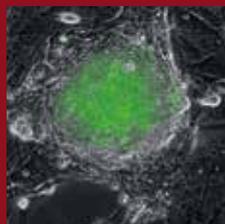
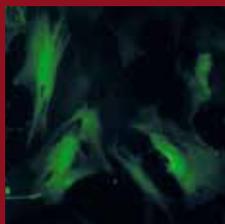
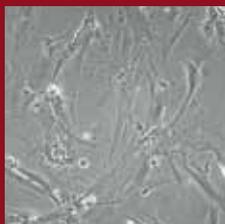


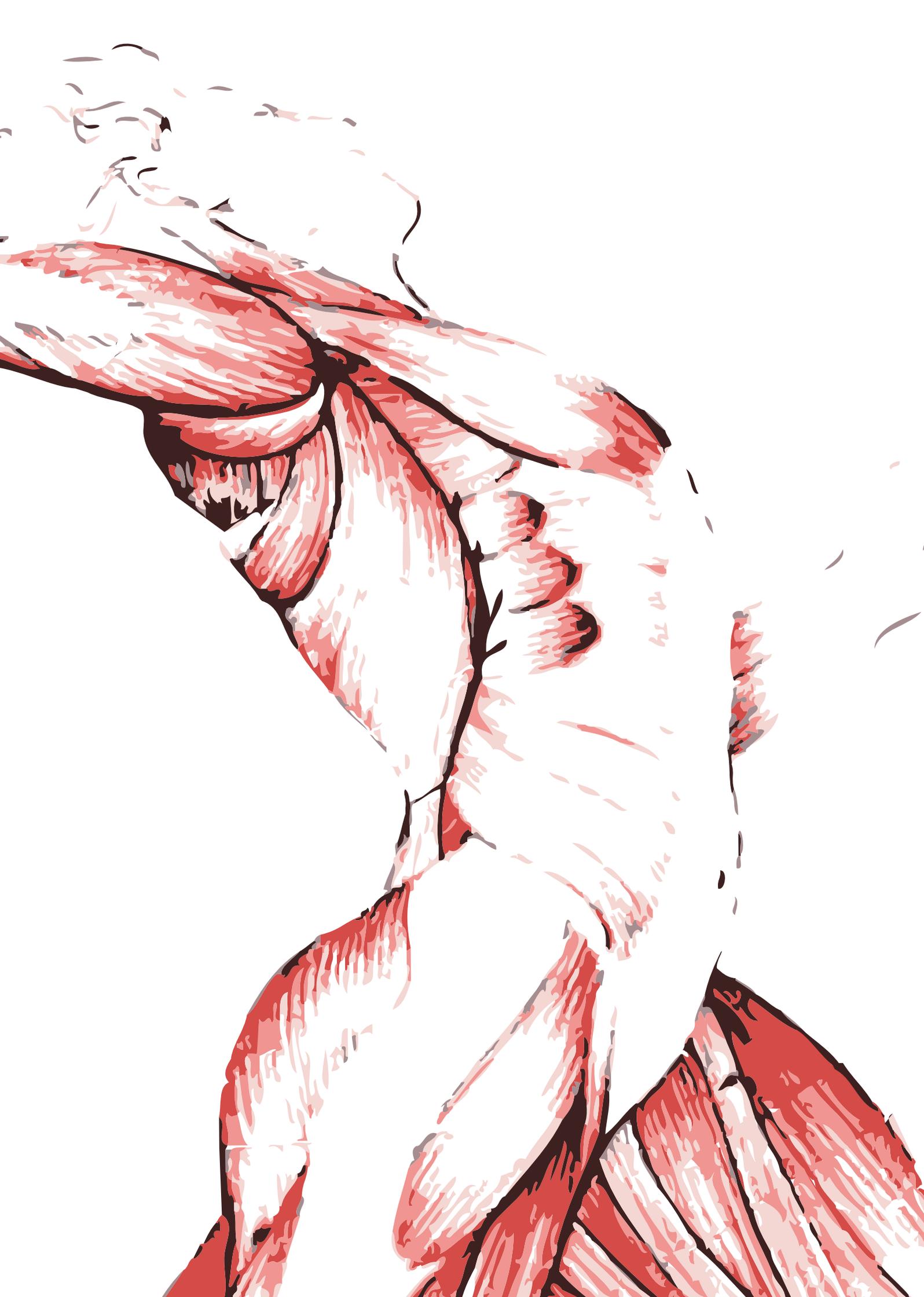


ISMULT: SCIENTIFIC WORKSHOP ON STEM CELLS FOR LIGAMENT AND TENDON TISSUE ENGINEERING AND REGENERATION

APRIL 18, 2013 - Pescara

*Auditorium Leonardo Petruzzi
via delle Caserme, 60*





ISMuLT: I SCIENTIFIC WORKSHOP ON STEM CELLS FOR LIGAMENT AND TENDON TISSUE ENGINEERING AND REGENERATION

APRIL 18, 2013 Auditorium Leonardo Petruzzi - via delle Caserme, 60 - Pescara

08:30 Registration

09:00 Welcome and introduction

Patrizia Accorsi (FF Director of the Transfusion Medicine Department, Pescara)

Rocco Erasmo (Director of the Orthopaedics and Traumatology Unit, Pescara)

I.S.Mu.L.T. presentation

Francesco Oliva (Vice President)

09:30 Human experimentation: where are we currently?

Nicola Maffulli (President of I.S.Mu.L.T. and President of E.F.O.S.T.)

I SESSION STEM CELLS FOR LIGAMENT AND TENDON TISSUE REGENERATION

Chairperson: **Anna C. Berardi / Nicola Maffulli**

10:00 The use of blood vessel-derived stem cells for ligament regeneration and repair

Johnny Huard

Effect on ligament marker expression by direct-contact co-culture of mesenchymal stem cells and anterior cruciate ligament cells

Jose Canseco

11:30 Coffee break

Novel mesenchymal stem cells based approaches for regeneration of injured skeletal tissues: bone, ligament and cartilage - from the bench to the clinic

Meir Liebergall

Ovine amniotic epithelial cells: In vitro characterization and transplantation into equine superficial digital flexor tendon spontaneous defects

Aurelio Muttini

Neoangiogenesis and tendinopathies

Michele Abate

13:15 Lunch

II SESSION BIOENGINEERING

Chairperson: **Francesco Oliva / Nicola Maffulli**

14:30 Cellular and molecular bioengineering: a tipping point

Helen H. Lu

Tissue Engineering Using Supercritical Based Technology

Ernesto Reverchon, Giovanna Della Porta

