2007Autumn PaduaMuscleDays
Muscle Recovery&Reconstruction

Terme Euganee&Palazzo Bo, Padova (Italy), October 14-16, 2007

Terme Euganee Conference Hall, Hotel Augustus, Viale Stazione 150 - 35136 Montegrotto Terme (Padova)
Phone +39 049 793 200 - Fax +39 049 793518 - http://www.hotelaugustus.com/english/pages/hotel_augustus.php
2007Autumn PaduaMuscleDays Openings: Archivio Antico of the Palazzo Bo, Padua University, October 15, 2007.
Via VIII FEBBRAIO, 3 - I-35100 Padova, Italy - Phone +39 049 8273300 - http://www.unipd.it/

Organizers

Ugo Carraro, Juan C Chachques, Giorgio Fanò, Helmut Kern, Urszula Slawinska

Topics

Exercise-related muscle adaptation & rehabilitation – Myogenic/neurogenic stem cells & muscle reconstruction
Cardiac Bio-Assists: from skeletal muscle options to stem cell approaches

Summaries

Adami Nicoletta
Nicoletta Adami (1), Donatella Biral (2), Sandra Zampieri (1), Ugo Carraro (1,2), Michael Vogelauer (3), Christian Hofer (4), Helmut Kern (3,4). (1) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology, c/o Department of Experimental Biomedical Sciences of the University of Padova, Italy; (2) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Sciences, University of Padova, Italy; (3) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Vienna, Austria; (4) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria. E-mail: nicolettaadami@libero.it

Preliminary results of "swinging" protocol (vibration) for muscle training in young sportsmen

Twelve young sportsmen were randomly divided in two groups, one performing usual muscle hypertrophy training, and the second performing in addition a new "swinging" protocol (vibration). Biopsies were harvested before and after the end of the protocol. Morphometric analyses show that: 1. No differences in tissue type distribution (myofibers, adipocytes, collagen and loose connective tissues) have been observed comparing biopsies before and after training; while 2. the distribution of muscle fiber diameter was increased of about 20 μm after vibration stimulation protocol, i.e., from 59.7+/-15.2 (μm, mean+/-SD) before to 73.8+/-13.7 (μm, mean+/-SD) after training. The new protocol seems to be able to increase the muscle mass over the values young sportsmen usually achieve. Molecular analyses of anti-atrophy master genes in the bioptic materials are in progress.

Baroni M David
Alberto Malerba (1), Libero Vitiello (1), Daniela Segat (1), Emanuela Dazzo (1), Marco Frigo (1), Ilaria Scambi (1), Paolo De Coppi (2), Luisa Boldrin (2), Laura Martelli (1), Alessandra Pasut (1), Chiara Romualdi (1), Rosa Grazia Bellomo (3), Jacopo Vecchiet (4), M. David Baroni (1). (1) Department of Biology and (2) Department of Pediatrics, University of Padova, Italy; (3) Physical Therapy Institute, (4) Clinic of Infectious Diseases, Department of Medicine and Aging, G. D'Annunzio University, Chieti, Italy. E-mail: mauriziodavid.baroni@unipd.it

Macrophage-secreted factors enhance the in vitro expansion of muscle precursor cells while preserving their myogenic potential

In this study we show that in the presence of macrophagic factors, small, round and poorly adhesive cells derived from adult rat muscle could be efficiently selected and expanded. These desmin- and MyoD-positive cells were highly myogenic and gradually became spindle-shaped and aligned before forming myotubes. Before myogenic commitment they showed differentiation plasticity and other properties already described in murine muscle stem cells and they could represent a new subpopulation of stem-like cells stimulated by macrophages. The same factors enhanced the in vitro proliferation of slow-growing myogenic cells. In neonatal and adult potentially myogenic cell preparations they induced the formation of large amounts of contracting myotubes and few foci of differentiation, respectively. Thus, the macrophage-sensitive stem-like cells appeared to be more abundant in developing muscles. We also measured a cell size decrease of the myogenic target cells exposed to macrophagic factors, which might be a marker of their activation. Finally, mMCM responsive cells might be important for in vivo muscle repair as the administation of macrophagic

factors increased ten folds the amount of rat regenerating muscle after extensive muscle ablation. We discuss the important hypothesis that macrophages can activate and preserve a subpopulation of myogenic stem cells among their multiple roles during muscle regeneration.

Bernareggi Annalisa
Annalisa Bernareggi (1,2), Paola Lorenzon (1), Fabio Ruzzier (1), Ricardo Miledi (2). (1) Department of Physiology and Pathology and Centre for Neuroscience B.R.A.I.N., University of Trieste, Italy; (2) Laboratory of Cellular and Molecular Neurobiology, Department of Neurobiology and Behavior, University of California, Irvine. E-mail: "Paola Lorenzon" <pielle@dfp.units.it>

Microtransplantation of acetylcholine receptors and calcium channels from rat skeletal muscle to frog oocytes
It is well known that after skeletal muscle denervation or before complete maturation of the fibres there is a large increase in the number and distribution of acetylcholine receptors (AChR) and the muscle develops sustained "fibrillatory" activity. However, the mechanisms underlying this muscle activity are not well understood. Taking advantage of the "microtransplatation technique", we compared the incorporation of AChRs and calcium channels in oocytes injected with membranes isolated from innervated, denervated (by transecting the sciatic nerve) and newborn rat skeletal muscle. AChR and calcium currents were recorded using the two microelectrodes voltage-clamp technique. The oocytes injected with rat skeletal muscle membranes incorporated functional AChR and calcium channels. The amplitude of ACh-currents generated by oocytes injected with membranes from innervated muscle was only a few nA, about 20 nA for newborn-muscle and higher for "denervated-muscle oocytes". In contrast, the calcium current was greater for innervated-muscle injected oocytes compared to denervated and newborn muscle membrane injected oocytes. Our results demonstrate that oocytes are able to incorporate functional AChR and calcium channels when injected with membranes isolated from skeletal muscle. Therefore, we propose the "microtransplantation technique" as a novel and useful approach to further investigate the mechanisms responsible for the properties of skeletal muscle cells in the absence of their motor nerve.

Biral Donatella
(1) Donatella Biral, (2) Helmut Kern, (3) Nicoletta Adami, (4) Simona Boncompagni, (4) Feliciano Protasi, (1,3) Ugo Carraro. (1) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Sciences, University of Padova, Padova, Italy; 2 Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital Vienna, Austria; (3) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology c/o Department of Biomedical Sciences, University of Padova, Italy; (4) IIM – Inter-university Institute of Myology, CeSI - Centro Scienze dell'Invecchiamento, University Gabriele d'Annunzio, Chieti, Italy. E-mail: d.biral@bio.unipd.it

Atrophy-resistant fibers in permanent peripheral denervation of human skeletal muscle
Human muscle fibers usually undergo severe atrophy/degeneration as a result of long-term peripheral denervation. However, some biopsies from paraplegic patients suffering complete Conus Cauda Syndrome display the presence of a small percentage of muscle fibers with a very large diameter (big fibers). The objective of the present study is to determine if these big fibers are the result of residual innervation/re-innervation, or if instead they are fibers resistant to atrophy. Human muscle biopsies were harvested from the *vastus l.* of Spinal Cord Injury (SCI) patients affected by complete lower motor neuron lesion (CLMNL). The specimens were either processed for light microscopy or embedded for electron microscopy (EM). Our results indicates that the big fibers are neither the results of residual innervation or sparse reinnervation. In spite of the fact that the extra-synaptic NCAM immunostaining disappear a few months after SCI, the big fibers are characterized by positive molecular markers of denervation, that is, the differential labeling of their dystrophin molecule by anti-C and anti-N terminals antibodies. Furthermore, the EM analysis shows these cells present the peculiar ultra structural disarrangements of the contractile apparatus and of the internal membrane systems characteristic of "peripheral denervation". No fibers presenting large areas of cross striation were found. The EM analysis provides the final evidence that these big fibers are muscle fibers which are indeed denervated, very different from normal and/or disused (e.g., upper motor neuron lesion) muscle fibers.

