinct tension patterns of the two ACL bundles through the range of motion of the knee, various graft tensioning protocols have been proposed to mimic the native ACL tension patterns. Based on the evidence from the literature, there is a general acceptance on fixing the posterolateral (PL) bundle at low and anteromedial (AM) bundle at high flexion angles. However, sparse attention is devoted towards the sequence of graft bundle fixation. We hypothesize that the force distribution in the graft bundles is dependent on the sequence of their fixation.

METHODS

Two biomechanical cadaveric studies that used a similar robotic testing system to investigate the biomechanics of double bundle ACL reconstruction were reviewed.[1,2] The study design and methodologies were similar except that the PL bundle graft was fixed first at full extension in one study,[1] while in another study the AM bundle graft was fixed first at 60° of flexion.[2] In both the studies AM and PL bundles were fixed at 60° of flexion and full extension respectively. The force distributions in the native ACL and ACL graft bundles were measured under an anterior tibial load of 134N. The differences in the in-situ forces between the graft bundles and the corresponding native ACL bundles were compared in the two different fixation sequences under the same testing conditions.

RESULTS

The sequence of graft fixation did not significantly affect the trend of the in-situ force-versus-knee flexion angle curves. However, the absolute values of in-situ forces significantly changed while using different sequence to fix the grafts. Fixing the AM bundle at 60° of flexion followed by the PL bundle at full extension overloaded the PL graft compared to the intact PL bundle (Figure 1). On the contrary, fixing the PL bundle at full extension then the AM bundle at 60° of flexion, overloaded the AM graft bundle compared to the intact AM bundle.

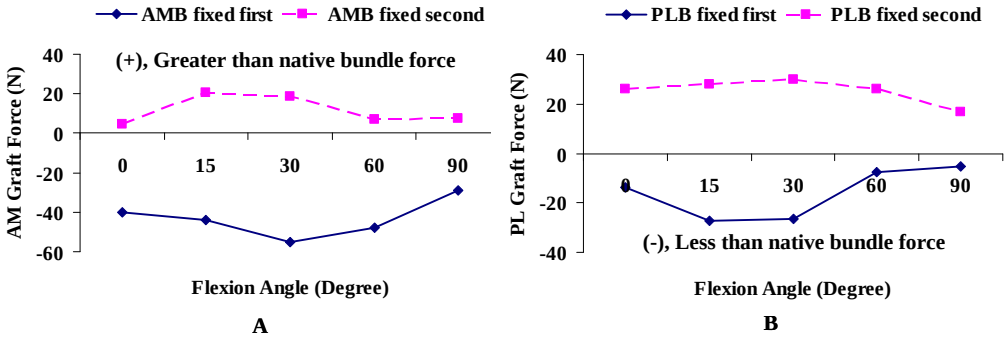

Figure 1: The difference in the in-situ forces between the graft and native bundles. (A) AM bundle (B) PL bundle.

DISCUSSION

Our findings demonstrated that the absolute values of the in-situ forces in the grafts are dependent on the fixation sequence. More specifically, overloading of the graft fixed last will occur compared to the corresponding native bundle. The trend of the tension patterns in the grafts along knee flexion were not significantly affected by the fixation sequence. In PL bundle, the peak value was around 0°-15° and reduced gradually thereafter. In the AM graft, the peak force was observed around 30°-60°. These results are consistent with previously reported data and have been the rationale for many graft fixation protocols that suggested tensioning the AM bundle between 30°-60° and the PL bundle between 0°-15° of knee flexion. There is a tendency that the last fixed bundle will result in a posterior tibial translation causing the previously fixed graft to become lax. Due to this phenomenon, the graft fixed last will be more profoundly loaded than the corresponding native bundle. This study supports our hypothesis that the sequence of graft fixing will affect the forces experienced by the individual graft bundles. To avoid overloading, graft failure or residual laxity, it is important to set up a reliable fixation protocol that includes not only an optimal initial graft tension and flexion angle but also the fixation sequence.

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TECHNIQUE AND PROBLEMS IN ANATOMIC DOUBLE BUNDLE ACL RECONSTRUCTION

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Among the factors that influence the results of ACL reconstruction, selection of graft materials, tibial and femoral graft position placement, orientation of the drill holes, fixation and tensioning of a graft are known to be important intra-operative considerations when performing ACL reconstruction surgery. Reconstructed anterior cruciate ligament grafts with an autogenous bone-patellar tendon-bone graft and a hamstrings graft have been shown to be revascularized and remodeled after the implantation and are therefore thought to be biologically suitable materials. Thus, the use of one of these graft materials has become a common procedure. The graft material, fixation technique, preconditioning, and tensioning will influence the early postoperative graft load and probably subsequent graft remodeling.

Regarding graft positioning, theoretically, more anatomically placed femoral graft positioning will also provide better biomechanical function and clinical result. We have been trying anatomic two bundle ACL reconstruction through two tibial and femoral tunnels since 2002. Femoral tunnels can be prepared through two tibial tunnels or far anterior medial arthroscopic portals. Femoral positioning of the posterolateral bundle should be more posterior and distal than conventional femoral drill hole placement. Once two bundle grafts are passed through drill holes they can be tensioned and fixed separately. Objective evaluation of instability is very important aspect of outcome measurement in this surgery. We have developed noninvasive in vivo measurement system using an electromagnetic sensor. Two year follow up of the anatomic double bundle reconstruction has shown favorable results comparing to conventional single bundle technique assessed by this measurement. Advantages and disadvantages and technical pitfalls of this procedure will be discussed both in basic and clinical profiles.

**Finalist of the Best Research Fellow Paper Award*

PERIOSTEUM-LIKE CELL SHEETS ENHENCED TENDON-BONE HEALING IN AN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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INTRODUCTION:

Tendon-bone healing using periosteum wrapped around tendon grafts was reported in our previous studies. The cambium layer of periosteum contains osteochondral progenitor cells that can differentiate into osteoblasts and chondrocytes during tendon-bone healing.[1] In order to promote tendon-bone healing, we developed functional cambium layer progenitor cell sheets that may be used as periosteum-like tissue transplants.[2] Naturally derived submucosal layer and acellular tissue matrices from small intestines can serve as cell sheets protectors for tendon-bone healing on anterior cruciate ligament reconstruction (ACL) in a rabbit model.

METHODS:

Polymerized fibrin-coated PE dishes were fabricated with fibrinogen monomers mixed with thrombin.[2] Periosteum progenitor cells (PPCs) derived from rabbit tibia periosteum were cultivated on a fibrin-coated surface for 10 days at 37°C. The laminated cell sheets were dissociated intact from the polymerized fibrin layer by proteases secreted from cells (shown as Figure 1-A). The small intestinal submucosa (SIS) was prepared from the intestines of rabbit by mechanical and chemical process (shown as Figure 1-B). Bilateral ACL reconstructions using the long digital extensor tendon grafts were performed on mature rabbits. A 2.3-mm diameter femoral and tibial tunnel was created according to the ACL footprints. The SIS only, PPCs only and PPCs plus SIS layers (PPCs-SIS) wrapped around the tendon grafts (shown as Figure 2) were pulled manually from tibial outlet through the drill hole to the joint and then to the femoral tunnel. The rabbits were sacrificed at 8 weeks postoperatively. Histology, total collagen, glycosaminoglycan (GAG) and biomechanics properties of ACL reconstruction tissue specimens were estimated in this study.

RESULTS:

Higher mature fiber cartilage formatted around the tendon-bone junction in PPCs-SIS than in PPCs and SIS was observed (shown as Figure 3). Fiber cartilage formed completely around the tendon-bone junction in PPCs was found (shown as Figure 3-B). There were less fiber cartilage formation in SIS (shown as Figure 3-A). The tendency of total collagen in ACL construction tissue was SIS > PPCs > PPCs-SIS (shown as Figure 4). GAG content of ACL construction tissue in PPCs-SIS was similar in PPCs, but higher than in SIS (shown as Figure 5). The tendency of tensile strength was PPCs-SIS > PPCs > SIS.

DISCUSSION:

In PPCs-SIS group, mature tendon bone junction was constructed by this bi-layer cell sheets design due to PPCs can enhance fiber cartilage formation and SIS can protect PPCs during healing. The periosteum-like PPCs-SIS can feasibly offer a new therapeutic strategy for novel approaches to augment tendon-bone junction healing.

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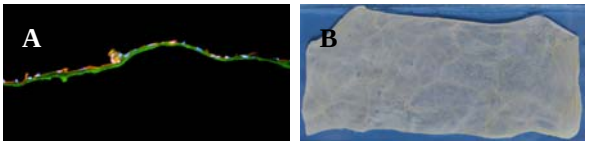

Figure 1. PPCs cell sheet and SIS before surgery. A: PPCs ; B: SIS.

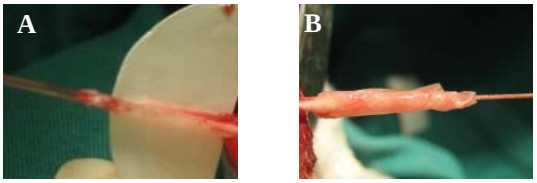

Figure 2. Process of cell sheet wrapped around the tendon grafts. A: Cell sheet wrapped around a tendon, B: Multi-layers cell shee swrapped around tendon.

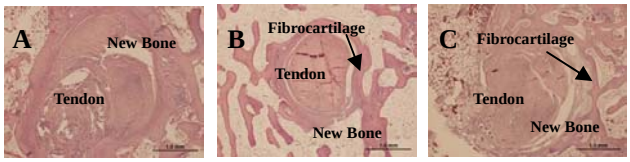

Figure 3. H-E staining of ACL constructions postoperative 8 weeks. A: SIS; B: PPCs; C: PPCs-SIS.

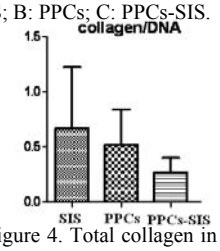

Figure 4. Total collagen in ACL construction tissue.

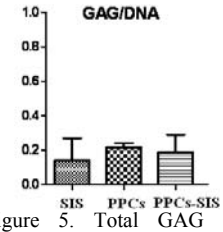

Figure 5. Total GAG in ACL construction tissue.

IMMUNOHISTOCHEMICAL AND GENE EXPRESSION ANALYSIS IN THE RUPTURED HUMAN ANTERIOR CRUCIATE LIGAMENT.

~Expression of activated Stat3~

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INTRODUCTION

We hypothesized that fibroblast-like cells activated by the IL-6 induced signal transducer and activator of transcription 3 (Stat3) may be involved in the response to injury of the ACL. The objectives of this study were to examine the distributions of Stat3-activated fibroblast like cells and the change of gene expression of ruptured ACL.

MATERIALS AND METHODS

Ruptured human ACL tissues were harvested from 105 patients undergoing primary ACL reconstruction. These specimens were divided into four phases based on the period from injury to surgery according to a previous study: phase I was less than 21days, phase II was 21 to 55 days, phase III was 56 to 139 days, and phase IV was more than 140 days. We performed immunohistochemical analysis using primary antibodies to the CD55, Stat3 and Phospho-Stat3, and gene expression assays of collagen types 1 (COL 1) and 3 (COL 3), biglycan, decorin, α-SMA, IL-6, TGF-β1, MMP-1, MMP-2 and TIMP-1 by quantitative RT-PCR.

RESULTS

The cells positive for CD55 were significantly increased in the remnant ACL in phase III and these cells existed merely in the synovial lining layer of the ruptured end in phase IV. P-Stat3 positive cells were significantly increased in both the ruptured end and midsubstance in phase III, being especially prominent in the synovial lining layer. In phase IV, the positive cells were maintained in the synovial lining layer in the ruptured end particularly.