Injectable polyethylene glycol-fibrinogen hydrogel adjuvant improves survival and differentiation of transplanted mesoangioblasts in acute and chronic skeletal-muscle degeneration

Cesare Gargioli, Stefano Cannata

16:30 Coffee break

Extracorporeal shock wave treatment (ESWT) improves in vitro function activities of healthy and ruptured human tendon-derived tenocytes

Vincenzo Visco

In vivo regenerative strategies: use of adult stem cells and platelet-rich plasma in lesioned flexor tendons

Marco Patruno

Successful recellularization of human tendon scaffolds using adipose-derived mesenchymal stem cells and collagen gel

Tiziana Martinello

An innovative stand-alone bioreactor for the highly reproducible transfer of cyclic mechanical stretch to stem cells cultured in a 3D scaffold

Emanuele Giordano, Marco Govoni

18:00 Closing remarks

18:30 C.M.E. evaluation test

ABSTRACTS

NICOLA MAFFULLI

*Centre for Sports and Exercise Medicine Queen Mary University of London,
Barts and The London School of Medicine and Dentistry*

Department of Musculoskeletal Medicine and Surgery, University of Salerno

Human experimentation: we are we currently?

Tendon injuries present a significant clinical challenge to orthopaedic surgeons. In the United States, approximately 200.000 patients undergo tendon or ligament repair per year. The search for a successful long-term solution remains the focus of intense research. Over the past few years, several exciting technologies have emerged, which, given time, may provide a realistic clinical management option. Mesenchymal stem cell based therapies show promise in improving outcomes.

Much of the work has been experimental, although early clinical use in equine strain-induced tendon injuries supports the efficacy of this strategy. While much has been studied about the mechanisms of action of implanted MSCs, the relative importance of the various mechanisms is still unknown. This talk presents a detailed review of new areas of research which hold promise for future success in managing tendon disorders.