Carraro Ugo
Laboratory of Translational Myology of the Interdepartmental Research Center of Myology c/o Department of Biomedical Sciences, University of Padova, Italy. E-mail: ugo.carraro@unipd.it

The cell and tissue degeneration/regeneration processes that characterize the 3-phase progression of human muscle changes in paraplegics with complete lower motor neuron lesion (CLMNL) are delayed/reversed by FES

Complete irreversible denervation of muscles results in a progressive loss of structural and functional properties of fiber, which finally ends in complete waste of muscle mass. In rodents repeated cycles of myofiber regeneration had been described during long-term muscle denervation and we recently shown that this also apply to human muscle in spinal cord injury (SCI). Enrolment in the EU Project RISE and follow-up of paraplegics with complete lower motor neuron lesion (CLMNL) were performed in Wilhelminenspital, Vienna, Austria, analyses of muscle biopsies in Padova, Italy. During the first two year post-SCI simple atrophy characterises the muscle biopsies. From three-year SCI the human denervated muscle presents severe atrophy, adipocytes and connective tissue (denervated degenerated muscle, DDM). Monoclonals against embryonic myosin show that regenerative events are present from 1- to 37-year post-spinal cord injury. In 2-year FES-trained muscles larger than pre-FES round myofibers are present. They are accompanied by regenerative events, but at a lower rate than in DDM (myofiber per cryosection unit area: 0.8+/-1.3 in FES vs. 2.3+/-2.3 in DDM, mean+/- SD, p = 0,011). The average diameter went from 15.4 to 27.0, a 76% increase after two years of FES. All together, microscopic and ultrastructural analyses demonstrate that daily Vienna-FES of lower motor neuron denervated muscles in CLMNL paraplegics is safe and that the cosmetic and cushioning effects are granted in all persons, whatever the time from spinal Cord Injury (SCI) at the beginning of the treatment. Supported by EU Commission Shared Cost Project RISE (Contract n. QLG5-CT-2001-02191) and Italian Ministry of University (MIUR) PRIN Project Contract n. 2004061452-002.

Celichowski Jan
Department of Neurobiology, University School of Physical Education, Poznań, Poland. E-mail: celichowski@awf.poznan.pl

The transformation of motor units evoked by increased and decreased activity
The plasticity of motor units (MUs) contractile properties in the rat medial gastrocnemius muscle evoked by the spinal cord transection and the locomotor training was studied. Effects of both, the total and left-side transection of the spinal cord were studied in increasing time distance after the injury. In all groups of spinal rats the twitch time parameters of investigated MUs were prolonged in comparison to control rats. Moreover, characteristic for fast MUs "sag" phenomenon disappeared, especially after the total spinal cord transection. Therefore, for fast/slow MUs classification a new method basing on an analysis of 20 Hz unfused tetanus was proposed. After the spinal cord total transection the increasing proportion of fast fatigable (FF) units beginning one month after the injury as well as disappearance of slow (S) units within the three months were indicated. No significant changes were found in twitch forces in all MU types of spinal animals. However, the maximal tetanic force decreased, and the twitch-to-tetanus ratio increased in spinal rats. The ability of fast MUs to potentiate the force was reduced. On the other hand, one month locomotor training on a treadmill effected in an increase of fast resistant (FR) and a decrease of FF MUs in studied muscle. The mean values of a fatigue index for particular types of MUs were not changes, but the force of the two types of fast units considerably increased whereas the twitch time parameters shortened. Concluding, fast units are more suitable for plasticity than slow ones and their properties can change in larger range.

Chachques Juan C
Chachques JC, Schussler O, Shafy A, Chachques E, Spadaccio C, Grandjean P, Carpentier A. Pompidou Hospital, Paris, France. E-mail: j.chachques@brs.ap-hop-paris.fr

From latissimus dorsi dynamic cardiomyoplasty to stem cell therapy
Cardiac Bioassist procedures have been conceived for patients presenting heart failure, refractory to medical therapy. Cardiomyoplasty (CMP) and aortomyoplasty were born in the 1980s from the use of skeletal muscle flaps from plastic and reconstructive surgery and by the demonstration of muscle-fatigue resistance induced by chronic electrostimulation. CMP augments ventricular contractility through the transposition of autologous latissimus dorsi muscle (LDM) around both ventricles and synchronization of LDM contractions with heart contractions. Following CMP, the LDM is chronically electrostimulated using specific electrodes and a pulse train generator. More than 2000 procedures have been performed worldwide. The most recent application of CMP is chronically depressed right ventricular function (e.g.: arrhythmogenic RV dysplasia), which represents an unresolved therapeutic challenge. When indicated, CMP could be considered as a biological bridge to heart transplantation, 26 patients have been transplanted within 2.3+ 3 years after CMP, long-term survival was similar to those for primary heart transplantation.
Stem Cell Therapy: the recent progress in cellular and molecular biology allows the development of new therapies for heart failure. One of the most innovative approaches consists in the transplantation of stem cells (from bone marrow, skeletal myoblasts, umbilical cord, etc) into the myocardium for heart muscle regeneration. This approach is called "cellular cardiomyoplasty". Adult myocardium cannot effectively repair itself after infarction due to the limited number of stem cells. Thus, most of the injury is irreversible. For this reason, cell transplantation strategies for heart failure have been designed to replace damaged cells with cells that can restore cardiac function, either in ischemic or non-

ischemic cardiomyopathies. The goal of stem cell transplantation is to grow new muscle fibers (cardiomyogenesis) and/or to develop new blood vessels (arteriogenesis/angiogenesis) in the damaged myocardium. This may potentially contribute to improve systolic and diastolic ventricular function, and to reverse the post ischemic remodeling process of the ventricular chambers. Tissue engineering using biological and synthetic matrix is now associated with cell therapy. The goal is to develop a bioartificial myocardium. The MAGNUM Clinical Trial (Myocardial Assistance by Grafting a New Upgraded bioartificial Myocardium) was initiated.

Didié Michael
M. Didié, C. Rogge, M. Tiburcy, T. Eschenhagen, W.-H. Zimmermann. Institute of Experimental and Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. E-mail: m.didie@uke.uni-hamburg.de

Tissue engineering approaches for myocardial support and regeneration
Heart disease is the number one cause of death in industrialized countries. New therapeutic strategies to support and regenerate diseased myocardium are inevitably called for. Over the last decade our group has developed a novel technology to construct engineered heart tissue (EHT). EHTs can be generated from immature cardiomyocytes of different species including chick, rat, mouse, and human. In either model spontaneously beating EHTs exhibited morphological and functional properties of native myocardium. In addition, we could demonstrate that rat EHTs can be used to partially repair myocardial defects in a rat model of infarction. Subsequent studies provided evidence that EHTs may not only be used to repair local heart defects but in fact be utilized as biological ventricular assist devices to potentially support globally failing hearts. A major obstacle when aiming to transfer tissue engineering approaches to a clinical application is the identification of a suitable cell source. In this context, adult cardiac precursor cells and embryonic stem cells of different origin appear instrumental. While the cardiac potential of adult stem cells has not been unambiguously documented, embryonic stem cells can undoubtedly differentiate into cardiac myocytes. Consequently, we used human embryonic stem cell derived myocytes to construct force-generating human EHTs. Whether the latter or other tissue engineering approaches will eventually make it to the clinic remains an exciting but nevertheless open question.

Drzymała-Celichowska Hanna
H. Drzymała-Celichowska, J. Celichowski. Department of Neurobiology, University School of Physical Education, Poznań, Poland. E-mail: celichowski@awf.poznan.pl

Differences between male and female motor units of the medial gastrocnemius muscle in rats
The study shows differences of contractile properties and proportions of the three types of motor units as well as estimation of the number of motor units located in the hind limb locomotor muscle – the medial gastrocnemius - of male and female rats. Experiments were performed on Wistar rats under general anesthesia. Functional isolation of motor units was achieved by electrical stimulation of single axons from the ventral roots of L4 – L5 spinal nerves whereas whole muscle force was measured during stimulation of the sciatic nerve. The number of motor units in the medial gastrocnemius was estimated by comparison of the whole muscle tetanic force to the mean tetanic force of its motor units. The studied motor units were classified as fast fatigable (FF), fast resistant (FR) and slow (S) type. Composition of motor units was different for male and female subjects, with higher number of the FF and lower number of S type units in male animals. The contraction and the half-relaxation times were significantly longer in three types of male motor units, what might be due to differences in muscle size. Slower contraction of male motor units likely corresponds to lower firing rates of their motoneurons. Higher force parameters (the twitch and the maximum tetanus forces) were observed for male motor units although these differences were significant only for FR units. The mass of the muscle was approximately 1.5 times greater in male rats. However, the mean ratio of motor unit tetanus force to the muscle mass was almost twice smaller in this group, what can be due to higher number of motor units in male muscles. Estimation of the force that can be generated by the three types of motor unit in the studied muscle revealed that in both sexes fast fatigable units are responsible for over 60% of the total force output whereas the percentage of the force output contributed by slow units in females is approximately twice that in males. In male animals the medial gastrocnemius muscle was innervated by 10% more motoneurones than in females, and contained 57 and 52 motor units, respectively. Finally, female muscles appeared to have higher fatigue resistance as an effect of higher proportion of resistant units (slow and fast resistant) and slightly higher values of the fatigue index in fast motor unit types. The motor unit action potentials in female rats had slightly lower amplitudes and shorter time parameters although this difference was significant only for fast resistant units. To summarize, the rat medial gastrocnemius muscle is sexually dimorphic, and proportions, the contractile properties and the number of its motor units in male and female muscles are different.