COL 1 was significantly higher in phase II than in phases I and IV. COL 3 was increased in phases II and III, but decreased in phase IV. Biglycan was significantly higher in phase III than in phases I and II, and decorin was increased from phase I to IV with a significant increase in phase IV. α-SMA was also significantly elevated in phase IV compared to in phase I. IL-6 was significantly elevated in phases I, II and III compared to in phase IV. TGF-β1 was significantly increased in phase IV more than in phases I and II. MMP-1 and MMP-2 were significantly increased in phase III compared to in phase IV with MMP-2 expression level consistently higher than MMP-1. TIMP-1 expression level was constant throughout all phases.

DISCUSSION

Our experiments confirm that activated fibroblast-like cells in which Stat3 was phosphorylated had increased in the ruptured ACL in phases III and IV. In these phases, the expressions of COL 1 were decreased, and MMPs and proteoglycans were elevated. Based on this study, the procedure for ACL healing may fail if it is conducted in the chronic phase after rupture. In addition to newer approaches using scaffolds loaded with cells and growth factors, inhibition of Stat3 signaling and activation these cells may eventually lead to improved rates of native ACL healing.

BIOMECHANICAL FUNCTION OF ANTERIOR CRUCIATE LIGAMENT REMNANTS: EFFECTS OF REMNANT PATTERN AND DURATION BETWEEN INJURY AND SURGERY ON KNEE STABILITY EVALUATED WITH A NAVIGATION SYSTEM

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INTRODUCTION

The biomechanical function of anterior cruciate ligament (ACL) remnants remains largely unknown. This study aimed to evaluate the biomechanical function of ACL remnants in antero-posterior (AP) and rotational knee stability in patients with a complete ACL injury. This was done using a navigation system and our own unique device [1]. We hypothesized that the ACL remnants would provide some AP and rotational stability to the knee in the period soon after ACL injury, but that this biomechanical function would be lost after a year. We also documented variations in the ACL remnant pattern of patients with either a complete or partial ACL injury.

METHODS

A prospective study was performed in 100 patients undergoing ACL reconstruction. ACL remnants were classified into 5 morphological patterns (Group 1: bridging between the posterior cruciate ligament (PCL) and tibia; Group 2: bridging between the intercondylar notch and tibia; Group 3: partial rupture of the posterolateral bundle; Group 4: partial rupture of the anteromedial bundle; Group 5: no substantial ACL remnants). Patients in Groups 1 and 2 underwent intraoperative arthrometry with a navigation system before and immediately after resection of the ACL remnant. Effects of chronicity (length of time from injury to surgery) and ACL remnant pattern on changes in knee laxity after debridement of the ACL remnant were investigated.

RESULTS

The percentage of patients in each ACL remnant pattern group was 18%, 12%, 14%, 6%, and 50% for Groups 1, 2, 3, 4, and 5, respectively. The 30 patients of ACL scar pattern Groups 1 and 2 underwent evaluation of the biomechanical function of their ACL remnant. At 30° of knee flexion, the AP knee laxity of patients who underwent ACL reconstruction within 1 year of injury was found to have increased by 2.22 mm after resection of the ACL remnant. In contrast, the AP knee laxity of patients who underwent surgery at more than 1 year after injury was found to have increased by only 0.17 mm. There was statistical significant difference ($P < .01$). However, chronicity did not influence either AP knee laxity evaluated at 60° of knee flexion or rotational knee instability (Table 1). We also investigated whether any changes in knee stability were related to ACL scar pattern, although no effects were found.

Table 1. Relationship between chronicity (duration between injury and surgery) and changes in knee stability following resection of the ACL remnant.

	Changes in AP knee laxity (mm)		Changes in rotational knee laxity (degree)	
Chronicity	30° of knee flexion	60° of knee flexion	30° of knee flexion	60° of knee flexion
≤ 1 year	2.22 ± 2.16	0.67 ± 0.91	0.61 ± 3.85	0.61 ± 3.05
> 1 year	0.17 ± 0.39	0.42 ± 0.79	-0.25 ± 1.22	0.17 ± 2.69
Significance	P = .0014	NS; P = .52	NS; P = .39	NS; P = .76

DISCUSSION

In the present study, we found that ACL remnants continued to contribute to antero-posterior knee stability evaluated at 30° of knee flexion for up to 1 year after injury, beyond which this biomechanical function was lost. We also found that ACL remnants provided no rotational knee stability at any stage after injury.

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IN-SITU FORCE IN THE THREE BUNDLES OF THE HUMAN ANTERIOR CRUCIATE LIGAMENT

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INTRODUCTION

In the current reconstruction technique of the anterior cruciate ligament (ACL), the anteromedial (AM) and posterolateral (PL) bundles of the ACL were independently reconstructed based on a biomechanical studies[1,2]. More recently, the triple-bundle ACL reconstruction technique has been developed[3,4] in which the three bundles of the ACL are individually reconstructed with tendon grafts. However, the detailed mechanical functions regarding the three bundles have not been quantitatively determined yet. Therefore, a 6-degree of freedom (6-DOF) robotic system, developed in our laboratory, was applied to the determination of the in-situ forces in the medial and lateral parts of the AM bundle, and PL bundle of the human ACL in response to externally applied anterior force to the knee.

METHODS

A novel robotic system[4], consisting of a 6-DOF manipulator, servo motor controllers, control computer, and a universal force-moment sensor (UFS) was utilized in the present study. A previous test revealed that the clamp-to-clamp stiffness of the system is better than 312 N/mm[4]. The system is controlled by a custom-made PID-hybrid position/force control software on the LabView (version 8.0, National Instruments). It is possible to simultaneously control the both displacement of, and force/moment applied to, the human knee joint in 6-DOF. Five human cadaveric knee specimens were utilized. With a laser digitization system, the knee joint coordinate system[5] was fixed to the knee. A three-dimensional path of the intact knee in response to the anterior force (up to 100 N) to the knee was recorded. Then the ACL was separated to medial anteromedial (AMM), lateral anteromediate (AML), and posterolateral (PL) bundles following a previous study[2]. After the AMM bundle was transected, the 3-D paths of intact knee motion were reproduced. The intact paths were similarly reproduced after the transection of the AML bundle and then the PL bundle. Under the principle of superposition[6], the bundle forces in response to 100 N of anterior force were determined.

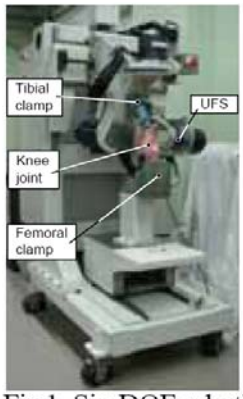

Fig.1. Six-DOF robotic system for the joint biomechanical tests

RESULTS & DISCUSSION

The average cross-sectional areas of the AMM, AML, and PL bundles at mid-substance of the ACL were 11, 11, and 16 mm^2, respectively. The anterior-posterior laxity between 100 N of anterior-posterior forces varied between 6 and 9 mm at flexion angles from 0 to 90 degree. The laxity became more than double after the transection of ACL. In response to 100 N of anterior force, the force in the PL bundle was larger than those in the AMM and AML bundles (Fig.2). As the flexion angle was more than 30 degree the force in the AMM bundle increased, but the force decreased as the flexion angle was more than 60 degree. The force in the AML bundle increased as flexion angle was more than 60 degree. The present study is the first one that quantitatively determined the mechanical functions of the three bundles of the human ACL. The joint loading tests have been successfully performed using the 6-DOF robotic system developed in our laboratory. As compared with a previous study performed by Sakane et al.[2] the force in the AM bundle (AMM+ AML) was slightly larger, possibly because the cross-sectional area of the transected AM bundle (11+11 mm^2) was larger than that of the PL bundle (16 mm^2). The obtained results of the reciprocal function of the AM and PL bundles in response to anterior force was similar to the previous finding[2]. More importantly, we found that the force in the AML bundle was not negligible in response to anterior force. In particular, we found that, as flexion angle increased more than 60 degree, the force in the AML bundle increased while those in the AMM and PL bundles decreased. This finding suggests that the P-AM bundle has a significant mechanical function for the anterior stability of the knee joint at deep flexion angles.

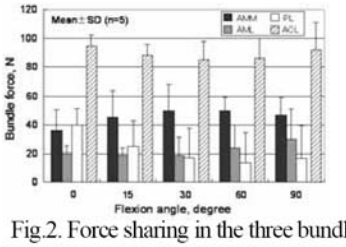

Fig.2. Force sharing in the three bundles of the ACL in response to 100 N of anterior load applied to the human knee

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ACKNOWLEDGMENT

The present study was financially supported in part by the Project-In-Aid for the Establishment of Strategic Research Centers (BERC, Kogakuin University) and Grant-In-Aid for Scientific Research (#20591766) both from the MEXT, Japan.

TRANSFORMING GROWTH FACTOR-β1 GENE TRANSFER THERAPY IMPROVES ACHILLES TENDON HEALING BY PROMOTING COLLAGEN FORMATION

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BACKGROUND:

Repaired Achilles tendons typically take weeks before they are strong enough to handle physiological loads. Collagen content and cross-linking are believed to be major determinants of tendon structural integrity and function. Gene therapy is a promising treatment for Achilles tendon defects.

OBJECTIVE:

In present study, we evaluated the effects of transforming growth factor (TGF)-b1 on the collagen content and cross-linking of Achilles tendons, and on the histological and biomechanical changes occurring during Achilles tendon healing in rabbits.

METHODS:

Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) were transduced with adenovirus carrying human TGF-β1 cDNA (Ad-TGF-β1). BMSCs were surgically implanted into the experimentally injured Achilles tendons, no cDNA (Ad-GFP), and the BMSCs without gene transfer and the intact tendon were used as control.
TGF-β1 distribution, cellularity, nuclear aspect ratio, nuclear orientation angle, vascular number, Collagen content and cross-linking and biomechanical features were measured at 1, 2, 4, and 8 weeks after surgery.

RESULTS:

Achilles tendons treated with TGF-b1-transfected BMSCs exhibited improved parameters and have higher concentrations of collagen I protein, more rapid matrix remodeling, and larger fiber bundles.

CONCULSIONS:

Thus treatment with TGF-β1 cDNA-transduced BMSCs grafts is a promising therapy for acceleration and improvement of tendon healing, leading to quicker recovery and improved biomechanical properties of Achilles tendons. In addition, TGF-b1 can promote mechanical strength in healing Achilles tendons by regulating collagen synthesis, cross-link formation, and matrix remodeling.

EFFECTS OF LOW-INTENSITY RESISTANCE TRAINING WITH RESTRICTED MUSCLE BLOOD FLOW ON TENDON AND LIGAMENT MATURATION -STUDY OF AFTER ACL RECONSTRUCTION-

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INTRODUCTION

Low-intensity resistance training combined with restricted venous blood flow (KAATSU) from the working muscles can result in significant and rapid increases in muscle hypertrophy[1]. Serum growth hormone was elevated after acute KAATSU resistance training. We hypothesized that the KAATSU resistance training encourages not muscle hypertrophy, but the maturation of the tendon and ligament after ACL reconstraction.

METHODS

Ten male patients (age 19-22), who had undergone ACL reconstruction with ST tendon, participated in this study. Four patients undergone rehabilitation with KAATSU training (KAATSU group) and six patients without KAATSU (control group). Rehabilitation with KAATSU had worked with trainer after 1-4 months after ACL reconstruction. We measured morphological changes of the regenerated ST were observed using MRI every month after operation. And we also established and measured T2 relaxation time to clarify the degree of maturation of the regenerated ST tendon through our explorative study. We examined the following conditions; repetition time 3000 ms, echo time 10, 20, 30 ms.