JOHNNY HUARD

Department of Orthopaedic Surgery University of Pittsburgh, School of Medicine

Biological Treatment based on Adult Stem Cells to Improve Musculoskeletal Tissues healing after Injury, Diseases & Aging

Members of my laboratory have isolated various populations of myogenic cells from the postnatal skeletal muscle of mice and humans on the basis of the cells' adhesion characteristics, proliferation behavior, and myogenic and stem cell marker expression profiles. Although most of these cell populations have displayed characteristics similar to those of satellite cells, we also have identified a unique population of muscle-derived stem cells (MDSCs). MDSCs exhibit long-term proliferation and high self-renewal rates and can differentiate toward various lineages, both in vitro and in vivo. The transplantation of MDSCs, in contrast to that of other myogenic cells, can improve tissue regeneration mainly through their ability to highly survive the microenvironment and their ability to secrete paracrine factors (pro-angiogenic) in response to local environmental cues. This survivability appears to be due to their increased resistance to oxidative and inflammatory stress. Although the origin of these MDSCs is still unclear, we have recently reported evidence that their origin is potentially associated with the blood vessel walls namely endothelial and perivascular cells. The presence of blood vessel derived stem cells in various tissues including ligament and meniscus and their potential use to repair these musculoskeletal tissues after injury will be discussed. I will also discuss the use of new approaches to deliver adult stem cells through the use of novel polymer and cells sheet to further improve these regenerative medicine applications designed to improve musculoskeletal tissue healing such as ligament healing (ACL, MCL), meniscus and articular cartilage. Further, I will discuss how genetic modification of adult stem cells to express angiogenic factor VEGF and anti-angiogenic factors can enhance the cells ability to regenerate these various musculoskeletal tissues. I will also outline in my presentation new results obtained with adult stem cell technology to delay aging in an animal model of accelerated aging (progeria). Finally, I will present new results on human muscle derived stem cells, which we believe will open new avenues for the use of adult stem cell-based gene therapy and tissue engineering to improve tissue regeneration after injury, disease and aging.

JOSE CANSECO

Jose A. Canseco, Koji Kojima, Ashley R. Penrose, Jason D. Ross, Haruko Obokata, Andreas H. Gomoll, Charles A. Vacanti

Harvard-MIT Division of Health Sciences and Technology

Laboratories for Tissue Engineering and Regenerative Medicine Brigham and Women's Hospital

Effect on Ligament Marker Expression by Direct-Contact Co-Culture of Anterior Cruciate Ligament Cells and Mesenchymal Stem Cells

Over 200,000 Americans sustain anterior cruciate ligament (ACL) injuries yearly, and a majority occurs in youngathletics patients. Current options for approaches often lead to donor-site morbidity, immune rejection and disease transmission. Our work aims to develop a technique to regenerate ACL from autogous mesenchymal stem cells (MSC) combined whit ACL fibroblast. Our research shows thata co-culture of ACL cells and MSCs in vitro has a potential beneficial effect on ligament regeneration not observed when these cells are cultured alone. Yorkshire pig ACL cells and MSCs were expanded in vitro anc co-cultured in well plates for 2 and 4 weeks at the following ACL/MSCs rations: 100/0, 75/25, 50/50, 25/75 and 0/100. Quantitative PCR resoultls suggest than Collagen type I and Tenascin-C expression significantly increases from 2 to 4 weeks in 50%/50% co-cultures ($p<0.05$), in ACL cells and 50%/50% co-culture compared to all other groups. Immunofluorescent staining for the same ligament markers supported our qPCR observations. Thus, we concluded that using 50%/50% co-cultures of ACL cells and MSCs, instead of either cell population independently, has the potential to enhance ligament marker expression and likely improve ligament healing.