Francini Fabio
Roberta Squecco (1), Giorgia Luciani (1), Lucia Formigli (2), Alessia Tani (2), Daniele Nosi (2), Silvia Nistri (2), Sandra Zecchi Orlandini (2), Daniele Bani (2), Fabio Francini (1). Departments of (1) Physiological Sciences and Anatomy, Histology, and (2) Forensic Medicine, University of Florence. E-mail: fabio.francini@unipd.it

Functional integration by gap-junctions between skeletal myoblasts and cardiomyocytes in coculture is favored by relaxin treatment

The success of cellular cardiomyoplasty as therapy for the repair of postischemic myocardium depends on the anatomical integration of the engrafted cells with the resident cardiomyocytes. Long-term success after cardiac allograft transplantation is limited by chronic inflammatory processes, despite prolonged effective immunosuppression.
Our aim was to investigate the interaction between undifferentiated mouse skeletal myoblasts (C2C12 cells) and adult rat or neonatal mouse ventricular cardiomyocytes in an in vitro model of coculture. The effects of the hormone relaxin, which has cardiotropic actions and binds to specific receptors in the heart, in influencing the intercellular coupling between the two cell types was also investigated. This was studied also in consideration of the suggested role for this hormone in the modulation of the growth and/or differentiation of cardiomyocytes during fetal and neonatal life. Connexin43 (Cx43) expression, Lucifer yellow microinjection, Ca2+-transient propagation, and electrophysiological analysis demonstrated that myoblasts and cardiomyocytes were coupled by functional gap junctions. We also showed that cardiomyocytes upregulated gap junctional communication and expression of Cx43 in myoblasts. Analysis of the gating properties of gap junctions by dual cell patch clamping showed that the co-presence of cardiomyocytes in the cultures significantly increased the transjunctional current between myoblast-myoblast cell pairs. Moreover, the coculture induced an asymmetric voltage dependence of the gap junction current being reduced its inhibition at negative potential of the paired myoblasts . This effect required direct cell-to-cell contact between the two cell types and was potentiated by treatment with relaxin.

Frati Giacomo
Roberto Gaetani (1), Juan C. Chachques (5), Federico Bizzarri (3), Luigi Muzzi (3), Patrizio Sartini (3), Giuseppe Pugliese (3), Lucio Barile (1,2), Elvira Forte (1), Vittoria Ionta (1), Isotta Chimenti (1,2), Alessandro Giacomello (1), Elisa Messina (1), Giacomo Frati (1,3,4,5). (1) Dept of Experimental Medicine, University of Rome "La Sapienza", Rome, Italy; (2) Division of Cardiology, Johns Hopkins University, Baltimore, Md, USA; (3) Division of Cardiosurgery, University of Rome "La Sapienza", Latina (RM), Italy; (4) Neuromed, Venafro (IS) Italy; (5) European Hospital G.Pompidou,University of Paris, France. E-mail: fraticello@inwind.it

Cardiospheres and tissue engineering for myocardial regeneration: potential for clinical application

Cardiovascular disease remains the leading cause for morbidity/mortality around the world. Cell transplantation into the damaged myocardium (cell therapy) for regeneration has received extensive attention and the accumulated evidence from both pre-clinical/clinical studies suggests that it has the potential to restore heart function. Cell transplantation, however, may not always be suitable (i.e for large myocardial damage with aneurysm, chronic transmural scar tissue, valve disease, aortic disease and congenital cardiac defects in infants). Moreover, the results from the known recent clinical trials evidence the need to improve the survival, the engraftment and the differentiation of the transplanted cells. Tissue engineering (TE) involves seeding a biodegradable scaffold with cells that grows into morphologically recognizable tissue both in vitro and in vivo. After the scaffold undergoes gradual biodegradation, the tissue formed from the seeded and infiltrated host cells retain the form of the scaffold. Recent advances in cell culture and TE have facilitated the development of suitable cell-engineered, biodegradable grafts. The optimal biomaterials and cell types, however, have not been identified. The improvement of the protocols for the Cardiac Stem Cells (CSCs) ex vivo expansion/differentiation, coupled with the application of the bio-scaffolds, will make autologous celluloplasty closer to the clinical translation. Our hypothesis is that autologous CSCs (isolated as Cadiospheres from human biopsy with the method established in our laboratory) in combination with the optimal biodegradable biomaterials, can be used to construct tissue-engineered cardiac patches which will be viable, differentiate and can save the survival and growth potential of the embedded cells. Our past experience with type I collagen matrix (4x3x1.5 mm) seeded with human stem cells, have shown that in infarcted mouse hearts, intramyocardial injections of this association, prevents myocardial wall thinning, limits postischemic remodelling and improves EF. So, this tissue engineered approach, seems to improve the efficiency of the cell treatment at the times (1 and six weeks) studied, corresponding to an improvement in an early post-infarct cardiac remodelling, vascularization and angiogenesis, but not in a consistent myocardial regeneration.

Gargiulo Paolo
Paolo Gargiulo (1), Thordur Helgason (1,2). (1) Department for Research and Development, Landspitali – University Hospital, Reykjavik, Iceland; (2) Reykjavik University, Reykjavik, Iceland. E-mail: paologar@landspitali.is

Changes of shape in denervated rectus femoris muscle influenced by FES
This work propose a method to reconstruct from CT referenced data, 3-D model of Rectus Femoris muscle from paraplegic patients with fully denervated and degenerated muscles (DDM) and use it to monitor changes of shapes induced by FES. Three patients with flaccid paraplegia and long term denervated muscle are stimulated in Iceland with long pulse and high power FES. The change of geometry of the growing denervated muscle is unknown and therefore 3-Dimensional reconstruction of the most influenced muscle by the stimulation allows a unique sight on the growing process and information on how this happens. Advanced segmentation methodology are developed to isolate Rectus Femoris muscle from other muscle bellies and to reconstruct the whole 3-dimensional shape. Complete and selective monitoring of the rectus femoris is in this way possible: volume and shapes can be compared during the therapy. The results show that degeneration of muscle shape in rectus femoris occurs in similar way among the patients. Indeed rectus femoris lose progressively strength and elasticity, in the degeneration process, visible in particular in certain area of the muscle volume. Beside, when the muscle is stimulated properly the shape is restored suggesting the reversibility of the degeneration process.

Genovese Jorge A.
Heart, Lung, and Esophageal Surgery Institute, University of Pittsburgh Medical Center and McGowan Institute for Regenerative Medicine, Pittsburgh, PA. USA. E-mail: "Genovese Jorge" <genovesej@upmc.edu>

Making cells for cardiac repair: stem and non-stem cell predifferentiation
During the last years there has been a real explosion of research and even application of stem cells in the cardiology field. Cellular cardiomyoplasty (CMP) is a stem cell based regenerative or reparative therapy which goal is limiting the consequences of a myocardial infarction or a non-ischemic cardiomyophaty. In any case a question remains: how differentiated should be the stem cell that will be applied in a sick heart? Perhaps, an intermediate option could result in a better cell integration, a higher environmental dependence, an improved survival and finally, in a more efficient functional restitution. The pre-differentiation process will let cells to express “in-vitro” some cardiac proteins that are the phenotypic expression of genetic changes toward the cardiomyocyte final stage. Avoiding the use of signals associated to cell transformation as many cytokines are; safer pre-differentiation models will be defined. In our experience, the modulation of nitric oxide sintetase or the application of an electric field can result in the expression of different cardiac proteins in a variety of stem cells. Pre-differentiation results in morphological changes: with multinucleated cells formation and cytoskeleton rearrangement. The expression of cardiac proteins related with cell contraction and intercellular communication is also early detected. Therefore myocardiocytic pre-differentiation of stem cells appear as a potentially powerful tool to obtain cells with still evolutive capacity and sensitivity to environment signals; presenting phenotypic and metabolic changes that could facilitate their homing, adaptation, survival, replication and function with the consequent improved therapeutic effect.

Gordon Tessa
Gordon T, Ly V, Hegedus, J and Tyreman N. Division of Physical Medicine and Rehabilitation/Centre for Neuroscience, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2S2. E-mail: tessa.gordon@ualberta.ca

Early detection of denervated muscle fibers in the G93A mouse model of amyotrophic lateral sclerosis
The cell adhesion molecule N-CAM is localized to the adult neuromuscular junction but is also expressed in the extrajunctional membrane of denervated muscles concurrent with extrajunctional acetylcholine receptors. Here we used NCAM immunohistochemistry to determine whether we could detect early denervation in hindlimb muscles of the G93A transgenic mouse model of amyotrophic lateral sclerosis (ALS). In denervated wild type mouse muscles, N-CAM immunoreactivity on the sarcolemma of all fiber types and within the sarcoplasm of only type IIA fibers was detected at 2 days: ~30% of the muscle fibers were fully circumscribed by N-CAM immunoreactivity and ~25% of fibers were incompletely circumscribed. The proportion of the latter fibers remained constant over the next 8 days as the proportions of the former fibers increased exponentially. Thereafter fully circumscribed muscle fibers increased to a maximum by 30 days with a concomitant fall in the incompletely circumscribed fibers. Hence early muscle denervation was detected by the incomplete circumscription of fiber membranes by N-CAM immunoreactivity with full circumscription and intracellular localization indicating more long-term denervation. In the G93A transgenic mouse, rapid denervation of fast-twitch muscles was readily detected by a corresponding proportion of muscle fibers with

positive N-CAM immunoreactivity. The proportions of incompletely and completely circumscribed muscle fibers corresponded well with the rate of decline in intact motor units and reduced muscle contractile forces. Progressively more fully circumscribed muscle fibers became evident with age. We conclude that the N-CAM immunoreactivity on muscle fiber membranes provides a sensitive means of detecting early muscle fiber denervation.