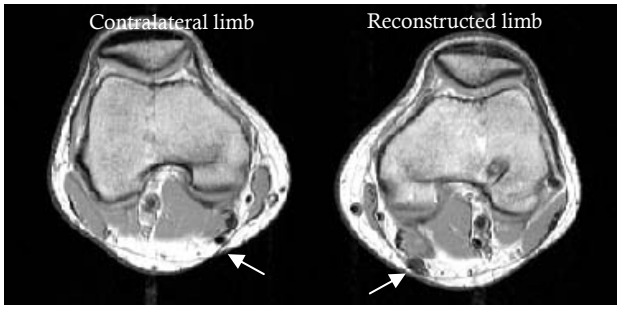

Fig.1 Typical MRI of the ST tendon (arrow). Tendon-like structure of the ST was confirmed at the ST tendon harvest site.

RESULTS

Cross-Sectional Area (CSA) of the regenerated ST tendon in the KAATSU group reached a maximum value at two months after surgery earlier than that of the control group (Fig. 2). The position of the musculotendinous junction shifted proximally in both groups. The T2 relaxation time of the regenerated ST tendon was gradually decreased after the operation (Fig. 3). Three months after the operation, T2 relaxation time was tend to be lower in the KAATSU group than that of the control group.

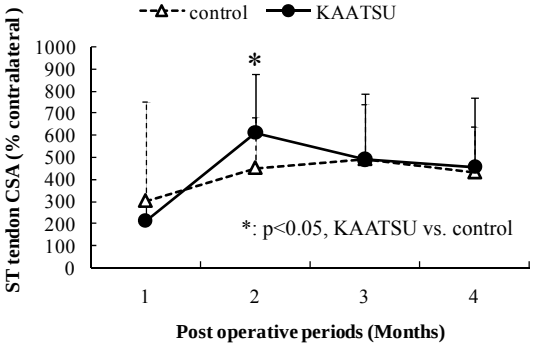

Fig.2 Cross-sectional area of the regenerated ST tendon. The value shows the ratio of CSA relative to the control

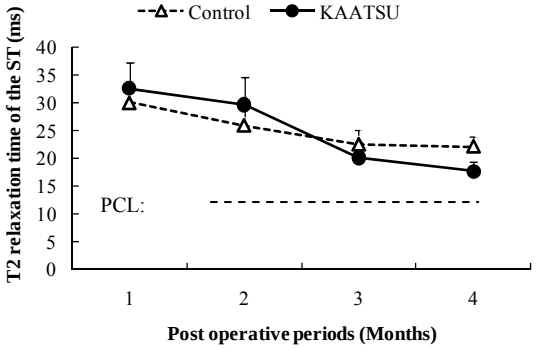

Fig.3 T2 value of the regenerated ST tendon, significant difference between Postoperative periods.

DISCUSSION AND CONCLUSION

The degree of maturation of the reconstructed ACL and the regenerated semitendinosus (ST) tendon after ACL reconstruction is invaluable information for determining appropriate loading controls for rehabilitation[2]. We got in KAATSU resistance training for early rehabilitation periods. It may be accelerated maturation of harvested ST tendon regeneration. Then KAATSU indicate the possibility that encourages not muscle hypertrophy, but the maturation of the tendon and ligament.

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**Finalist of the Best Poster Award*

LOCAL ADMINISTRATION OF TGFB1/VEGF$_{165}$ GENE TRANSDUCED MESENCHYMAL STEM CELLS ON PROPERTIES OF ACHILLES ALLOGRAFT REPLACEMENT OF ACL IN RABBITS

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INTRODUCTION

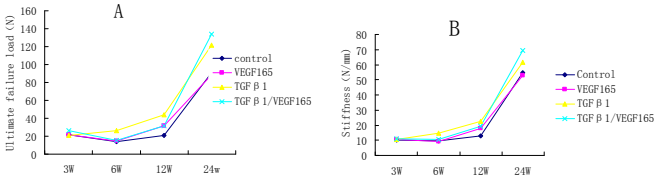
Local administration of VEGF significantly increased revascularization in the graft in ACL reconstruction, but it did not significantly affect the mechanical properties of the graft after implantation[1, 2]. Studies shows that TGF-β1 playing a role in mechanical regulation of local collagen type I and type III synthesis in tendon-related connective tissue in vivo. Mesenchymal stromal cells (MSCs) can accelerate the remodeling of the graft in ACL reconstruction. Based on our current knowledge of ligament formation, we have hypothesized that the introduction of TGFβ1/VEGF$_{165}$ gene and MSCs would beneficially affect the healing process of the ACL graft.

METHODS

A total of 144 skeletally-mature, female New Zealand White rabbits were used in this study approved by the Institutional Animal Care and Use Committee. The MSCs were harvested through gradient centrifugation and adhering to the plastic culture wall. The third passage cells were used in our study and divided into fore groups: control group and three adenovirus-infected groups (the MSCs were transfected with Ad-TGFβ1, Ad-VEGF$_{165}$ or Ad-TGFβ1/VEGF$_{165}$). The ACL was replaced with a Achilles tendon allograft, then the MSCs of each group (2×10^6 cells) with 100μl fibrin sealant was applied on the allograft. At 3, 6, 12 and 24 weeks after ACL reconstruction, animals were sacrificed and specimens were collected for histological observation and biomechanical analysis.

RESULTS

At 3, 6, 12 and 24 weeks after surgery, histological observation showed that implatation of VEGF$_{165}$ or TGFβ1/VEGF$_{165}$ transfected MSCs accelerated cellular infiltration and newly formed vessels into the ACL graft and enhanced collagen deposition in the wound. At 6, 12 and 24 weeks after surgery, biomechanical properties of the TGFβ1 groups were significantly better than control group on the ultimate failure load and the stiffness (P<0.05). At 12 weeks after surgery, the ultimate failure load and the stiffness of the VEGF$_{165}$ group or the Co-expression of TGFβ1/VEGF$_{165}$ group were higher than those of the control group, while there were no significant differences (P>0.05), but at 24 weeks, TGFβ1/VEGF$_{165}$ group had the maximum ultimate failure load and stiffness among all the fore groups (Fig. A and B)



DISCUSSION

The results suggest that TGFβ1 gene transfected MSCs could accelerate the remoedling of the reconstructed ligament. The cross-talk between TGFβ1 and VEGF$_{165}$ has positive consequences, TGFβ1/VEGF$_{165}$ gene transfected MSCs could significantly promote angiogenesis of the reconstructed ligament and enhance the mechanical properties of the reconstructed ligament. Co-expression of these genes is more powerful and efficient than single gene therapy.

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**Finalist of the Best Poster Award*

THE ASSEMBLY OF HESC-MSC AND KNITTED SILK SCAFFOLD COMBINED WITH COLLAGEN MATRIX DEVELOP TO ENGINEERED TENDON UNDER MECHANICAL STRESS

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INTRODUCTION

ESC is one of the most attractive seed cells for tissue engineering [1]. However, there is no efficient protocol to induce differentiation of ESC to teno-lineage. Previous study [2] has showed that the knitted silk scaffold combined with collagen matrix is a promising candidate for tendon tissue engineering. Thus, we aim to develop engineered tendon using hESC and silk + collagen scaffold under mechanical stress.

METHODS

Human ESC-derived mesenchymal stem cells (hESC-MSCs) were obtained and seeded on the silk + collagen scaffold. We applied dynamic mechanical stress on these constructs *in vitro* for 14 days using a bioreactor; the one without mechanical stress was used as control. Cell morphology change was examined by SEM. Gene expression was detected by real-time PCR. Constructs were implanted into nude mice for 4 weeks, receiving natural dynamic load. Histological, biochemical analysis and ultrastructural morphology were used to evaluate the *in vivo* results.

RESULTS

The histological and SEM results analysis showed tendon-like tissue was formed in vitro in the constructs with mechanical stress. The fluorescent detective assay showed that cells under mechanical stress *in vivo* displayed spindle-shaped morphology and aligned regularly, Tendon-specific genes expressions were significantly higher by the mechanical stress. Ultrastructural morphology showed mechanical stress group formed significant larger collagen fibers compared with the control group. Moreover constructs receiving mechanical stress had more collagen deposited,.

DISCUSSION

It was found that hESC-MSC displayed tendon-like tissues under mechanical stress when seeded on the silk + collagen scaffolds. Gene expression and histological evaluation demonstrated hESC-MSC differentiated into teno-lineage. Ultrastructural detection showed the mechanical stress enhanced the collagen fibers development. In conclusion, engineered tendon is successfully fabricated by human ESC combine with mechanical stress and the collagen sponge -knitted silk scaffolds. The engineered tendon developed in this study is promising in restoring or replacing the damaged tendon in future clinical trial.

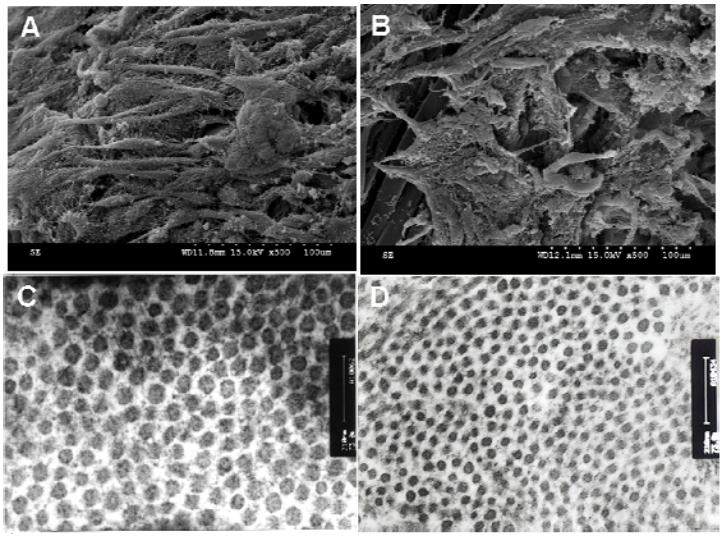

Fig. SEM observations showed the cell morphology and direction on the combined scaffolds at 14 days under dynamic stress (A) or not (B). TEM images of engineered tendon implanted in nude mice receiving dynamic stress (C) or not (D) for 4 weeks.

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**Finalist of the Best Poster Award*

AN OBSERVATIONAL STUDY TO IDENTIFY THE PRESENCE OF ACHILLES TENDINOPATHY AND ULTRASOUND DETECTED CHANGES IN ELITE FOOTBALLERS AND AGE AND GENDER-MATCHED CONTROLS

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OBJECTIVES

The primary objective of this study was to determine and compare the prevalence of AT symptoms and ultrasound defined signs of tendinopathy in elite male footballers and controls. The study also aimed to observe the prevalence of asymptomatic ultrasound signs in footballers.

METHODS

This was a cross-sectional observational design, investigating both tendons in 25 male elite footballers [mean(sd) age 22.8 years (3.6), height 1.81m (0.07), weight 77.5kg (6.9)] and 25 male age matched controls [mean(sd) age 22.3 years (1.5), height 1.76m (0.05), weight 74.3kg (10.1)] between April and May 2009. Participants in the football group were recruited from an English Premier League club via the club's medical team, while control subjects were selected if they trained for less than 3 hours per week on average. All participants completed a questionnaire regarding their present or past history of Achilles tendinopathy, and were scanned using grey-scale and power Doppler ultrasound for evidence of tendon hypoechoicity, neovascularisation and abnormal thickening.