MEIR LIEBERGALL

Chairman, Orthopedic Surgery Complex Hadassah

University Hospital Jerusalem, Israel

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Novel mesenchymal stem cells based approaches for regeneration of injured skeletal tissues: bone, ligament and cartilage - from the bench to the clinic

Injuries of mesenchymal tissues such as bone, ligament and cartilage are common, painful and debilitating, affecting population worldwide, and are significant financial burden. These tissues are considered ideal candidates for biological therapies based on the utilization of the mesenchymal stem cell, which is the common progenitor cell of these tissues. However following two decades of research and thousands of basic studies, only a hand-full of clinical studies were reported in this field. The three corners paradigm of tissue regeneration - carriers, signals and cells, is well based. Whereas carriers were thoroughly investigated, both biologic and synthetic, our knowledge on the cell-signal axis is still lacking and requires further intensive research efforts. Current understanding of physiologic response to injury recognizes the bidirectional nature of the cell-signal axis - signals serve to activate cells, and cells secrete signals and promote secretion of others that in turn induce regenerative environment.

Our efforts in the past decade focused on both aspects of the cell-signal axis. From the signal path we have linked a specific signal molecule to mesenchymal stem cell recruitment to the site of injury and were able to show in a rat model, significant regeneration, both anatomical and functional, of injured ligament and cartilage. From the cell end we have completed a clinical trial aimed to prevent delayed union of complicated fractures by direct delivery of freshly isolated autologous mesenchymal stem cells into the fracture.

Our current research efforts, revealing most probably the tip the iceberg regarding the complex and fascinating interactions in the cell-signal axis during mesenchymal tissues regeneration, emphasize our limited knowledge of the physiologic response to injury, and encourage us to further investigate the cell-signal axis. Once better understanding of these complex interactions is reached, we foresee a future treatment platform aimed to augment and facilitate the physiologic processes of stem cell recruitment and activation toward regeneration of the injured tissue.

AURELIO MUTTINI

Aurelio Muttini, Luca Valbonetti

*Dipartimento di Scienze Biomediche Comparate
Università degli Studi di Teramo Stem TeCh*

Ovine amniotic epithelial stem cells: in vitro characterization and transplantation into equine superficial digital flexor spontaneous tendinopathy

Ovine amniotic epithelial cells (oAECs) collected from slaughtered sheep were evaluated for their phenotype, stemness and ability to differentiate towards into tenocytic lineage. Then, fifteen horses with acute, spontaneous superficial digital flexor tendon lesions were treated with one intralesional injection of oAECs under US guidance. Tendon recovery under controlled training was monitored with clinical and ultrasonography (US) evaluations. Histological and immunohistochemical examinations were performed in one horse died after 60 days. In vitro expanded oAECs showed a constant proliferative ability, a conserved phenotype and stable expression profile of stemness markers (Oct 4, Tert, Nanog, Sox2). Furthermore differentiation into tenocytes was also regularly documented when oAECs were xeno co-cultured with equine tenocytes. oAECs transplantation procedure was well tolerated and twelve horses resumed their previous activity. US controls showed the progressive infilling of the defect and early good alignment of the fibers. The low immunogenicity of oAECs was demonstrated by the low expression of MHC 1 and MHC2. Additionally cells were able to survive in the healing site. In addition, oAECs supported the regenerative process producing ovine collagen type I into newly deposited extracellular matrix, amongst the equine collagen type I fibers that resulted aligned along the healthy portion of tendon. Thus oAECs can be proposed as a new approach for the treatment of spontaneous equine tendon.

MICHELE ABATE

*Department of Medicine and Science of Aging,
University "G. d'Annunzio" Chieti-Pescara*

Neovascularization and tendinopathies

The etiology of tendon disorders is believed to be multifactorial, with a combination of extrinsic and intrinsic risk factors. It is well known that well structured long-term exercise within the "physiologic window" reinforces the tendon as synthesis prevails over degradation.

However, when repeated loading deviates from normal limits by differences in magnitude, frequency, duration, and/or direction, overuse injury may develop.

The pathogenetic cascade is very complex and involves tenocytes apoptosis, hypoxia, "smoldering" disorganized fibrillogenesis, collagen fiber disruption, hyaline and mucoid degeneration, and neovessel proliferation, usually with an absence of inflammation in the advanced stages.

The role of neovascularization in the pathogenesis of tendinopathies is still debated and under investigation. It is well known that healthy tendons are relatively avascular even if neovessels have been reported in asymptomatic athletes and in subjects after strenuous exercise. On the other hand, hypervascularity (induced by the expression of an angiogenic factor, vascular endothelial growth factor [VEGF]) has been detected in symptomatic tendon disorders in a percentage varying from 47% to 100%. A strict correlation between vasculo-neural ingrowth and pain has been reported by Scandinavian authors; however, other researchers have observed that neovascularization per se is related more to the abnormal morphology and tendon size rather than symptoms. Moreover, the presence of neovessels has no firmly confirmed prognostic value.