Grandjean Pierre A
Bakken Research Center, Maastricht, The Netherlands. E-mail: pierre-andre.grandjean@medtronic.com

Heart failure monitoring: applications in cardiac bioassist
Heart failure remains an unresolved and growing medical challenge for which many new therapies have been developed or are under investigation. Ambulatory assessment of cardiac function i.e. during daily life as well as during hospital tests could enable optimization and customization of existing treatments as well as a more accurate evaluation of clinical benefits of emerging new therapies. With this aim, we developed two ambulatory approaches: 1) daily measurement of transthoracic impedance to detect fluid overload indicating impending pulmonary edema; 2) continuous measurement of right ventricular (RV) intracardiac pressure for hemodynamic assessment of cardiac function (i.e. RV systolic and diastolic pressure, pulmonary artery diastolic pressure, heart rate, patient activity etc). For both approaches, data are collected via an implantable device. Transmission of the information collected can be performed from patient's home to a protected website accessible by the cardiologist or treating physician or in the physician's office. Data available on website allow clinician to optimize patient treatment, aiming at reducing heart failure hospital admissions which is a major costs in healthcare expenditures. Although these devices have been primarily designed for patient clinical management, these technologies can also be used for the assessment of investigational new heart failure therapies. These new diagnostic and clinical management and assessment tools as well as clinical experience gained to date will be described.

Helgason Thórdur
Thordur Helgason (1,2), Paolo Gargiulo (1). (1) Department for Research and Development, Landspitali – University hospital, Reykjavik, Iceland. (2) Reykjavik University, Reykjavik, Iceland. E-mail: thordur@landspitali.is

Monitoring bone mineral density of femur during electrical stimulation therapy of denervated degenerated thigh muscles.
In the frame of the RISE (QLG5-CT-2001-02191) project and with the aim to reverse muscle degeneration three patients with denervated and degenerated muscles (DDM) where treating themselves at home with electrical stimulation for up to one hour for each muscle, once a day, six days a week for three and a half year. A quantitative spiral computer tomography (QCT) was made in regular time intervals of four months. The rectus femoris muscle and the femur bone where segmented from the rest of the tissue. The CT data from these two organs was then analyzed on tissue density. Result show an increase in tissue density of the muscle correlated with increase in muscle volume. Mechanical properties and form of the muscle changed at the same time. Femur bone remodulation took place at the same time with a slight increase in overall bone density but uneven spatial distribution. Difficult and time consuming set up of the stimulating equipment is a severe drawback of the method and is the main cause for poor compliance in some cases. To overcome these obstacles an implantable stimulating system is suggested. Drawback of such a system is a need for surgery. Both protocol and devices for a less invasive implantable devices need to be developed.

Herreros Jesus
Jesus Herreros, Juan Jose Gavira, Maitane Perez-Ilzarbe, Diego Martínez Caro, Gloria Abizanda, Juan C. Chachques, Felipe Prosper. University of Navarra. Pamplona. Spain. E-mail: "Jesus Herreros" <jherreros@unav.es>

Cellular cardiomyoplasty with skeletal myoblasts
Transplantation of autologous skeletal myoblasts (SM) has emerged as a potencial means to restore left ventricular function. Our aim was to compare surgical versus percutaneous administration of SM and to evaluate the number of cells and the schedule of administration in Goettingen minipigs with myocardial infartion (MI). The results were correlated with the clinical results at 1-year of autologous SM associated to CABG in patients with MI without viability.
Two months after MI, pigs received media alone (n=4), SM by surgical injection (n=6) or percutaneous approach (n=6). In order to study the number of cells, 20 pigs received by percutaneous approach media alone (n=5), 390x106 SM 8 weeks after MI (n=5), 390x106 SM 8 and 16 weeks after MI (n=5) and 390x106 SM 8, 16 and 24 months after MI (n=5). Twelve patients with a previous MI underwent CABG + SM cultured with autologous serum (220x106 cells). The results were compared with a historical control group (n=14) of patients with the same characteristics treated with CABG alone. In pigs, SM transplant was associated with an increase in left ventricular ejection fraction ($p<0,01$),

increased vasculogenesis (p< 0,05), decreased fibrosis (p<0,05), and reduced collagen type I/III ratio, as compared with control animals. No differences were found between groups receiving surgical or percutaneous SM. The increase in left ventricular ejection fraction was higher in pigs receiving 3 doses of 390x106 SM (p<0,003) than in tose receiving 2 doses (p<0,005) or 1 dose (p<0,07). In the clinical study, SM was not associated with adverse events or an increased incidence of arrythmias. The left ventricular ejection fraction (p<0,01) and the regional contractility index p<0,0001) improved at 12 months in the SM group, without significant improvement in the control group. Fluorine 18FDG and Nitrogen 13Amonia PET showed an increase in viability and perfusion globally and in segments treated with SM (p<0,01 and p<0,004). Treatment with SM is safe, feasible and is associated with an improvement in left ventricular function, perfusion and viability. The beneficial effect is higher after high doses of SM or repeated injections. The increased vasculogenesis and decreased fibrosis are associated with beneficial effect, without differences between surgical and percutaneous approach.

Jakubiec-Puka Anna
Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland. E-mail: annajakubiec@o2.pl - ajpe@nencki.gov.pl

Effect of passive movement on the 2-month-denervated fast and slow rat muscles
We investigated whether and how a passive movement can affect a progress of denervation atrophy in rat fast (EDL – extensor digitorum longus) and slow (soleus) muscles. To address this question, a locomotor training on a treadmill was applied to rats, starting either 7 days or 1 month after the sciatic nerve cut in the right hindlimb. Two months after denervation, the muscles were examined using electron microscopy, and immunohistochemical and biochemical methods. Locomotor training caused slight increase in weight and significant increase in fiber diameter of both muscles. The training positively affected factors that were believed to be the reasons of atrophy irreversibility: it increased the number of capillary blood vessels and muscle nuclei. The contractile apparatus was also improved as more regular organization of sarcomeres and the hexagonal arrangement of myosin filaments were seen. Moreover, the amount of myosin heavy chains (which decreases after denervation) increased following training. The effects were more evident in the animals longer trained. Such beneficial influence was visible in both soleus and EDL. However, essential difference between these muscles was observed in the degree of damage and regeneration: in soleus the number of severely damaged muscle fibers was reduced, as compared with untrained muscles, while in EDL it was evidently increased. Similar differences concerned the amount of regenerating fibers. Thus, passive movement seems to attenuate some of the pathological processes within denervated muscle, but it also can cause damage of muscle fibers, particularly in fast muscle.

Jarvis Jonathan C
JC Jarvis, Z Ashley, S Salmons. Department of Human Anatomy and Cell Biology, The University of Liverpool, UK. E-mail: jcj@liverpool.ac.uk

Stimulation of long-term denervated lower hind limb muscles in the rabbit
To investigate the response of muscle to stimulation several months after injury in long-term denervated rabbit muscle. After selective denervation of the motor branches of the left common peroneal nerve, a telemetric stimulation system allowed us to deliver adjustable stimulation to the denervated muscles. In a terminal procedure the muscles were characterised physiologically then removed for analysis. One group underwent denervation alone up to 51 weeks. Other groups were stimulated after 10 or 39 weeks of denervation, for 2, 6 or 10 weeks.
1. Denervated muscles showed atrophy, fibrosis and disruption of ultrastructure, but little necrosis and/or regeneration. Rabbit muscles therefore differ from rat muscles—which undergo necrosis within a few months of denervation—rather, they resemble human muscles in the first year post-injury.