RESULTS

The football group experienced a significantly ($p<0.001$) greater lifetime history of AT symptoms. Longitudinal Achilles antero-posterior thickness measures, adjusted for height, were all significantly greater ($p<0.001$) in the footballer tendons than the control tendons. Abnormal tendon thickening, hypoechoic areas and neovascularisation were significantly greater in the football group ($p<0.002$). In the footballers, 65% of tendons had asymptomatic US signs.

CONCLUSION

This study suggests that elite footballers experience significantly more symptoms and ultrasound signs of AT, and have significantly thicker Achilles tendons than the normal population. This indicates that elite football is a risk factor for Achilles tendinopathy and that clinicians should be vigilant for this condition in this group of players. The high percentage of footballers with asymptomatic US signs, and previous evidence of the high rate of progression from asymptomatic to symptomatic signs in Danish footballers, suggests that US screening may be beneficial for elite Premiership footballers.

**Finalist of the Best Poster Award*

NEOVASCULARIZATION IS AN ESSENTIAL FACTOR IN MEDIAL COLLATERAL LIGAMENT (MCL) HEALING.

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INTRODUCTION

It is still unclear which types of cells contribute to the development of ligament healing and how the healing process originates. In this study, to investigate the hypothesis that neovascularization is essential for supporting endogenious ligament healing, we used an anti-angiogenic agent in MCL injured animal models.

MATERIALS & METHODS

24 immunodeficient rats were used in this study. After creating MCL injury, $1x10^5$ human peripheral blood CD34 positive cells, as known to endothelial/ hematopoietic progenitor-enriched cell population, were locally transplanted in the experimental knee. These rats were divided into two groups; one group receiving soluble (s) Flt1 (VEGF receptor 1), known to inhibit proliferation of endotherial cells (ECs) (20μg/kg, subcutaneous) once daily for 14 days (sFlt group), and the other receiving PBS only (PBS group) (n=12 in each group). On week 2 (n=6 in each group) and week 4 (n=6 in each group) after cell transplantation, macroscopic, histological and immunohistological assessment were performed in each group.

RESULTS

Vascular staining with isolectin B4 (marker for rat EC, but not human) using tissue samples at week 2 post-injury demonstrated inhibited neovascularization around the peri-injury site in the sFlt1 group compared with the PBS group. Neovascularization assessed by capillary density was significantly inhibited in the sFlt group compared with the PBS group (sFlt1, 221.7±21.191; PBS, 303.2±20.299 /mm^2, respectively. $P<0.05$). Macroscopic inspection demonstrated the ligament significantly healed in 33 percent of rats (2 of 6) at week 2 and all rats (6 of 6) at week 4 in the PBS group compared with 0 percent of rats (0 of 6) at week2 and 33 percent of rats (2 of 6) at week 4 in the sFlt1 groups. Histological evaluation with H&E staining demonstrated a fibrous continuity at week 2 and almost complete healing excepting a small number of inflammatory cells at week 4 in PBS group. In contrast, the laceration site could easily be observed in spite of the existence of inflammatory cells at week 2, and the healing process had not yet been completed at week 4 in the sFlt1 group.

DISCUSSION

These results indicated that inhibited intrinsic neovascularization leads to delaying ligament healing. We suggested that microenviromental neovascularization is essential to support endogenous ligament healing.

**Finalist of the Best Poster Award*

STRESS CHANGES OF LATERAL COLLATERAL LIGAMENT UNDER VARIOUS MOTIONS

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INTRODUCTION:

This study aimed to create a three-dimension finite element model of knee ligaments and to analyse the stress changes of lateral collateral ligament (LCL) with and without displaced movements.

METHODS:

A four-major-ligament contained knee specimen from an adult died of skull injury was prepared for CT scanning with the detectable ligament insertion footprints, locations and orientations precisely marked in advance. The CT scanning images were converted to the 3-dimensional model of knee with the 3-D reconstruction techniques and transformed into finite element model by the software of ANSYS. The natural stress changes of LCL in five different knee flexion angles(0°,30°, 60°, 90°, 120°) and these under various motions of anterior-posterior tibial translation, tibial varus rotation and internal-external tibial rotation were measured.

RESULTS:

During knee flexion in neutral rotation, our study suggested the stress of LCL decreased about 19.9%-29.0% with knee having each 30° bending from full extension to 120° flexion. It was further found that no matter which movement was carried out to displace the knee, the increased stresses on the LCL peaked in full extension and dropped gradually with the knee bending toward 120°. The maximum increase rates reached to 87%-113% in varus motion in early 30° of knee flexions. The increased stress values were smaller than the the peak value of initial stress at 0° when knee bending was over 60° of flexion in anterior-posterior tibial translation and internal-external rotation.

DISCUSSION:

We conclude that LCL is vulnerable to varus motion in almost all knee bending postions and susceptible to anterior-posterior tibial translation or internal-external rotation in early 30° of knee flexions.

ACKNOWLEDGEMENT:

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HISTOLOGICAL EVALUATION OF PATELLAR TENDON AND ITS ENTHESIS IN TRAINED, UNTRAINED AND DETRAINED RATS: EXPERIMENTAL STUDY, PRELIMINARY RESULTS

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OBJECTIVE

The mechanical function of tendon depends on precise alignment of collagen fibers and on expression of appropriate proteoglycans. We evaluated the histologic changes in the patellar tendon and its enthesis in trained, untrained and detrained rats.

MATERIALS AND METHODS

Twenty seven male Sprague-Dawley rats aged 9 weeks were randomly divided into 3 groups: 20 adult male rats were trained on a treadmill for 10 week at ~ 60% VO2Max. At the end of training, 10 rats were euthanized immediately (trained group), while 10 were caged without exercise for a further 4 weeks before being euthanized (de-trained group). Seven sedentary rat were used as controls. Tendon insertion, methacromasia, fibres organization and thickness of enthesis and tendon were evaluated by of histomorphometry.

RESULTS

Histological evaluation showed normal enthesis in all three groups. In the detrained group there was a disorganized distribution of fibers associated to low captation of coloring. Methacromasia with Toluidin Blue, a staining that binds to the proteoglycans, was more evident in the trained group versus the control group. A lower degree of methacromasia was observed in the detrained group versus the control group. Fibers organization was different in all groups: completely closely and aligned bundles in the trained group, whereas in the detrained group and in the control group we observed a partial or complete disorganization of the fibers, with less disorganization in the detrained group.

CONCULSION

Moderate physical activity causes the activation of extra cellular matrix-specific metabolic factors. Unique results were obtained in detrained rats, that showed histopathomorfologic data with worse quality versus the control group. Probably, non continuous or suddenly interrupted sport activity can be deleterious for the structure of tendons.

SERUM CONCENTRATIONS OF THE NEUROTROPHIN BDNF AND THOSE OF TNF-RECEPTOR1 ARE CORRELATED IN INDIVIDUALS WITH ACHILLES TENDINOSIS BUT NOT IN HEALTHY CONTROLS

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INTRODUCTION

We have recently shown that, unexpectedly, the neurotrophins brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are expressed in the tenocytes of the Achilles tendon (Bagge et al 2009). The tenocytes also expressed the neurotrophin receptor p75 (Bagge et al 2009). In other tissues, neurotrophins such as these have marked trophic effects and are involved in pain and inflammation (Nockher and Renz 2005). Furthermore, it is well known that marked interactions can occur between different signal substances in many tissue types. For example, TNFalpha and IL-6 enhance BDNF secretion in human peripheral blood monocytes (Schulte- Herbruggen et al 2005).

AIMS

The aims of this study were (1) to see if there were correlations between serum BDNF and TNFreceptor1 concentrations in individuals with Achilles tendinosis as well as in healthy controls, and (2) to see if there were correlations between BDNF and TNFalpha in tendon tissue from individuals with Achilles tendinosis.

METHODS

Serum from Achilles tendinosis patients (n=27) and age-matched healthy controls (n=27) was analyzed for BDNF and TNFreceptor1 concentrations via ELISA. Furthermore, homogenized tendon tissue samples from Achilles tendinosis patients (n=12) were analyzed for BDNF and TNFalpha levels via ELISA.

RESULTS

The serum concentrations of BDNF and TNFreceptor1 were correlated ($p = 0.010$, $r = 0.489$) in the Achilles tendinosis group. In contrast, serum BDNF and TNFreceptor1 concentrations were not correlated in the control group. There was marked inter-individual variability in the levels of these substances in both groups, but especially for the tendinosis group

There was no correlation between between BDNF and TNFalpha levels in the tendon tissue samples of Achilles tendinosis patients.

IMPLICATIONS

This study shows that serum BDNF and TNFreceptor1 concentrations are correlated in Achilles tendinosis patients but not in healthy controls. The results are of interest, as interactive effects are known to occur for these substances in other situations. Furthermore, TNFalpha is known to have damage-modifying effects, and BDNF is known to be related to physical activity levels and also to be involved in pain signalling mechanisms. The implications of the correlation between BDNF and TNFreceptor1 in tendinosis patients but not in controls should be further investigated, and the reasons for the marked inter-individual variability should be explored.

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WILL MULTIPLE FREEZE/THAW CYCLES CHANGE THE TENSILE PROPERTIES OF HUMAN PATELLAR TENDONS?

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INTRODUCTION:

Soft tissue allografts, such as the bone-patellar tendon-bone (BPTB) graft, have been used frequently for anterior cruciate ligament (ACL) reconstruction [1]. After initial tissue recovery, the allografts are frozen and thawed multiple times as part of the processing steps in preparation for use in the operating room. Additionally, the graft may be thawed in the operating room and surgeons may choose not to use it due to improper sizing, change in surgical procedure, and/or other variables. The graft is then refrozen for use for another patient. Thus, the research question is whether an allograft tissue that has been subjected to multiple freeze/thaw cycles would have altered biomechanical properties. To address this, we chose to measure and compare the biomechanical properties of the human BPTB graft after 1, 4, and 8 freeze/thaw cycles.

METHODS:

Whole patellar tendon and tibial bone section specimens were obtained from 21 human donors (age range 27-79 years with mean ± standard deviation equal to 63 ± 14 years). For each donor, three or four samples with approximately 10 mm width were excised, along with the corresponding patellar and tibial bone ends. Specimens were then randomly selected and subjected to either 1, 4, or 8 freeze-thaw cycles. A freeze/thaw cycle was defined as freezing at -20±10°C for at least six hours, and thawing at 22±3°C for at least 3 hours. Afterward, each specimen was thawed and tensile tested. First, the cross-sectional area of the PT was measured [2]. Reflective markers were then placed evenly along the tendon for the determination of tissue strain. The specimen was placed in custom clamps and then mounted on a materials testing machine within a physiological saline bath held at 37°C. A 2 N preload was first applied and the gauge length was established. The specimen was preconditioned for 100 cycles between 50 N and 250 N. Afterward, it was returned to its gauge length and then loaded to failure at a constant crosshead speed of 50 mm/min. A load-elongation curve representing the structural properties of the BPTB was obtained and the stiffness (slope of the linear portion of the curve) and ultimate load were determined. A stress-strain curve representing the mechanical properties of the PT could also be obtained by dividing the load of each specimen by its respective cross-sectional area and calculated strain from as the change in distance between the surface markers divided by their initial distance, and the tangent modulus (slope of the linear portion of the curve) and stress at failure were calculated. Statistical analysis between the three groups was done using a repeated measures ANOVA with a Bonferroni post-hoc test. Significance was set at $p < .05$.

	Freeze/Thaw Cycles		
	1	4	8
Stiffness (N/mm)	242 ± 45	237 ± 41	230 ± 40
Ultimate Load (N)	1264 ± 338	1150 ± 318	1156 ± 347
Tangent Modulus (MPa)	905 ± 335	836 ± 371	913 ± 259
Stress at Failure (MPa)	26 ± 8	22 ± 7*	23 ± 7

Table 1. Parameters representing the structural properties of the BPTB and mechanical properties of the PT.