In conclusion, better designed studies are necessary to establish the role of neovascularization in the pathogenesis of tendinopathies and to evaluate its clinical and prognostic value.

HELEN H. LU

Biomedical Engineering, Columbia University New York

Cellular and molecular bioengineering: a tipping point

In January of 2011, the Biomedical Engineering Society (BMES) and the Society for Physical Regulation in Biology and Medicine (SPRBM) held its inaugural Cellular and Molecular Bioengineering (CMBE) conference. The CMBE conference assembled worldwide leaders in the field of CMBE and held a very successful Round Table discussion among leaders. One of the action items was to collectively construct a white paper regarding the future of CMBE. Thus, the goal of this report is to emphasize the impact of CMBE as an emerging field, identify critical gaps in research that may be answered by the expertise of CMBE, and provide perspectives on enabling CMBE to address challenges in improving human health. Our goal is to provide constructive guidelines in shaping the future of CMBE.

ERNESTO REVERCHON

Reverchon E., Della Porta G.

Department of Industrial Engineering, University of Salerno

Tissue Engineering Using Supercritical Based Technology

A review of the supercritical technologies for the fabrication of scaffold to be used in tissue engineering will be proposed. [1-4]. Tissue regeneration is aimed at repairing damaged tissues; several techniques and materials have been proposed to produce synthetic scaffolds that can mimic the extracellular matrix of the organ to be repaired. The scope is to induce adhesion, growth, migration and differentiation of autologous cells: all these steps are promoted by structural, micrometric and nanometric characteristics of the scaffold environment. The limits of traditional techniques used to produce scaffolds are organic solvent residues and limited process flexibility; therefore, supercritical CO₂ based techniques are emerging in this field to overcome these limitations: a comparison with traditional techniques and analysis of their potential and the obtained results will be proposed.

- 1 Pisanti P, Yeatts AB, Cardea S, Fisher JP, Reverchon E. Tubular perfusion system culture of human mesenchymal stem cells on poly-L-lactic acid scaffolds produced using a supercritical carbon dioxide-assisted process. *J Biomed Mater Res A*. 2012; 100(10):2563-72.
- 2 Reverchon E., Baldino L., Cardea S. De Marco I. Biodegradable synthetic scaffolds for tendon regeneration. *Muscle, Ligaments and Tendons Journal*, 2012; 2(3): 181-186.
- 3 Della Porta G., Falco N., Giordano E., Reverchon E., PLGA Bioactive Scaffold by Supercritical Emulsion Extraction for Myoblasts Growth, *Journal of Biomaterial Science*, in press 2013.
- 4 Della Porta G., Nguyen B., Campardelli R., Reverchon E., Fisher J., Synergistic effect of sustained release growth factors from PLGA microspheres and dynamic bioreactor flow on hMSC osteogenic differentiation in alginate scaffolds, *J Biomed Mater Res* submitted 2013.

CESARE GARGIOLI / STEFANO M. CANNATA

Claudia Fuoco¹, Roberto Rizzi², Claudia Bearzi², Stefano M. Cannata¹, Roberto Bottinelli^{3,4}, Dror Seliktar⁵, Giulio Cossu⁵ and Cesare Gargioli²

Autologous progenitor cells in a hydrogel form an artificial and functional skeletal muscle in vivo

Extensive loss of skeletal muscle tissue results in incurable mutilations and severe loss of function. In vitro generated artificial muscles undergo necrosis when transplanted in vivo before host angiogenesis may provide the amount of O₂ required for muscle fibre survival. Skeletal muscle tissue engineering has met with limited success, due to the complex tissue architecture and the presence of a dense microvascular network without which muscle fibers do not survive once implanted in vivo. Here we report a novel strategy exploiting the good survival and differentiation of mouse mesoangioblasts in a recently discovered biomaterial, PEG-Fibrinogen, and their ability, once engineered to express Placenta derived Growth Factor and embedded in this material, to attract host vessels and nerves while myotubes begin to form. Mesoangioblasts, embedded into PEG-Fibrinogen hydrogel, generate an additional muscle on the surface of the Tibialis Anterior. This strategy opens the possibility of in vivo autologous muscle creation for a large number of pathological conditions.