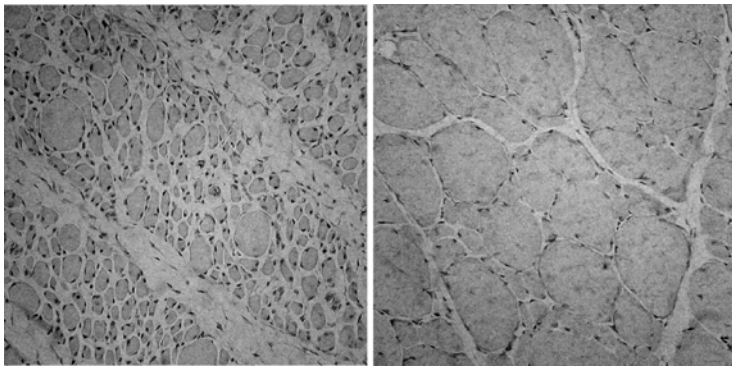

Figure 1. Rabbit TA muscles. Left: 10-week denervation. Right: denervated for 10 weeks then stimulated for 10 weeks.

2. Weight and CSA of stimulated muscles was almost normal. The histological appearance showed a substantial improvement (Figure 1). 3. Stimulation increased force-generating capacity, but not commensurate with the increase in mass. Denervated muscles were slow with or without stimulation This loss of functionality could be related to ultrastructural abnormalities, interfering with excitation-contraction coupling. In conclusion, contractile activity induced by daily electrical stimulation cannot reverse all the deleterious effects of denervation. There is, however, improvement in the volume and quality of the muscle and a substantial recovery in force-generating ability.

Jarvis Jonathan C
Department of Human Anatomy and Cell Biology, The University of Liverpool, UK. E-mail: jcj@liverpool.ac.uk

Skeletal muscle, myoblasts, and cardiac assistance
The Liverpool group helped to provide the theoretical foundation for cardiac assist from skeletal muscle, showing that the continuous work output from suitably adapted skeletal muscle could approach the levels available from cardiac muscle. Our recovery model in pigs demonstrated that an intrathoracic skeletal muscle ventricle could be formed in a single operation and provide immediate assistance to the heart by counterpulsation. Our model made use of a homograft lining wrapped in skeletal muscle. There is much research activity and media interest in tissue engineering with very high goals, such as the formation of endothelialised pumping chambers, or even complete hearts. A more realistic approach might be to produce a tissue engineered sac that could be powered by skeletal muscle, allowing for the possibility that tissue engineered myocardium might replace the stimulated skeletal muscle in the future. In any case, a thorough understanding of the response of skeletal muscle cells and cardiomyocytes to their pattern of use – activation and loading – is essential. The study of normal development demonstrates that it is not enough to modify the expression of a few target genes by modifying the cellular environment in order to make a pumping chamber. Muscle cells contain complex adaptive circuitry that underpins the crucial epigenetic influences on normal development. Considerable progress has been made over the last few decades in understanding the response of healthy skeletal muscle to changes in activity pattern or loading. At the level of gene expression, on the other hand, changes in response to activity take place within hours. Such changes measured with quantitative PCR, can provide an early indication of the eventual functional outcome of particular exercise or stimulation regimes.

Kern Helmut
Kern H (1,2), Vogelauer M (1), Forstner C (1), Mödlin M (1), Mayr W (3), Hofer C (2). (1) Department of Physical Medicine and Rehabilitation, Wilhelminenspital Wien, Vienna, Austria; (2) Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria; (3) Center for Biomedical Engineering and Physics, Medical University Vienna, Austria E-mail: helmut.kern@wienkav.at

Clinical results of the European research project "RISE": FES restors denervated human muscle
The aim of the EU project RISE was to restore and long term maintain denervated human muscles in patients with total denervation of their lower limbs due to a complete conus cauda lesion. Depending on the time elapsed since the accident, there is a progressive muscle tissue degeneration with an extent that varies greatly, being influenced by secondary illnesses such as polytrauma, metabolic crisis, long comatose states or local destruction of tissue. The absence of the motoneurone and the degeneration of the muscle tissue are the reasons that long pulse durations and high intensities are necessary for activating the denervated muscle. In the course of the European research project RISE we could demonstrate, by applying this highly intensive transcutaneous electrical stimulation protocol, a significant structural and functional regeneration of the long-term denervated, degenerated muscle (DDM). Using an increased number of stimuli per day, thus increasing the amount of the muscle activity, we were able to improve muscle bulk,

force, perfusion and excitability of the patients thigh muscles. The method was not only capable of increasing the size of the denervated muscles as measured by CT-scans, it could also restore the capacity of the muscle to generate tetanic contractions, a precondition for inducing recovery of muscle mass. Measuring the muscle cross sectional area with computed tomography (CT) the m. quadriceps area increased by of 35%. The electrically induced contraction force was improved by 828% in patients which were paralyzed up to 2 years. This is also confirmed by the perfusion, which was increased by 100% - 480%. Regarding the excitability, the mean MFCV was increased from 1.6 up to 2.4 m/s and the refractory period decreased from 5.3 to 3.1 ms. Training over a longer period of time made it first possible to induce knee extension by direct electrical stimulation of the denervated quadriceps muscle. Later with further improvement of muscle function 25% of the patients could perform stand up and walking exercises. The above mentioned improvements are also an important contribution for preventing secondary diseases like decubital ulcers in patients with longterm denervation of the legs.

Mayr Winfried
Center of Biomedical Engineering and Physics, Medical University of Vienna, Austria.
E-mail: winfried.mayr@meduniwien.ac.at

Equipments for FES of denervated muscles: Demands and technical answers
Traditional functional electrical stimulation (FES) targets neural structures and indirect control of muscle contraction. The recent EU FP5 project RISE has demonstrated the feasibility and effectiveness of the novel application FES of denervated muscles. The main difference between nerve and direct muscle stimulation is the required impulse width of the applied stimuli. Nerves are stimulated with less than 1 ms, muscles with 30 to 200 ms at comparable intensity levels. The resulting charge per impulse and average electrical power levels are critical in applications of both surface electrode based and implantable stimulation systems. In non-invasive systems up to 30 mC of impulse charge and 25 W of power may occur. To minimize the risk of skin damage a large contact surface and holohedral current distribution are essential in design and handling of the electrodes. Stimulators must guarantee charge balance and zero DC at the outputs and must include all thinkable safety monitoring, operation and handling features to avoid failures in daily home based routine treatment. A suitable dual-channel system was developed in RISE and successfully tested in a patient study for a period of more than 2 years. Implanted systems are not yet developed to a level that justifies clinical application.

Messina Elisa
Lucio Barile(1,2), Giacomo Frati (1,3, 4), Roberto Gaetani (1), Elvira Forte (1), Vittoria Ionta (1), Isotta Chimenti (1,2), Federico Bizzarri (3), Luigi Muzzi (3), Patrizio Sartini (3), Giuseppe Pugliese (3), Alessandro Giacomello (1), Elisa Messina (1). (1) Dept of Experimental Medicine, University of Rome "La Sapienza", Rome, Italy; (2) Division of Cardiology, Johns Hopkins University, Baltimore, Md, USA; (3) Division of Cardiosurgery, University of Rome "La Sapienza", Latina (RM), Italy; (4) Neuromed, Venafro (IS) Italy. E-mail: elisa.messina@libero.it

Cardiospheres for myocardial regeneration: research and development
Currently, cellular cardiomyoplasty is being actively explored as means of regenerating damaged myocardium using several different cell types. Cardiac Stem Cells (CSCs) seem a logical cell source to exploit for cardiac regeneration therapy. Their presence into the heart, the frequent co-expression of early cardiac progenitor transcription factors, and the capability for ex vivo and in vivo differentiation toward the cardiac lineages offer promise of enhanced cardiogenicity compared to other cell sources. Recently, we have successfully isolated CSCs from small biopsies of human myocardium and expanded them ex vivo by many folds without losing differentiation potential into cardiomyocytes and vascular cells, bringing autologous transplantation of CSCs closer to clinical evaluation. These cells are spontaneously shed from human surgical specimens and murine heart samples in primary culture. This heterogeneous population of cells forms multi-cellular clusters, dubbed cardiospheres (CSs), in suspension culture. CSs are composed of clonally-derived cells, consist of proliferating c-Kit positive cells primarily in their core and differentiating cells expressing cardiac and endothelial cell markers on their periphery. Although the intracardiac origin of adult myocytes has been unequivocally documented, the potential of an extracardiac source of cells, able to repopulate the lost CSCs in pathological conditions (infarct) cannot be excluded and will be discussed in this presentation. Preliminary results will be presented concerning some of the expression markers and functional properties of these stem/progenitor cells in their different features and maturation phases. Considerable expansion of the CSs, resulting in >1 million human CSs within a 30-day period. Floating CSs can be also expanded as monolayers in fibronectin to upwards of 7-70 million cells in about 45 days from a single endomyocardial biopsy specimen weighting 20 mg on average. The delivery of human CSs or of CSs-derived cells into the injured heart of the SCID mouse resulted in engraftment, myocardial regeneration and improvement of left ventricular function, with respect to control groups receiving PBS and/or an alternative cell source (fibroblasts), at least within 1 month after cell transplantation. The modulation of the CSCs differentiation with minimal cells manipulation is another challenge to be considered for clinical translation. In this regard, the effect of extremely low frequency magnetic field to CSCs has become to be studied in our Lab and preliminary results will be showed. Our method for ex vivo expansion of resident CSCs for subsequent autologous transplantation back into the heart, may give these cell populations, the resident and the expanded, the combined ability to mediate myocardial regeneration to an appreciable degree, and may very well change the way in which cardiovascular disease is approached in the future.