RESULTS:

There were no clear trends in the stiffness and ultimate load of the BPTB graft or the tangent modulus or stress at failure of the PT tissues (Table 1). Further, no significant differences were found between the freeze thaw groups ($p>0.05$). The lone exception was that values for stress at failure for the 4 freeze/thaw group were significantly lower than those for the 1 freeze/thaw group ($p<0.05$).

DISCUSSION:

The results of this study suggest that multiple freeze/thaw cycles has little or no effect on the structural properties of the BPTB graft or mechanical properties of the PT tissue. The data on the biomechanical properties of the BPTB were found to be consistent with those in the literature [3, 4]. Thus, the data obtained support the notion that the BPTB allograft could be re-frozen and still be used. However, in order to put this conclusion in a more definitive basis, additional studies to separate the age and gender dependence will need to be done. In the end, the selection of BPTB allografts for ACL reconstruction after multiple freeze/thaw cycles could be done on a scientific basis.

ACKNOWLEDGEMENTS:

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ARBITRARY STARTING POINT OF SEPARATION AFFECT THE MORPHOLOGY OF THE TWO BUNDLES OF ANTERIOR CRUCIATE LIGAMENT AT INSERTION SITES

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INTRODUCTION

In previous reports about the anatomical studies of ACL, the two bundles were separated with the knee in flexion, in which position a mark line could be found and detected from the anterolateral aspect of ACL. However, it has been described in none of the reports regarding from which point on this mark line the separation started. Because the ACL is a rotating fiber structure in knee flexion, changes of the starting point on the mark line at the beginning of separation may result in different morphology at insertion. So the purpose of this study was to explore if it was the case.

METHODS

Ten cadaver knees were used to separate the ACL fibers into two bundles from three different starting points that located respectively at the proximal one, two and third fifth of the inter-bundle mark line. (Fig 1,2) The dividing lines between the two bundles at both insertions and the area of the AM bundle resulted from these different separation manners were compared, with data collected respectively in group Ⅰ, Ⅱ, and Ⅲ. (Fig 3,4)

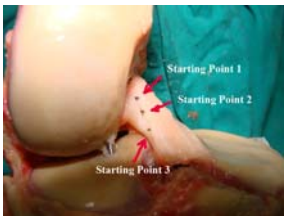

Fig 1

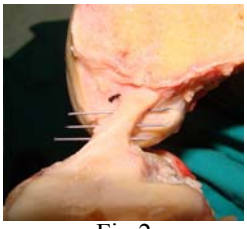
Fig 2

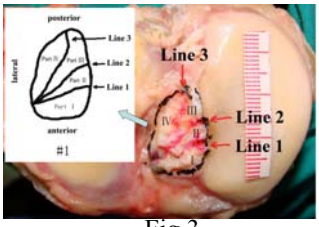

Fig 3

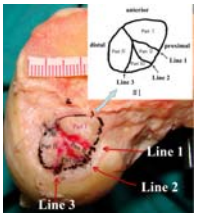

Fig 4

RESULTS

The angle of the dividing line and the long axis of the femoral footprint was 67.24° ± 11.94°, 91.01° ± 11.16°, and 116.03° ± 9.01° , and the percentage of the AM bundle area in the whole femoral footprint were 38.64 ± 5.55, 52.22 ± 6.76, and 65.09 ± 4.53, respectively in group Ⅰ, Ⅱ, and Ⅲ. (Fig 5,6) At the tibial insertion sites, the angle between the dividing line and the sagittal plane was 110.17° ± 13.26°, 127.72° ± 8.94°, and 149.28° ± 18.80°, and the percentage of the AM bundle area in the whole footprint were 25.72 ± 3.82, 40.41 ± 3.73, and 60.56 ± 6.59 respectively in group Ⅰ, Ⅱ, and Ⅲ. (Fig5,7) There were significant differences between the angle or the area data of each of the two groups at either the tibial or the femoral insertion site.

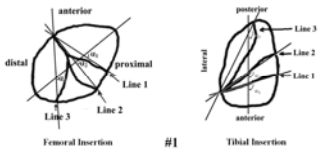

Fig 5

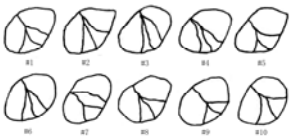
Fig 6

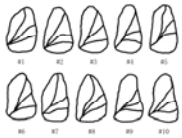
Fig 7

DISCUSSION

In anatomical study of ACL, changes of the point that separation started from resulted in obviously different bundle morphology at the insertion. The morphological diversities at the insertion sites regarding the two ACL bundles in previous studies may be due to normal anatomical variance and artificial factors during separation. Reducing the influence of the artificial factors during anatomical study is certainly necessary for accurate description of the normal anatomy of the ACL at insertion.

EFFECT OF CTGF ON THE TENOGENIC DIFFERENTIATION OF RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN VITRO

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INTRODUCTION

Bone marrow-derived mesenchymal stem cells (BMSCs) isolated from adult bone marrow have been shown to have multi-differentiation potential. The application of BMSCs for bone and cartilage repair has been studied for many years and has been used as a new treatment in clinic. Recent investigations have tried to use BMSCs for tendon repair, but ectopic calcification was reported in some cases (Seib et al., 2009). In order to promote tendon repair with BMSCs, the in vitro differentiation of BMSCs to tenocytes before transplantation is suggested. Connective Tissue Growth Factor (CTGF) was reported to be an effective induction factor for fibroblastic differentiation of human BMSCs (Lee et al., 2006). In this study, we investigated the effect of CTGF on the tenogenic differentiation of rat BMSCs in vitro.

METHODS

BMSCs were isolated from bone marrow tissue of GFP (Green Fluorescent Protein) rats by density gradient centrifugation method. Chondrogenic, osteogenic and adipogenic differentiation assays were used to demonstrate the multi-differentiation potential of the isolated BMSCs. BMSCs at passage 3 were cultured with different concentrations of CTGF (0ng/ml, 25ng/ml, 50ng/ml, and 100ng/ml) for 2 weeks (5 wells / group). Afterwards, mRNA expression of scleraxis, tendomodulin, tenascin C and type I collagen (COL1A1) from 4 wells in each group was examined by qRT-PCR. The remaining sample (n=1) from each group was used for immunocytochemistry staining of tenomodulin. The protein expression of tendomodulin in cells treated with CTGF (50ng/ml) for two weeks was also examined by Western blotting. Kruskal-Wallis test was used to compare the difference in relative mRNA expression among different groups. If there was a difference, Mann-Whitney U test was used for 2-group comparison. All the data analysis was done using SPSS (SPSS Inc, Chicago, IL, version 16.0) , $P<0.05$ was regarded as statistically significant.

RESULT

Multi-differentiation assays have confirmed that BMSCs have osteogenic, chondrogenic and adipogenic differentiation potentials. There was increase in the mRNA expression of tendomodulin, scleraxis and tenascin C after treatment with CTGF for 2 weeks. The mRNA expression of tendomodulin significantly increased by 11.00, 5.06 and 5.89 folds for the low, medium and high dose groups respectively compared to that in the control group ($P<0.05$). The mRNA expression of scleraxis significantly increased by 2.83, 1.39 and 2.28 folds for the low, medium and high dose groups respectively compared to that in the control group ($P<0.05$). The mRNA expression of tenascin C significantly increased by 2.47, 3.46 and 3.67 folds for the low, medium and high dose groups respectively compared to that in the control group ($P<0.05$). The mRNA expression of COL1A1 significantly increased by 2.97, 2.34 and 2.47 folds for the low, medium and high dose groups respectively compared to that in the control group ($P<0.05$). Immunocytochemistry staining showed that tenomodulin expression was detected in the three treatment groups but not in control group. This was further confirmed by Western blotting which showed higher protein expression of tenomodulin in the medium dose (50ng/ml) group compared to that in the control group.

CONCLUSION

CTGF significantly increased scleraxis, tenomodulin, tenascin C and COL1A1 mRNA expression of rat BMSCs in vitro.

DISCUSSION

CTGF may be used to promote in vitro tenogenic differentiation of rat BMSCs before transplantation for tendon repair.

ACKNOWLEDGMENT

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MEASUREMENT OF NORMAL TIBIALIS ANTERIOR MUSCLE ARCHITECTURE BY ULTRASOUUND IN ELITE ATHLETES AND CONTROLS-A CROSS-SECTIONAL STUDY

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BACKGROUND

There are currently no published data describing the normal muscle architecture values for functionally significant muscles such as Tibialis Anterior in athletic populations. These data would allow investigation of sport-specific training adaptations, architectural response to injury and recovery.

OBJECTIVES

To compile a set of normal values for muscle architecture of the Tibialis anterior muscle in elite athletes and sedentary controls. Secondly, to compare the architecture of athletes across different events. Thirdly, to provide additional evidence for reliability of ultrasound in measuring architectural parameters.

METHOD

The superficial pennate portion of Tibialis Anterior from both legs in 15 elite athletes [mean (sd) height: 1.78m (0.08), weight: 77.4kg (8.7), age: 25.5yrs (3.6), 10 males] and 16 sedentary controls [mean (sd) height: 1.70m (0.12), weight: 66.9kg (8.72), age: 21.6yrs (1.7), 6 males] was imaged in vivo using ultrasound. Images were analysed using Image J software to obtain values for muscle thickness, pennation angle and fascicle length.

RESULTS

All analyses were adjusted for gender and leg length. Athletes had significantly larger pennation angles [adjusted mean (SE) athletes: 12.21° (0.40), controls: 10.27° (0.39), p=0.002] and thicker muscles than controls [athletes: 1.12cm (0.03), controls 1.02cm: (0.03), p=0.024]. No significant differences were found for fascicle lengths between athletes and controls [athletes: 5.54cm (0.23), controls: 5.73cm (0.22), p=0.577]. Sprinters had larger pennation angles than fencers [sprinters: 12.92° (0.43), fencers: 10.34° (0.67), p=0.004] and controls [controls: 10.24° (0.36), p=0.000]. Fencers had longer fascicle lengths than sprinters [fencers: 6.55cm (0.39), sprinters: 5.15cm (0.25), p=0.008].

CONCULSION

This study describes normal muscle architecture for Tibialis Anterior in elite athletes and sedentary controls. This information needs to be built on using larger subject numbers in order to provide a base for examining architecture in muscular injuries.

Key Terms: Muscle architecture, athletes, Tibialis Anterior, ultrasound

**Finalist of the Best Poster Award*

AN EXPERIMENT OF HUMAN ACELLULAR DERMAL MATRIX ON THE ROTATOR CUFF REPAIR IN A CANINE MODEL

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INTRODUCTION

Currently, there is no gold standard for the treatment of cuff tears, even though several types of surgical options are recommended for massive rotator cuff tears. This outcome has fueled the continued investigation into methods to augment rotator cuff repairs. The tissue engineered augmentation of rotator cuff repair tissue has focused on the use of biodegradable scaffolds. These scaffolds are intended to provide an absorbable matrix to guide, and in some cases promote, the ingrowth of host tissue that will eventually modulate into a connective tissue of similar biological and biomechanical characteristics to the native rotator cuff tendon. Our purpose was to determine whether rotator cuff tendon defects can be reconstructed with acellular dermal matrix (ADM) grafts that may serve as a biodegradable scaffold.