- 1 Department of Biology, Tor Vergata Rome University, Rome, Italy
- 2 IRCCS MutiMedica, Milan, Italy
- 3 Department of Physiology and Interuniversity Institute of Myology, University of Pavia, Italy

- 4 Fondazione Salvatore Maugeri (IRCCS), Scientific Institute of Pavia, Italy
- 5 Faculty of Biomedical Engineering, Technion Israel Institute of Technology, Haifa, Israel
- 6 Department of Cell and Developmental Biology, UCL, London, UK

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La Sapienza University of Rome, Italy*

² *Physical Medicine and Rehabilitation Unit, Sant'Andrea Hospital,
La Sapienza University of Rome, Italy*

Extracorporeal Shock Wave Treatment (ESWT) Improves In Vitro Functional Activities of Ruptured Human Tendon-Derived Tenocytes

Focused extracorporeal shock-wave therapy (ESWT) has been recently used worldwide in the treatment of different pathologies -including tendinopathies- with promising results, although our knowledge of the specific mechanisms by which it induces therapeutic effects remains largely limited. In particular, an elucidation of the biological machinery underlying the clinical effectiveness of ESWT in musculoskeletal disorders might hold important clues for ameliorating tendon repair strategies. For better understanding the cellular and molecular events involved in tendon degeneration, several in vitro models of cultured tenocytes have been yet established (1–4), but an extensive report able to define the specific effects of shock-wave treatment on human cultured tenocytes derived either from healthy or ruptured tendons is still lacking. Therefore, we analysed, for the first time, the ESW application on primary cultured human tenocytes established from human healthy semitendinosus tendons collected from patients undergoing arthroscopic anterior cruciate ligament (ACL) reconstruction (5). Then, we also evaluated the ESWT-mediated effects on human tenocytes explanted by ruptured Achilles tendons, in order to verify possible differences in the behaviour of the healthy and ruptured tendon-derived cultures (6).

To molecularly characterize both kinds of cell cultures, RT-PCR was successfully used to measure the simultaneous gene expression of typical tenocyte markers, including Scleraxis (Scx), Tenomodulin (Tnm), Tenascin-C (Tn-C) and Type I and III Collagens (Col I and Col III) (6). An energy flux density of 0.14 mJ/mm² was then applied to the cultures, primarily considering that medium energies showed a clinical efficacy in vivo, and we investigated their behavior over a 12 days period following the exposure (5,6). We showed that ESWT significantly interferes with the overall cell morphology, by impairing the typical cell de-differentiation previously documented by several authors (2,3).

To evaluate the proliferative rate of our tenocyte cultures in response to ESWT, we performed a BrdU assay (5-bromo-2'-deoxyuridine, indicating DNA synthesis), focusing on the possible differences between cells derived from healthy compared to pathological tendons. Our results indicated that ESWT significantly enhances the proliferation of tendon-derived cells, when compared to untreated controls ($p < 0.05$), although those effects were mainly observed in tenocytes explanted from ruptured versus healthy tendons ($p < 0.05$) (5,6).

A further ESW-mediated functional activity of the cells was found measuring a significant increase in collagen (mainly type I) synthesis by stimulated tenocytes compared to control cells ($p < 0.005$) (5). Lastly, in order to validate the reliable repairing capacity of the exposed cultured tenocytes, we performed a classical scratch test of cell migration, able to mimic a possible ESWT-induced in vitro wound repair (6). The shock wave exposure induced a typical migratory phenotype in the majority of the cultured tenocytes and this effect was significantly more evident for cells derived from ruptured tendons ($p < 0.05$).

Our findings suggest that human cultured tenocytes are metabolically "activated" by ESWT and significantly induced to proliferate, migrate and synthesize collagen, compared to untreated controls. Nevertheless, the shock wave efficacy was more evident in tenocytes explanted from ruptured than from healthy tendons and this may provide a possible explication of ESW-mediated therapeutical benefits in patients affected by chronic tendinopathies.