Meznaric-Petruša Mija
Mija Meznaric-Petruša (1), Neža Župančič (2), Jana Frelih (2), Zvonka Rener-Primec (2), Barbara Garavaglia (3), Massimo Zeviani (3) , Mihael Rogač (2), Corrado Angelini (4), David Neubauer (2). (1) Medical Faculty Ljubljana, Institute of Anatomy, Ljubljana, Slovenia; (2) University Clinical Centre Ljubljana, Children's Hospital, Department for child, adolescent and developmental neurology, Ljubljana, Slovenia; (3) National Neurological Institute "Carlo Besta", Milano, Italy; (4) University of Padova, Department of Neurosciences, Padova, Italy. E-mail: marija.meznaric-petrusa@mf.uni-lj.si

How informative is in children muscle biopsy to diagnose mitochondrial disease?
Muscle biopsy can contribute up to 4 points to total score of MDC, but is an invasive procedure. We analysed muscle morphology at 38 paediatric patients, aged from 1 month to 17 years, with encephalomyopathies or isolated muscle symptoms, in whom mitochondrial disease was suspected mostly on the basis of lactic acidemia and/or brain imaging studies. The diagnosis of mitochondrial disease was established biochemically in 25 patients; in three patients, in addition, mutations of TK2 gene (two patients), ANT1 and COX assembly SCO2 gene were discovered (in one patient each). Ragged red fibres and ragged blue fibres were observed in two single patients and virtually total absence of cytochrome c-oxidase in other two single patients; mild generalised reduction of cytochrome c-oxidase was present in three patients; two patients with cytochrome c-oxidase abnormalities had vacuolar myopathy with lipid accumulation. By EM mitochondrial abnormalities were detected in six patients, in others EM was not performed. Only coarse distribution of enzyme product was seen on SDH stain in other (14) patients. However, coarse distribution of oxidative enzyme product was present also in patients with normal activity of respiratory chain enzymes and PDHc and was therefore considered as unreliable abnormality. In three of the latter patients muscle biopsy was helpful, since CMD was diagnosed in two and muscle glycogenosis in one. In conclusion, in our series of infantile patients with mitochondrial cytopathy typical morphological mitochondrial abnormalities were present in about half of patients. The most useful morphological investigations were cytochrome c-oxidase histochemistry and electron microscopy.

Musarò Antonio
Department of Histology and Medical Embryology, University of Rome "La Sapienza", Rome, Italy. E-mail: antonio.musaro@uniroma1.it

Stem cell, muscle regeneration and the role of tissue niche
Muscle regeneration is a coordinate process in which multiple factors are sequentially activated to maintain and preserve muscle structure and function upon injured stimuli. However muscle regeneration is severely compromised in several muscle diseases, including muscular dystrophy, due to also alteration in satellite cell activation and reduction in satellite cell pool in pathological muscle. In this context stem cell-based therapy represents a promising approach t. However, it has been proposed that recruitment of stem cells into pathological muscle is a limiting factor to sustain an efficient mechanism of muscle regeneration and repair. Nevertheless, it is also possible that the recruitment and mobilization of stem cells is not the only limitation for stem cell-mediated muscle regeneration in dystrophic muscle. Among potential parameters that have impeded the generation of satisfactory protocols for stem cell therapy is that dystrophic milieu triggers activation of deleterious signal transduction pathways. One of the pathogenic factor associated with muscular dystrophy is chronic inflammation that affects the functional performance of skeletal muscle. Inflammation is clearly a critical component of muscle physiology and is an important phase in the regenerative process. Inflammatory responses resulting from tissue injury or infection generally resolve in beneficial self-limiting, healing process. To date much evidence reveals that the magnitude of the inflammatory response is crucial to keep the organism homeostasis and deregulation of it can promote disease. Thus, the inflammatory response must be resolved to allow muscle repair. We have previously reported that transgenic expression of a locally acting IGF-1 isoform (mIGF-1) safely enhanced and preserved muscle fibres integrity in senescent and dystrophic muscle, suggesting that mIGF-1 acts as a survival factor by prolonging the regenerative potential of skeletal muscle through increases in satellite cell activity. More recently we characterized the specific effect of mIGF-1 on the modulation of the inflammatory response during muscle injury and in mdx dystrophic muscle. In particular we observed that local expression of mIGF-1 transgene accelerates the regenerative process of injured skeletal muscle and protect dystrophic muscle, modulating the inflammatory response and limiting fibrosis. At the molecular level, mIGF-1 expression significantly down-regulated proinflammatory cytokines, such as TNF-alpha and IL-1beta, and modulated the expression of CC chemokines involved in the recruitment of monocytes/macrophages. Analysis of the underlying molecular mechanisms revealed that mIGF-1 expression modulated key players of inflammatory response, MIF, HMGB1 and NFkB. The rapid restoration of injured mIGF-1 transgenic muscle was also associated with connective tissue remodelling and a rapid recovery of functional properties. By modulating the inflammatory response and reducing fibrosis, supplemental mIGF-1 creates a qualitatively different environment for sustaining more efficient muscle regeneration and repair.

Patruno Marco
Martinello T (1), Sacchetto R (1), Maccatrozzo L (1), Toniolo L (2), Reggiani C (2), Mascarello F (1), Patruno M (1). (1) Department of Experimental Veterinary Sciences and (2) Department of Human Anatomy & Physiology, University of Padova, Italy. E-mail: marco.pat@unipd.it

Embryonic chick cocultures of neuronal and muscle cells
The aim of our study was to set up an effective experimental cocultures model, between cells of spinal cord and myotubes of adductor muscle, from chick embryos. We obtained neuronal cells from chick spinal cord explants at Embryonic Day 5 (ED 5) by means of an enzymatic digestion. Small spinal cord fragments were added in cultured muscle cells committed to the differentiative program. Myoblasts were isolated from the chick adductor muscle at ED12. The validation of the experimental model was confirmed by a remarkable spreading pattern of neuronal cells, labeled with a NF200 antibody, and high concentration of myotubes, marked by α-actinin antibody. The indication of neuronal contacts was highlighted by α-bungarotoxin. Our communication reports one of the few morphological description of muscular and neuronal coculture preparation, performed on chick embryos. Moreover, the effects of innervation on myogenic mechanisms were assessed by Real-Time PCR and immunohistochemistry. The experimental model presented in this work might be a useful tool in order to study the cascade of myogenic positive and negative signals activated by paracrine neuronal factors.

Pellacani Andrea
Pellacani A (1,2), Caserta G (1), Landi A (1), Bortolotto SK (2), XiaoLian Han (2), Hussey A (2), Morrison WA (2). (1) Policlinico of Modena, Modena Italy; (2) Bernard O'Brien Institute of Melbourne, Australia.

Engineering muscle tissue in load and unload model
We developed a new model for muscle regeneration, by space and time maintaining the volume of an experimentally muscle defect. The addition of matrices (Matrigel, Myogel, Plasma Clots, Curaspon) and cultured myoblasts into defect helped to maintain total muscle volume, providing a barrier against muscle collapse. 14 days group: Matrigel plus myoblasts restored 28.64 % of muscle tissue in the biopsy site, resulting the best group compared to all the other ($P< 0.05$). Plasma clots resulted the best angiogenetic matrix ($P< 0.05$), but not capable to produce good results in terms of myoblasts new formation. It could be postulated that once providing a minimal amount of blood supply to cells surviving, the local signalling is the key point to obtain a better quality of tissue regeneration. A neutral to positive induction of matrigel induced macrophages on myoblast (satellite cells or cultured myoblasts) was proved. 30-90 days group: We couldn't see any advantage by adding cultured myoblasts to the biopsy site at 30 and 90 days, both in the loaded and unloaded groups. The 30 days loaded group had a muscle and connective tissue percentage respectively higher and lower ($P<0.05$) compared to the unloaded. The 90 days loaded group had a statistically higher percentage of adipose tissue compared to the unloaded group ($P<0.05$). Our model seems really good for preliminary matrices testing up to 30 days. The main limit is the difficult to define the biopsy site after 30 days, which could be nevertheless considered a good time for small animal biology. The ideal matrix for myoblasts growth is the one which can let nutrients easily reach the cells, non collapsible, whit a precise myoblasts molecular targeting, in which cells are physiologically orientated since in vivo culturing.