METHODS

Human derived dermal tissue was minimally processed to remove epidermis and all cellular components but maintain the extracellular structure. The tissue was washed by cryopreservation solution and then freeze-dried. Product was not chemically cross-linked. A canine model for a full-thickness infraspinatus tendon tear was used. Tendon was excised from the bony interface to the myotendinous junction, and a human acellular dermal matrix (ADM) graft was using to bride the defect. Animals were sacrificed after 6 and 12 weeks, and shoulders were evaluated histological analysis and Magnetic Resonance Image (MRI) finding.

RESULTS

Histologically, cell infiltrations were noted in experimental specimens taken by 6 weeks. The graft material was intact and filled with numerous elastic fibers and blood vessels. Extensive host cellular infiltration was evident along populated. Calcification or infection was not evident. By 12 weeks, histologic evidence of native cell infiltration and neotendon development was observed. The ADM graft was incorporated into a structure resembling control specimen. Also, Experimental specimens on the rotator cuff defect revealed similar as normal tendon structure by the magnetic resonance imaging (MRI) study.

DISCUSSION

The ADM was incorporated into the defect areas by cellular infiltration, alignment of collagen fibers, and blood vessel ingrowth. This graft material exhibits key biologic factors of the remodeling process when used as an augmentation device in rotator cuff repair. Further clinical studies are necessary to evaluate the potential applications of ADM augmentation for rotator cuff repair.

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**Finalist of the Best Poster Award*

EFFECT OF CYCLIC STRETCHING ON THE TENOGENIC DIFFERENTIATION OF RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS *IN VITRO*

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INTRODUCTION

Bone marrow-derived mesenchymal stem cells (BMSCs) isolated from adult bone marrow have been shown to have multi-differentiation potential. The application of BMSCs for bone and cartilage repair has been studied for many years and has been used as a new treatment in clinic. Recent investigations have tried to use BMSCs for tendon repair, but ectopic calcification was reported in some cases (Seib FP et al., 2009). In order to promote tendon repair with BMSCs, the in vitro differentiation of BMSCs to tenocytes before transplantation is suggested. Recent study suggested that cyclic stretching promoted the synthesis of collagen types I and III and tenascin-C by the rat BMSCs (Lei Zhang et al., 2008). In this study, we investigated the effect of cyclic stretching on the tenogenic differentiation of rat BMSCs in vitro.

METHODS

BMSCs were isolated from bone marrow tissue of GFP (Green Fluorescent Protein) rats by density gradient centrifugation method. Chondrogenic, osteogenic and adipogenic differentiation assays were used to demonstrate the multi-differentiation potential of the isolated BMSCs. BMSCs at passage 3 were seeded at the density of $2.5\times10^4/cm^2$ in silicon chamber. After overnight, Group A was cultured in silicon chamber for 24h as control without cyclic stretching, and for Group B, a cyclic 10% uniaxial stretching at 1 Hz was applied for 24h. Afterwards, mRNA expression of scleraxis, tendomodulin, tenascin C, type I collagen (COL1A1) was examined by qRT-PCR. Mann Whitney U test was used to compare the difference in relative mRNA expression between the two groups. If there was a difference, Mann-Whitney U test was used for 2-group comparison. All the data analysis was done using SPSS (SPSS Inc, Chicago, IL, version 16.0)，$P<0.05$ was regarded as statistically significant.

RESULT

Multi-differentiation assays have confirmed that BMSCs have osteogenic, chondrogenic and adipogenic differentiation potentials. Results showed that cyclic stretching could change the alignment of BMSCs, whichwas perpendicular with the stretch direction. The mRNA expression of tendomodulin, scleraxis and type I collagen (COL1A1) were significantly increased by 2.56, 2.45 and 1.80 folds respectively, compared to that in the control group ($P<0.05$), while the mRNA expression of tenascin C was increased by 0.76 fold compared to that in the control group ($P>0.05$).

CONCLUSION

Cyclic stretching could significantly promote the tenogenic differentiation of BMSCs in vitro.

DISCUSSION

Tensile load using may be applied to promote in vitro tenogenic differentiation of rat BMSCs before transplantation for tendon repair.

ACKNOWLEDGMENT

This work was supported by equipment / resources donated by the Hong Kong Jockey Club Charities Trust and the Restructuring and Collaboration Fund from University Grant Council.

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**Finalist of the Best Poster Award*

DYNAMIC STRAIN-MEDIATED TENDINOGENIC DIFFERENTIATION OF BONE MARROW STROMAL CELLS ON SMALL INTESTINAL SUBMUCOSA MEMBRANE

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INTRODUCTION

Tendon regeneration requires the recruitment of stem cells and their differentiation into mature committed cells. Previous studies indicated that bone marrow stromal cells (BMSCs) can form tendon-type tissue [1]. However, BMSCs form bone rather than tendon-like tissue when undifferentiated cells are transplanted into mice [2]. Finding the optimal conditions that will induce BMSCs differentiation into tendon-forming cells will be crucial for the development of BMSCs-based tendon repair. In the study, we investigated the possibility of BMSCs differentiated into tenogenic cells by small intestinal submucosa (SIS) membrane strain.

METHODS

BMSCs were isolated from bone morrow of one-week SD rats. The differentiation induction media were used to confirm BMSCs differentiation for osteogenesis and adipogenesis. The expressions of cell surface markers were determined by flow cytometry. BMSCs were seeded on SIS membrane and cultured for 2d, and then subjected to the dynamic strain stimulation (frequency 0.02Hz, time 3h/d, strain 5%) for 5d. SEM was used to examine the morphology of the cells. The contents of scleraxis and tenomodulin in culture supernatant were tested by ELISA.

RESULTS AND DISCUSSION

The cultivated cells had adipogenic and osteogenic differentiation abilities. Flow analysis showed that the undifferentiated cells were CD34- CD45- and CD90+, expressing the surface markers of BMSCs. SEM showed that the differentiated cells were spindle-shaped with fibroblast-like colonies covering the surface of the SIS membrane, and there were more aligned cells along the direction of the strain in strained group than in non-strained group (Fig.1). The contents of scleraxis and tenomodulin were much higher in strained group than in non-strained group (Fig.2). The products of these proteins in supernatant are important components of tendon ECM, and their synthesis is crucial for normal tendon development and for repair of tendon laceration. These findings suggest that the mechanoactive stimulation produced by SIS membrane strain can induce BMSCs differentiation into tenocytes.

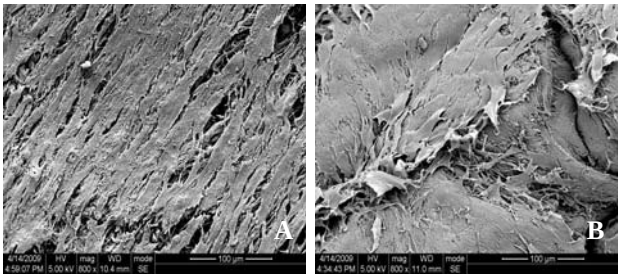

Fig.1 Morphological changes of BMSCs. Strained (A) and Non-strained (B).

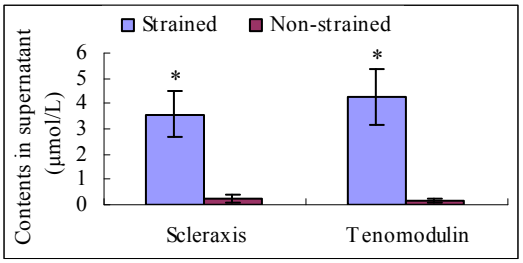

Fig.2. Contents of scleraxis and tenomodulin in supernatant. * P ＜0.05 when compared with control.

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ACKNOWLEDGEMENT

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**Finalist of the Best Poster Award*

REGULATORY EFFECT OF COLLAGEN V ON THE FIBRILLEGENESIS OF TENOCYTES IN A TISSUE ENGINEERING MODEL

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OBJECTIVE

The presence of uniformly small collagen fibrils in tendon repair is believed to play a major role in suboptimal tendon healing. Collagen V is significantly elevated in healing tendons and related to fibrillogenesis. The present study aimed to investigate the effect of particular subunits of collagen V on the fibrillogenesis of tenocytes.

METHODS

Specific siRNA oligonucleotides were used against different procollagen subunits of type V. Their effects on cell proliferation, apoptosis, collagen synthesis, fibril organization and fibril diameter were analyzed using a tissue engineered tendon model.

RESULTS

The results showed that siRNA against the a1 type V collagen gene (COL5A1 siRNA) and siRNA against the a2 type V collagen gene (COL5A2 siRNA) had different effects on collagen I and proteoglycan decorin gene expression. Transmission electron microscopy showed that tissue engineered tendon treated with COL5A1 siRNA had smaller collagen fibrils with abnormal morphology, the tendon treated with COL5A2 siRNA had smaller collagen fibrils but similar in morphology to those of the control group. More importantly, the engineered tendon formed by the coculture of COL5A1 siRNA treated tenocytes and normal tenocytes with a ratio of 1:0.5 or 1:1 had better fibrogenesis and larger collagen fibrils than that of normal tenocytes.

CONCLUSION

Our studies demonstrated that tissue engineered tendon is a novel and useful model for biological investigation. The type V procollagen a1 and a2 chains have different effects on regulating tendon matrix gene expression. And an optimal level of collagen type V is vital in regulating collagen fibrillogenesis. This may provide a basis for future development of novel cellular- and molecular biology-based therapeutics for tendon diseases.

Keywords: Tissue engineered tendon, Collagen type V, Fibrillogenesis

**Finalist of the Best Poster Award*

STROMAL CELL-DERIVED FACTOR 1 ENHANCES THE REGENERATION OF TENDON USING A KNITTED SILK SCAFFOLD COMBINED WITH COLLAGEN MATRIX

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INTRODUCTION

In recent years, the efficacy of stem cell and silk scaffold for tendon tissue engineering was investigated in vitro and in animal models, and the results indicate that silk is a suitable biomaterial for tendon tissue engineering[1], but a bioactive silk scaffold remains a signification challenge. Stromal cell–derived factor 1 is a dominant chemokine in bone marrow and the local delivery of SDF-1 into injured tissue promotes the repair of lesions in the heart, brain[2] and bone.The purpose of this study was to find a bioactive scaffold for tendon tissue engineering and the mechanism of the SDF-1/CXCR4 axis in tendon repair.

METHODS

SDF-1 regulates CXCR4 expression of MSCs, tendon cells and fibroblast was detected at the mRNA level.The in vitro migration of cells in response to recombinant human SDF-1 alpha (rhSDF-1) was assessed using Transwell. Collagen gel containing stromal cell–derived factor 1 (SDF-1) were prepared and the release of SDF-1 from these gel and their effect on healing of tendon wounds in rat were evaluated. Histological analysis was conducted to evaluate in vivo results. Transmission electron microscopy (TEM) was used to assess collagen fibril diameter and alignment. Gene expression was detected by real-time PCR. Different types of cells were injected into the repaired sites to evaluate the mechanism of induced cells for tendon repair.

RESULTS

Higher expression of cxcr4 were found in the repaired tendons. Real-time PCR results showed that the MSC has the heighest CXCR4 expression and fibroblast has the lowest CXCR4 expression. However, SDF-1 up-regulated CXCR4 expression in MSCs and fibroblasts. In vitro Transwell assay showed the rhSDF-1 (200 ng/ml) induced the migration of MSCs, tenocytes and fibroblast. In vivo injection of cells showed that more MSCs were induced into the repaired sites. The histology of SDF-1 treated groups showed that more cells and denser connective tissues filled and wrapped the scaffold at 1 weeks.More collagen fibers were formed in the SDF-1 treated group than in the scaffold alone group at 4 weeks. The expression levels of the Collagen and tendon specific genes in silk tcollagen+SDF-1 treated Achillesn tendon were higher than those of silk collagen treated Achillesn tendon.