MARCO PATRUNO

Patrino M.*, Bronzini I.*, Maccatrozzo L.*, Perazzi A.**, De Benedictis G.M.**,
Iacopetti I.**, Martinello T*

* *Department of Comparative Biomedicine and Food Science, University of Padova, Italy*

** *Department of Department of Animal Medicine, Production and Health, University of Padova, Italy*

In vivo regenerative strategies: use of adult stem cells and platelet-rich plasma in lesioned flexor tendons

Injection of mesenchymal stem cells (MSC) and/or platelet rich plasma (PRP) into the core of a damaged tendon aims to take maximal advantage of reparative mechanisms that occur naturally in the animal body. In this study we examined the effects of autologous MSC derived from peripheral blood (PB-MSC), PRP and a combination of both for improving the regeneration of experimentally lesioned digital flexor tendons of sheep. Success of applications was evaluated at 30 and 120 days post treatments comparing clinical, histo- and immunohistochemical features.

Concerning tendon morphology and extracellular matrix composition significant differences were found between all treated groups and their corresponding controls (placebo). However, our results indicate that the combined use of PRP and MSC did not produce a synergistic regenerative response whereas stressed the predominant positive effect of MSC on healing of tendons.

TIZIANA MARTINELLO

T. Martinello, I. Bronzini, V. Vindigni, L. Maccatrozzo, F. Bassetto, Marco Patrino

Department of Comparative Biomedicine on food Science, University of Padova. Italy

Successful recellularization of human cadaveric tendons with adipose-derived mesenchymal stromal cells

The regenerative capability of tendons is limited and depends on the severity of the considered pathology. Our research group investigated partial and full thickness tendon injuries that are very often observed in sport horses (the former) and human subjects (the latter). New methodologies were studied such as the applications of natural scaffolds associated with cell-based therapies.

The aim of this study was to develop a recellularized biocompatible scaffold from human cadaveric tissue and recellularize it with mesenchymal stem cells isolated from adipose tissue. Our results showed that it is possible to introduce proliferating cells in the core of a decellularized tendon treating the scaffold with a collagen gel. This method resulted efficient since the correct extracellular matrix of scaffold was maintained and because of the injected mesenchymal stromal cells expressed collagen type I and COMP proteins.

EMANUELE GIORDANO / MARCO GOVONI

Marco Govoni, Fabrizio Lotti, Luigi Biagiotti, Maurizio Lannocca, Gianandrea Pasquinelli, Sabrina Valente, Claudio Muscari, Francesca Bonafè, Claudio M. Caldarera, Carlo Guarnieri, Silvio Cavalcanti and Emanuele Giordano

*University of Bologna, Health Science and Technology
Interdepartmental Center for Industrial Research (HST-CIRI)*

An innovative stand-alone bioreactor for the highly reproducible transfer of cyclic mechanical stretch to stem cells cultured in a 3D scaffold.

Bioreactors play a key role in the construction of cell-based products or biological grafts where they have been used also to transfer to the cells physical stimuli, such as mechanical stress, to promote cellular proliferation and differentiation. In bioreactors used to apply a mechanical stretch to the cells, the stimulation operates in an open-loop condition, without an accurate control of the force stressing the cells. To overcome such limitation, we have developed a novel bioreactor able to transfer a controlled pulsating stress to the cells, intended for the commitment of competent cells towards the muscle phenotype. This bioreactor allows the application of an auto-regulated cyclic stretch to an elastic biopolymer (scaffold) where the cells are seeded and cultured. The appealing innovation of this device is the facility to maintain constant the force applied on the scaffold during the trial by automatically adapting the stretching. This feature avoids that changes in time of scaffold elasticity, caused by several factors such as fibres-cells interactions, relaxing or degradation of the fibres, cell proliferation, etc. may modify the dynamic culture conditions and thus the results of the experiment.

In order to investigate the efficiency of this novel bioreactor system we applied a controlled mechanical stimulus on elastic scaffold bearing rat Mesenchymal Stem Cells (MSCs) and observed the proliferation and differentiation of these cells in dynamic conditions by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and immunohistochemical analysis. The obtained results were compared with a static model, prepared at the same conditions of cell concentration, used as control.

Con il contributo di:



Laura Bassi
Onlus



FONDAZIONE
A T A L M I