Pietrangelo Tiziana
Tiziana Pietrangelo (1), Rosa Mancinelli (1), Luana Toniolo (2), Carlo Reggiani (2), Giorgio Fanò (1). (1) Dept. of Basic and Applied Medical Science, Center for Excellence on Aging (CeSI), University G d'Annunzio, Chieti, Italy; (2) Dept. of Human Anatomy and Physiology, University of Padova, Italy. E-mail: tiziana@unich.it

Physical activities against Sarcopenia: an integrated experimental approach
Sarcopenia is a physiological status referred to reduction of skeletal muscle strength in older people that has a multifactorial origin linked to: oxidative damage of fibers (Fulle et al, Exp. Gerontol 40:189, 2005), mitochondrial damage (Brunk UT, Eur J Biochem, 269(8):1996-2002, 2002), reduced levels of GH, IGF-1, steroids and reduced myogenesis (Beccafico et al., ANYAS (270906) 345-352, 2007). Sarcopenia results in muscle atrophy and reduced mobility of subjects, but it is counteracted and delayed by physical activity. In our study, we analysed the effects of three different physical activity programs on 65-85 years old people in order to identify the cellular and molecular pathways by which these training projects act against sarcopenia status. The training typologies were: a) endurance training (cycloergometer, 1th-4th week: 30 min 60-70 HRmax, 5th-8th week: 40 min 60-70% HRmax, 9th-12th week: 40 min 80% HRmax); b) resistance training (1th-4th week: 3 series 12 repetitions 60-70 maximal strength, 5th-8th week: 3 series 10 repetitions 75-80 max strength, 9th-12th week: 3 series 6-8 repetitions 80-85% max strength); c) local (vastus lateralis muscle) vibrational energy application (1th-8th week: once a week 15 min 300Hz, 9th-12th week: 3 times a week, 15 min, 300 Hz, without voluntary muscle contraction). A nedlee biopsy (50-70 mg) was taken on vastus

lateralis muscle before and after the training and each biopsy was divided in three part in order to give different experiments. We analysed (i) the specific tension development of single fibers and the expression of myosin heavy chain proteins, (ii) the transcriptional profile and (iii) the regenerative capacity of satellite cells. The preliminary results show that each type of training are able to increase the muscle strength (inferior legs) and fatique resistance on each subject. The single fiber strength development did not change with any training protocol even if the endurance training significantly increase the cross sectional area of fibers and significantly reduced the myogenicity and fusion index of satellite cells. Considering the gene expression profiles, each physical activity share a stimulation of a specific metabolic pathway (both endurance and vibrational training increase the aerobic metabolism while the resistance training stimulates the creatine metabolism), it increases the folding of sarcomeric and cytoskeletal proteins and regulates specific responses to reactive oxygen species production. Moreover, endurance and resistance training seemed to silence the Foxo activation due to ageing but local vibration training seem do not interfere with this atrophic signal. In conclusion, our results suggest that the training protocols have been effective on elderly people because each of them are able to stimulate a specific metabolism. Considering this coherence between exercise typology and metabolisms, we think that also the other results are specific of training type.

Poltronieri Carlo
Carlo Poltronieri, Susanna Sivieri, Marco Patruno, Daniela Bertotto, Claudia Simontacchi, Giuseppe Radaelli. Department of Experimental Veterinary Sciences and Department of Human Anatomy & Physiology, University of Padova, Italy. E-mail: giuseppe.radaelli@unipd.it

Tissutal expression of IGF-I and myostatin during development and growth of sea bass (Dicentrarchus labrax).
The distribution of IGF-I and myostatin was investigated during development and growth of sea bass (Dicentrarchus labrax) by a combined approach of Real-Time PCR, in situ hybridization and immunohistochemistry. Real-Time PCR showed a lower expression of IGF-I and MSTN mRNAs in young larvae if compared to animals aged 25, 40 and 80 days. In adults, Real-Time PCR showed a higher expression of IGF-I in liver, compared to other examined organs. In the same animals, the expression of MSTN mRNA was higher in muscle and almost absent in other anatomical districts. In situ hybridization revealed IGF-I mRNA in several tissues, whereas MSTN mRNA was mainly evidenced in skeletal musculature. IGF-I immunoreactivity was detected in developing intestine already at hatching. Also in skeletal muscle, skin and yolk sac, immunoreactivity to IGF-I and MSTN was already evident at hatching. From day 4, both IGF-I and MSTN peptides were present in liver and from day 10 in the kidney and heart musculature. During larval stages a faint immunostaining to IGF-I and MSTN was also detected in pancreas. These results suggests, on the basis of a combined approach of Real-Time PCR, in situ hybridization and immunohistochemistry that IGF-I and MSTN are involved in the regulation of somatic growth in the sea bass.

Protasi Feliciano
Simona Boncompagni (1), Helmut Kern (2), Katia Rossini (3), Christian Hofer (2), Winfried Mayr (4), Ugo Carraro (3), Feliciano Protasi (1). (1) IIM - Interuniversitary Institute of Miology, Dept. of Basic and Applied Medical Science, Center for Excellence on Aging (CeSI), University G. d'Annunzio, Chieti, Italy; (2) Ludwig Boltzmann Institute of Electrostimulation and Physical Rehabilitation, Department of Physical Medicine, Wilhelminenspital, Vienna, Austria; (3) C.N.R. Institute of Neuroscience, c/o Department of Biomedical Science, University of Padova, Italy; (4) Center of Biomedical Engineering and Physics, Medical University of Vienna, Austria. E-mail: fprotasi@unich.it

Reconstruction of denervated human muscle by functional electrical stimulation (FES) in complete absence of innervation
The relative importance of muscle activity versus neurotrophic factors in the maintenance and differentiation of muscle fibers has been greatly debated. Muscle biopsies from spinal cord injury patients - who were trained with an innovative protocol of functional electrical stimulation (FES) for prolonged periods of time (2.4 to 9.3 years) - offered the unique opportunity of studying the reconstruction of the ultrastructure of denervated fibers under the sole influence of muscle activity. FES stimulation induced surprising recovery of muscle structure, mass and force even in patients that had been denervated for prolonged periods before the beginning of FES training (up to 2 years) and whose muscles had almost completely lost muscle specific internal organization. 90% (or more) of the fibers analyzed by electron microscopy showed a striking recovery of the ultrastructural organization of myofibrils and Ca2+ handling membrane systems. This findings demonstrate that denervated fibers have the ability to recover if appropriately stimulated and that this functional/structural restoration follows a pattern that mimics some aspects of normal muscle differentiation. Most importantly, the recovery occurs in complete absence of motor and sensory innervation and of nerve-derived trophic factors, i.e. solely under the influence of muscle activity induced by electrical stimulation.

Schussler Olivier
Schussler O (1), Coirault C (2), Louis-Tisserand M (1), Al-Chare W (1), Oliviero P (2), Menard C (4), Michelot R (1), Heude D (5), Salomon DR (3), Carpentier A (1), Lecarpentier Y (2,6), Chachques JC (1). (1) Laboratory of Biosurgery, Pompidou Hosp, Paris University; (2) INSERM U689, Paris; (3) The Scripps Research Institute, Department of Molecular and Experimental Medicine MEM 241 La Jolla CA, USA; (4) CNRS FRE 2593 Montpellier; (5) Laboratory of Pathology Pompidou Hospital; (6) Department of Cardiorespiratory Evaluations, Bicetre Hospital, France.

Coupling Adhesion Peptides to Collagen Scaffold for the Development of Engineered Artificial Myocardium
Cardiac tissue engineering is an emerging field that holds great promise for replacement therapy of diseased myocardium or repair of cardiac malformations. Tissue engineering is an emerging field based on the use of a combination of cells, biomaterials and suitable biochemical factors to improve or replace biological functions. Collagen matrix (CM) seeded with neonatal cardiomyocytes represents the unique scaffold in which contractile activity has been demonstrated in vitro. However, contractility is unpredictable and terminal cell differentiation has been achieved only in the presence of the tumor extract: Matrigel™ that may compromise nutriment diffusion and clinical use. We hypothesized that improving cell matrix interactions by coupling adhesive peptides RGD+ to interact with integrin adhesion molecules on cardiomyocytes would enhance cell survival and differentiation without requiring Matrigel.
Parameters of contractility (spontaneous or under electrostimulation) during contraction and relaxation phases were mesuared by using a force length microtransducer under isotonic or isometric conditions. Contractile performance, cardiomyocyte viability and differentiation were analyzed after 8 days. RGD- scaffolds (n=11) were used as controls. In both RGD- and RGD+ constructs, active force (AF) reached a maximum value at 0.17 Hz electrostimulation frequency and tended to decrease with increased stimulation frequency. At optimal stimulation frequency, RGD+ had nearly 3-fold increase in both maximum extent of shortening and maximum shortening velocity. By classical and confocal microscopy, cardiomyocytes changed their morphology in RGD+ scaffolds with reorganization of contractile apparatus and development of cross-striation. In conclusion, we report a novel method of engineering a highly effective and safe collagen scaffold for engineering bioartificial myocardium