DISCUSSION

The present study is the first demonstration that SDF-1 can improve the repair of tendon wounds. In this study, SDF-1 enhanced the regeneration of tendon injury by migrating more MSCs and fibroblasts into the silk scaffolds, which may be one of the reasons for the change in the different levels of connective tissue density and collagen fibersfibril diameter, increasing the tenocytes migration into the repaired cites may improve the tendon regeneration. In conclusion, interaction of SDF-1 and CXCR4 could play important roles in the migration of MSCs and fibroblasts to impaired sites in the tendon, and could serve as an optimal bioactive scaffold for tendon regeneration.

EFFECT OF GDF-7 ON THE TENOGENIC DIFFERENTIATION OF RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS *IN VITRO*

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INTRODUCTION

Recent investigations have tried to use BMSCs for tendon repair, but ectopic calcification was reported in some cases (Seib FP, 2009). In order to promote tendon repair with BMSCs, the in vitro differentiation of BMSCs to tenocytes before transplantation is suggested. Growth and Differentiation Factor-7 (GDF-7) was reported to promote tendon healing and increase tensile strength of tendon regenerate (Violini S, 2009; Vishal Mehta, 2005). In this study, we investigated the effect of GDF-7 on the tenogenic differentiation of BMSCs in vitro.

METHODS

BMSCs were isolated from bone marrow tissue of GFP (Green Fluorescent Protein) rats by density gradient centrifugation method. Chondrogenic, osteogenic and adipogenic differentiation assays were used to demonstrate the multi-differentiation potential of the isolated BMSCs. BMSCs at passage 3 were cultured with different concentrations of GDF-7 (0ng/ml, 12.5ng/ml, 25ng/ml, and 50ng/ml) for 2 weeks (5 wells / group). Afterwards, mRNA expression of scleraxis, tendomodulin, tenascin C and type I collagen (COL1A1) from 4 wells in each group was examined by qRT-PCR. The remaining sample (n=1) from each group was used for immunocytochemistry staining of tenomodulin. The protein expression of tendomodulin in cells treated with GDF-7 (25ng/ml) for two weeks was also examined by Western blotting. Kruskal-Wallis test was used to compare the difference in relative mRNA expression among different groups. If there was a difference, Mann-Whitney U test was used for 2-group comparison. All the data analysis was done using SPSS (SPSS Inc, Chicago, IL, version 16.0)，$P<0.05$ was regarded as statistically significant.

RESULT

Multi-differentiation assays have confirmed that BMSCs have osteogenic, chondrogenic and adipogenic differentiation potentials. There was increase in the mRNA expression of tendomodulin, scleraxis and tenascin C after treatment with GDF-7 for 2 weeks. The mRNA expression of tendomodulin significantly increased by 3.07, 3.41 and 2.85 folds for the high, medium and low dose groups respectively, compared to that in the control group ($P<0.05$) while there was no significant difference among three dose groups. The mRNA expression of scleraxis significantly increased by 2.56, 1.50 and 2.13 folds for the high, medium and low dose groups respectively compared to that in the control group ($P<0.05$) while there was no significant difference among three dose groups. The mRNA expression of tenascin C significantly increased by 1.88, 2.86 and 2.45 folds for the high, medium and low dose groups respectively compared to that in the control group ($P<0.05$) while there was no significant difference among three dose groups. The mRNA expression of COL1A1 significantly increased by 1.92 and 2.45 folds for the low and medium dose groups respectively compared to that in the control group ($P<0.05$) while there was no significant difference among three dose groups. The mRNA expression of COL1A1 increased by 1.50 fold for the high dose group compared to that in the control group ($P>0.05$). Immunocytochemistry staining showed that tenomodulin expression was detected in the three treatment groups but not in control group. This was further confirmed by Western blotting which showed higher protein expression of tenomodulin in the medium dose (25ng/ml) group compared to that in the control group.

CONCLUSION

GDF-7 significantly increased scleraxis, tenomodulin, tenascin C and COL1A1 mRNA expressionof rat BMSCs in vitro.

DISCUSSION

GDF-7 may be used to promote in vitro tenogenic differentiation of rat BMSCs before transplantation for tendon repair.

ACKNOWLEDGMENT

This work was supported by equipment / resources donated by the Hong Kong Jockey Club Charities Trust and the Restructuring and Collaboration Fund from University Grant Council.

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METABOLISM OF EXTRACLLULAR MATRIX IN FLEXOR TENDON WITH STRESS DEPRIVATION

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INTRODUCTION

Stress deprivation after musculoskeletal tissue injuries or repair typically results in a rapid reduction in mechanical properties and mass of the affected tendons and ligaments[1, 2]. Knowledge of the specific effects on tendon metabolism with stress deprivation is limited[3, 4]. In this study, we investigated the effects of stress deprivation on the expression of extracellular matrix, matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteases (TIMPs) in canine flexor tendon *in vivo*.

METHODS

Twenty mixed-breed adult dogs were used this study, which was an adjunct to other IACUC approved studies. As a part of those studies, one fore paw in each dog was splinted in wrist flexion and a sling was used to maintain the paw underneath the chest without weight bearing. The other fore paw in each dog moved freely. Ten dogs were treated for 21 days. Another ten dogs were treated for 42 days. After the dogs were sacrificed, ten mm long segments were harvested from flexor digitorum profundus (FDP) tendons of the third digits of both fore paws. The tendon pieces were snap-frozen in liquid nitrogen and stored at –80°C until the time of RNA extraction. Total RNA was extracted with TRIzol® Reagent (Invitrogen) from the tendon segment according to the manufacturer's protocol. Genomic DNA was removed by DNase treatment and RNeasy Mini Kit (QIAGEN). Total RNA was reverse transcribed into single-stranded cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche). Quantitative RT-PCR was performed with LightCycler® to measure the gene expressions of collagen I, collagen III, decorin, fibronectin, MMP2, MMP3, MMP13, MMP14, TIMP1 and TIMP2. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was served as the reference house-keeping gene. Tensile testing was performed on FDP tendons from the 4th digits of both the suspended and non-suspended fore paws with MTS servohydraulic testing machine.

RESULTS

Stress deprivation decreased the modulus of the tendons which were in the limbs suspended for 21 and 42 days. The modulus difference was statistically significant for tendons in the limbs suspended for 42 days ($p < 0.05$).

In consistence with the decrease of modulus, stress deprivation resulted in a significant reduction of expression of collagen I, collagen III, decorin, and fibronectin in flexor tendon comparing to the tendon with free motion ($p<0.05$) (Fig. 1). The suspension for 42 days resulted in further reduction in the expression of collagen I, collagen III, decorin and fibronectin. There was statistical significance in the expression of decorin and fibronectin in flexor tendons between 21 and 42 days.

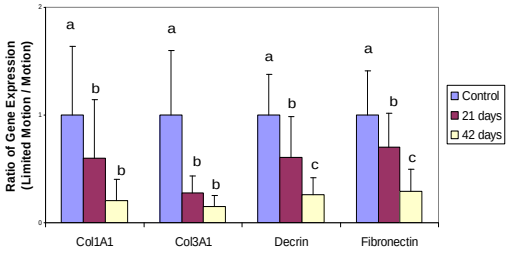

Fig. 1. Expression of the extracellular matrix proteins in flexor tendons with or without the limited motion for the difference periods in vivo. The statistical significance of expression of each gene was identified between the groups labeled with the different words.

The expression of MMP2, MMP3, MMP13 and MMP14 in the tendons suspended for 21 days was significantly increased compared to that in the contralateral tendons ($p<0.05$) (Fig. 2). The expression of MMP2 and MMP13 maintained a high level in the tendons suspended for 42 days. However, the expression of MMP3 and MMP14 in the tendons suspended for 42 days returned to the same level as those in the control tendons. Stress deprivation resulted in a reduction of the expression of TIMP1 and TIMP2 at 42 day ($p<0.05$) but not at 21 day.

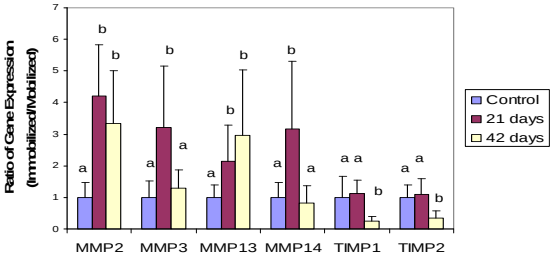

Fig. 2. Expression of MMPs and TIMPs in flexor tendons with or hout the limited motion in vivo. The meaning of labels is same as Fig 1.

DISCUSSION

This study is the first to systematically investigate the expression of multiple genes related to metabolism of tendon with stress deprivation. The gene expression in this study was detected from the intact FDP tendons. Although the injury of the neighbor digits may affect the gene expression in the intact tendon, we believe the major effects on the alteration of gene expression resulted from the mechanical loading.

Our study found that mechanical loading correlates with the mechanical properties and the expression of extracellular matrix proteins, MMPs and TIMPs in flexor tendon. The reduction in mechanical properties of tendons results from the decrease in anabolism of extraxcellular matrix proteins. Meanwhile, it also is associated with the enhancement of catabolic process of extracellular matrix proteins by increasing the expression of MMPs and decreasing the expression of TIMPs.

ACKNOWLEDGEMENTS

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CARPAL TUNNEL SIZE AND SHAPE ALTERATION INDUCED BY TRANSVERSE CARPAL ARCH DEFORMATION

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INTRODUCTION

The carpal tunnel is formed by carpal bones and the transverse carpal ligament. Carpal tunnel morphology plays a critical role in regulating biomechanical and physiological states of the carpal tunnel contents. Surgical treatment of carpal tunnel syndrome involves the transaction of the transverse carpal ligament, leading to an increase in cross-sectional area (CSA) (Kato et al. , 1994) and a decrease in pressure (Gelberman et al. , 1981). Manipulation of carpal tunnel morphology by myofascial manipulation (Sucher et al., 1993) and tunnel traction (Porrata et al., 2007) has been attempted as a non-surgical, therapeutic approach to improve carpal tunnel syndrome. Our recent study showed that carpal arch narrowing was effective in forming a palmar arch and in increasing tunnel CSA (Li et al., 2009). The purpose of this study was to investigate the change of carpal tunnel morphology in response to the widening and narrowing of the transverse carpal arch (TCA).

METHODS

The carpal tunnels of eight cadaveric wrists were evacuated. The widening (+3 mm) and narrowing (-3 mm) of TCA at the hamate level were implemented by a deformation device (Fig. 1). A silicone mold of the carpal tunnel was created for each of the three TCA conditions (i.e. narrowing, no change, widening) (Pacek et al., 2009). Each silicone mold was digitized and reconstructed. Cross-sectional area (CSA) and shape index at the hamat level were calculated.

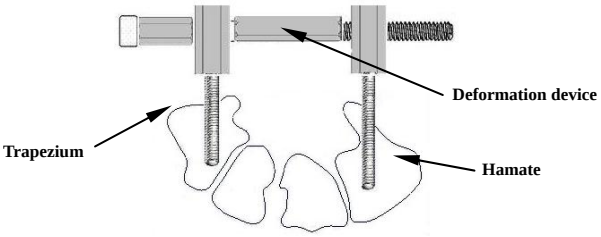

Fig. 1. Schematic of the carpal tunnel deformation device attached to the trapezium and hamate.

RESULTS

Deformation of the TCA in the inward or outward direction led to a change in the cross section of the carpal tunnel (Fig. 2). With the intact TCA, the CSA of the carpal tunnel was 167.6 ± 22.2 mm2 . The CSA increased to 180.9 ±24.9 mm2 when the TCA was narrowed by inward deformation. The CSA decreased to 150.6 ±22.9 mm2 when the TCA widened by outward deformation. The carpal tunnel shape indices (the ratio of depth to width) were 0.49 ± 0.04, 0.56 ± 0.07, and 0.41 ± 0.04 for the intact, narrowing, and widening conditions, respectively.

Fig. 2. Representative carpal tunnel cross sections for the narrowing (a), intact (b), and widening (c) carpal arch conditions.

(a) Narrowing carpal arch (b) Intact carpal arch (c) Widening carpal arch

DISCUSSION

The inward deformation of the carpal tunnel resulted in an increase in CSA, which is consistent with our previous findings (Li et al., 2009). The increased cross sectional area may be attributed by the increased depth/width ratio. The carpal tunnel tends to be more rounded by inward deformation of the transverse carpal arch. Conversely, outward deformation of the carpal arch led to a more flatted tunnel and a decreased cross sectional area. The result that carpal tunnel size can be expanded by shortening carpal arch width suggests that mobilization of the carpal bones to decrease carpal arch width may be used as a strategy to increase carpal tunnel volume, decrease carpal tunnel pressure, and improve carpal tunnel syndrome.

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INDUCED PLURIPOTENT STEM CELLS AS A MODEL TO TREAT AND STUDY HUMAN OSTEOARTICULAR DISEASES

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Induced pluripotent stem (iPS) cell technology has revolutionized the stem cells field and threatens to change Biology in general as well. The simple over-expression of a combination of transcription factors highly enriched in embryonic stem cells (ESC) can reprogram the nucleus of a somatic cell to an ESC-like pluripotent stage. These cells possess a very similar if not identical genetic program to ESC, can form teratomas when injected in immune-compromised mice, and in the case of mouse iPS can also give rise to entire individuals. Although many hurdles need to be overcome, iPS cells have the potential to provide patient specific tissues for transplantation purposes that are devoid of immunological and ethical concerns. In addition, iPS can be used as a model to study human genetic diseases in vitro, in particular those for which appropriate animal models don't exist. Herein we present our most recent work on iPS with particular emphasis on genetic diseases of different kind including osteoarticular. We also discuss the potential use of iPS for tissue replacement therapies of genetic or degenerative osteoarticular diseases.

HIGH EFFICIENCY DIFFERENTIATION OF NEURONAL CELLS FROM MOUSE EMBRYONIC STEM CELLS BY GRADUAL MEDIUM REPLACEMENT

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INTRODUCTION

The study of induction of Embryonic stem cells (ESCs) into neurons is not only valuable for the potential application in cell transplantation, but also an important *in vitro* model for studying mammalian nerve system development. However, the yields of neuronal cells are not very high, no better than 50%~70%. Here we report a new method of high efficiency derivation of neuronal cells from mESCs by gradual medium replacement.

METHODS

The mESCs were cultured on a feeder layer of PMEFs in an mESC growth medium that is consisted of high-glucose Dulbecco's modified Eagle's medium, 15%FBS, β-mercaptoethanol, nonessential amino acids, recombinant human leukecythemia inhibit factor. Cultures were maintained at 37ºC in a 5% CO_2 incubator. Then the mESCs were dissociated by trypsinization and plated on un-coated cell culture surface in a growth medium (NSCM) containing DMEM/F12, 5% fetal bovine serum, and 5% horse serum. Two days after plating, this culture medium was gradually switched to a serum-free defined medium consisted of DMEM/F12, 1% N2, 2% B27, 20 ng/ml bFGF. It took 5 days to completely change the medium into the serum-free defined medium and the cells were maintained for up to 10 days. The following conditions were varied to optimize the neural induction: initial amount of the serum-free defined medium at 10%, 12.5% and 15%; plating density of mESCs at 1.0×10^4/ml, 1.0×10^5/ml or 1.0×10^6/ml; total duration of culture in the serum-free defined medium.

RESULTS

The optimal concentration of the first addition of the serum-free medium was 12.5%, and the best mESC plating density was 1.0×10^5/ml. The culture medium was completely changed to the serum-free culture medium on the fifth day after the first medium replacement.Ten days after the initial induction cells showed significant proliferation and the formation of extensive neurite-like processes. Most cells expressed NSC marker nestin on the 4^{th} day after initial medium replacement. Neuron-specific markers including NeuN, TUJ1, and NCAM1 were expressed by the majority of cells on the 10^{th} day. The expression of GFAP, a marker for astrocytes, was not observed. Western blot analysis demonstrated the absence of neuron-specific type-III β-tubulin (TUJ1) in mESCs and its significant increase in differentiated cells at day 4 and day 10 after initial induction ($P < 0.05$). flow cytometry study with Neu-N indicated that about 90% of induced cells were neurons. Microarray analysis of gene expression patterns of the cells 4 and 10 days after the initial induction showed the expression of cell-type specific markers at different stages of the neural induction. Glial markers and markers for other germ layers (endoderm and mesoderm) were absent, suggesting that the final cellular population was mostly neurons from the ectoderm.

CONCLUSION

Using a gradual medium replacement protocol, we were able to efficiently induce very high ratio of mouse ESCs into neurons. The present study provides a simple, efficient, convenient method for differentiating mESCs into neuronal cells. This is the highest ratio ever reported.

DOES THE SITE OF MAXIMUM NEOVASCULARISATION CORRELATE WITH THE SITE OF PAIN IN RECALCITRANT MID-TENDON ACHILLES TENDINOPATHY? A PROSPECTIVE OBSERVATIONAL STUDY

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BACKGROUND
Neovascularisation and tendon changes such as localised thickening and focal hypo-echoic areas have been suggested as an important source of pain in symptomatic tendons, however, current evidence is still inconclusive. Investigation of this correlation between neovascularisation and site of pain was performed to inform clinical examination and enhance therapeutic potential.
OBJECTIVES
To measure the correlation between the sites of maximum clinically palpated tenderness and maximum ultrasound-determined neovascularisation in patients with recalcitrant mid-tendon Achilles tendinopathy. Secondary relationships between sites of tenderness, subjectively defined pain, neovascularisation and thickening were also assessed
METHODS
A total of 29 tendons from 26 patients with recalcitrant (> 3 months) tendinopathy of the main body of the Achilles tendon were included in the study. All patients had failed to improve after a minimum of 4 weeks routine eccentric loading exercises of the gastro-soleus complex. A dedicated measuring device, with established high intra-observer reliability, was used to measure the sites of maximum clinically palpated tenderness. All measurements were recorded as a vertical distance from the plantar aspect of the heel of the affected foot.
RESULT
Significant correlations were found between site of pain and tenderness ($r = 0.91$; $P < 0.001$), site of pain and site of maximal tendon thickening ($r = 0.84$; $P < 0.001$), site of pain and site of maximum neovascularisation ($r = 0.70$; $P = 0.001$), site of tenderness and site of tendon thickening ($r =,0.91$; $P = 0.001$),site of tenderness and site of maximum neovascularisation ($r = 0.85$; $P < 0.001$), and the site of tendon thickening and site of maximum neovascularisation ($r =0.86$; $P = 0.001$).
CONCLUSION
Sites of subjectively defined pain, clinically palpated tenderness, tendon thickness and neovascularisation have a significant degree of dependence, hence enhancing the utility of clinical examination.

Keywords: Achilles tendinopathy; neovascularisation; pain.

THE EFFECT OF ALENDRONATE ON THE MECHANICAL STRENGTH OF THE TENDON GRAFT TO BONE TUNNEL COMPLEX AFTER ACL RECONSTRUCTION

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INTRODUCTION:

The outcomes of ACL reconstruction depend not only on initial graft fixation but also on the remodeling of tendon graft and graft osteo-integration for long-term success. Prevention of excessive local bone loss due to disuse and trauma and increase in bone formation at the tendon/bone interface are of prime importance to stable tendon graft fixation to bone and hence strength of ACL reconstruction complex. This study aims to investigate the effect of alendronate on improving the mechanical strength of tendon graft to bone tunnel complex after ACL reconstruction.

METHODS:

Unilateral ACL reconstruction with flexor tendon autograft was performed in 26 rats. The rats were injected subcutaneously either with alendronate 300μg/kg or saline once/week starting from the day after surgery for 6 weeks (n=13 for each group). After 6 weeks, the ACL reconstruction complex was harvested for μCT analysis followed by biomechanical pull-out test.

RESULTS:

Three samples and five samples from saline and alendronate groups were damaged during sample preparation and hence were not included for biomechanical test. Both mechanical pull-out strength and stiffness in the alendronate group were higher than those in the saline group though they were not statistically significant (Ultimate strength: 8.03 ± 3.89 vs 7.06 ± 2.90; p=0.551; stiffness: 13.38±6.67 vs 12.57± 5.60; p=0.783). Graft mid-substance failure occurred in all samples in both groups. Preliminary μCT analysis on limited samples showed that the averaged tunnel diameter in femur and tibial metaphysis in the aldendronate group was smaller than that in the saline group. While the peri-tunnel BMD lost at week 6 compared to that at week 0 in the saline group, the peri-tunnel BMD at the femur and tibia at the metaphyseal region in the alendronate group increased.

DISCUSSION:

Our results showed that alendronate might have effects on the improvement of peripheral BMD and tunnel healing as well as biomechanical strength of the ACL complex. The insignificant result in biomechanical test is likely to be due to large sample variation. As all the samples in both groups failed at the graft mid-substances, studies at earlier time points are needed to dissect the effect of alendronate on ACL reconstruction.

ACKNOWLEDGEMENT:

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Important Notes

For Feb 5, 2010

Banquet

Starting time: 6:45pm

Venue: Maxim Palace, New Town Plaza, Phase I, Shatin

Cuisine: Cantonese

- board the shuttle bus at the entrance of the symposium venue by 6:15pm
- useful numbers: Miss Mandy Hui 6354 5497, Miss Stella Au 9357 1953
- the return bus will have stops at Regal Riverside Hotel and Hyatt Regency Shatin Hotel
- the restaurant is just above the Shatin MTR Station

For Feb 6, 2010

Associative program

Venue: Li Ka Shing Medical Sciences Building, 4/F

Rm 406 gathering place for lab tour

Rm 407 Research forum

NOTE: The venue is different from that of Feb 5.

NOTES

International Symposium on Ligaments and Tendons (ISL&T-X)

Main Program- February 5, 2010
Postgraduate Education Centre
Kai Chong Tong

8:00-8:30am	Registration and Light breakfast
8:30-8:40am	Opening remarks
8:40-9:55am	Session 1: Tendinopathy-Pathogenesis and Treatment
9:55-10:25am	Break and Poster Session 1
10:25-11:25am	Session 2: Tendon Development and Tendon Cell Differentiation
11:25-12:30pm	Session 3: Translational Research for Tendons and Ligaments
12:30-1:30pm	Lunch
1:30-2:35pm	Session 4: Functional Tissue engineering and Repair of Ligaments and Tendons
2:35-3:40pm	Session 5: Tissue Mechanics
3:40-4:10pm	Break and Poster Session 2
4:10-5:45pm	Session 6: ACL reconstruction-Biology of healing and in vivo knee kinematics
5:40-5:45pm	Closing remark
6:15pm	Departure of shuttle bus to banquet venue
6:45pm	Banquet

Associative Program- February 6, 2010
4/F Li Ka Shing Medical Sciences Building
Rm 406 & Rm 407

9:15-9:45am	Mentor Group: (Rm 407) Translational Research for clinical and industry needs Cheng Kung Cheng
9:45-10:15am	Mentor Group: Skills on writing papers Savio L-Y. Woo
10:15am-12:15pm	Option A: Lab Tour (Rm 406)
10:15am-1:15pm	Option B: Research Forum (Rm 407)