Sferrazza Rossella
Rossella Sferrazza (1), Vincenzo Vindigni (1), Nicoletta Adami (2), Sandra Zampieri (2), Ugo Carraro (2), Francesco Mazzoleni (1). (1)Unit of Plastic and Reconstructive Surgery of the University of Padova Interdepartmental Research Institute of Myology, c/o Department of Surgical and Medical Specialties, Padova, Italy; (2) Laboratory of Translational Myology of the Interdepartmental Research Center of Myology c/o Department of Biomedical Sciences, University of Padova, Italy. E-mail: francesco.mazzoleni@unipd.it

A rat model of a vascularized muscle construct
Skeletal muscle regeneration is a powerful, naturally-occurring process of tissue reconstruction that follows upon myofiber damage secondary to myotoxic injury which, instead, does not normally affect the tissue circulation and scaffold. Myofiber regeneration can occur after muscle damage. Healing of muscle necrosis may occur either as a repair process, which ends in a scar or by myofiber regeneration. In a rat model of full-thickness rectus abdominis muscle ablation, the ablated rectus abdominis was autografted in situ and marcaine was injected into both the autograft and the surrounding abdominal wall muscle. Three weeks after surgery, serial cross sections of the reconstructed abdominal wall were stained with haematoxylin-eosin or embryonic myosin antibody, a well-characterized molecular marker of early myogenesis in development and regeneration. Autografting of the middle third of the rectus abdominis muscle resulted in scar formation. The few muscle cells in the graft core were scanty myoblasts that could be detected only by monoclonal embryonic myosin antibody. Though negative for myofiber regeneration, the results in both cases confirmed the mechanical patency of the patches with regard to abdominal organ support. Myofibers were successfully regenerated in some of the graft by injecting marcaine into both the autograft and the surrounding muscles. In this case three weeks after surgery, the patch was paved with young, centrally nucleated myofibers intermixed with young myofibers and myotubes expressing embryonic myosin. Since muscle regeneration seemed to be the result of successive waves of coordinate migration of angioblasts and then satellite cell–derived myoblasts from the muscles surrounding the patch, we setted up a more robust model of muscle reconstruction by implanting a free soleus into an isolated intestine loop. Before suturing the transplated soleus to the abdominal wall, the transplant was wrapped with vascularized homentum. Two weeks later, the free transplanted muscle show a pripheral lkayer of dying/surviving myofibers, while the muscle core presented well developed MHC-emb positive myofibers in spite of the absence of the nerve. A new vascular pedicle was entering the isolated soleus from the homentum, thus allowing regeneration of infracted myofibers in the free transplant, at an extent, that compar well with in situ marcaine-injected ischaemic soleus. The results strongly suggest that vascularization of an artificial scaffold and successive coordinate proliferation of seeded (stem)cells may routinely be achieved in the near future.

Shafy Abdel
Shafy A, Goussef N, Grandjean PA, Alshamry A, Chachques JC. Laboratory of Biosurgical Research, Pompidou Hospital, University of Paris France, and, Emirates World Heart Foundation, UAE.

Electrostimulated «dynamic» cellular cardiomyoplasty
Atrial synchronized biventricular pacing associated with cellular cardiomyoplasty (CMP) was investigated by our group to transform passive cell therapy into « dynamic cellular support ». The principles of electrophysiological conditioning of muscle fibres was applied by our group in myoblast-based cellular CMP since skeletal myofibers express predominantly fatigue sensitive fast myosin, which is not suitable for cardiac work. Atrial synchronized biventricular pacing is indicated in heart failure patients to correct conduction disorders associated with chronic systolic and diastolic dysfunction. We explored the possibility to electrostimulate the ventricles following autologous skeletal myoblast implantation to create an active cellular cardiac support, since skeletal myoblasts are known not to contract spontaneously. Twenty two adult sheep were included. All animals underwent myocardial infarction by ligation of 2 coronary artery branches (distal LAD and D2). After 3 weeks, autologous cultured myoblasts were injected in the infarcted areas. Atrial synchronized biventricular pacing (BIV) was performed using epicardial electrodes, with a pulse amplitude of 5 Volts and a pulse width of 0.5 milliseconds. Ventricular electrostimulation includes depolarization of the implanted cells. Echocardiographic studies performed at 2 months showed a significant improvement in ejection fraction (47±6 vs 36±4 %) and a limitation of LV dilatation (49±7 vs 69±2 ml) in cell treated BIV vs control group. Viable cells were identified in the infarcted areas. Differentiation of myoblasts into myotubes was significantly improved in the group associating cell therapy with BIV, immunocytological studies showed enhanced expression of slow myosin heavy chain (MHC). Electrostimulation induced the synchronus contraction of transplanted myoblasts, thus improving ventricular function. Histochemical studies after pacing demonstrated an important number of myotubes and enhanced expression of slow MHC which is better adapted at performing cardiac work. Heart failure patients with conduction defects and necessity of myocardial regeneration should benefit from simultaneous cardiac resynchronization and cellular CMP.

Sławińska Urszula
Nencki Institute of Experimental Biology PAS, 02-093 Warsaw, Poland. E-mail: u.slawinska@nencki.gov.pl

Changes in contractile and structural properties of fast muscles after spinal cord transection in rats
Complete spinal cord transection at low thoracic level in adult rats severely impairs locomotor hindlimb movements. The structural and contractile properties of hindlimb muscles are considerably changed. The aim of our study was to investigate whether in spinal rats the fast muscle transformation depends on their function. The effects of total spinal cord transection (T9/T10) was investigated in two antagonistic muscles: medial gastrocnemius (MG; fast extensor) and tibialis anterior (TA; fast flexor) that are composed in similar proportions of three different types of muscle fibres. The contractile properties and the content of myosin heavy chain (MHC) isoforms in both muscles were studied one, three and six months after spinal cord total transection. We demonstrated that during the first month of reduced muscle activity caused by spinal cord transection the significant changes occurred in muscle contractile properties that remained up to six months after the injury. Both muscles became slower and weaker but unexpectedly more fatiguable. However, the changes were more pronounced in MG than in TA muscle. Moreover, the fast extensor (MG) muscle was composed of higher proportion of fast IIx and IIb MHC isoforms only while the fast flexor (TA) muscle of higher proportion of type IIx muscle fibres while type IIb was significantly reduced. Our results suggest that the atrophy and phenotype transformation of the rat muscles depend not only on their primary fibre composition but also on the muscle function and position i.e. whether they were chronically lengthened or shortened as a result of spinal cord injury. Supported by grant from Polish MNiSW no. 2 P05A 092 27.

Trainini Jorge
Trainini Jorge, Barisani José, Mouras Jorge, Christen Alejandra, Chada Salvador. E-mail: "Jorge Trainini" jctrainini@hotmail.com

Hemodinamic and functional effects of dynamic aortomyoplasty in patients with advanced heart failure
The goal of our study was to evaluate the benefits of aortic counterpulsation obtained by dynamic aortomyoplasty in patients with heart failure refractory to pharmacological treatment and contraindications to heart transplant. In this study we compared preoperative and postoperative data of 15 selected patients submitted to dynamic thoracic aortomyoplasty. In this surgical technique the right latissimus dorsi muscle(LDM) flap is wrapped around the ascending aorta. The LDM flap was electrically stimulated during diastole, following a muscle-conditioning protocol, to obtain diastolic augmentation. At 12-month follow-up, we evaluated, invasively and noninvasively, the hemodynamic, clinical and functional effects of aortomyoplasty. When comparing preoperative data with 12-months' follow-up data, we observed

a significant decrease in the number of hospitalizations ($p<0.0001$), improvement of NYHA functional class ($p<0.001$), reduction of left ventricular diastolic diameter ($p<0.05$) and left atrium diameter ($p<0.05$), decrease of pulmonary artery systolic pressure ($p<0.01$) and Wedge pressure ($p<0.01$). We observed a significant increase in the walking test ($p<0.001$), peak oxygen consumption ($p<0.05$), shortening fraction ($p<0.01$), left ventricular ejection fraction ($p<0.01$) and cardiac output ($p<0.01$). Dynamic biologic chronic counterpulsation assistance with electrostimulated LDM in heart failure (dynamic aortomyoplasty) in selected patients with severe heart failure, resulted in an important improvement of hemodynamic parameters, heart functional data and clinical functional class. A larger clinical experience, with a longer follow-up would be useful to evaluate the clinical relevance of this surgical approach.

Trainini Jorge
Trainini Jorge, Lago Noemí, Mouras Jorge, Bordone Javier, Chachques Juan, Barisani José, Masoli Osvaldo, de Paz Jorge. E-mail: "Jorge Trainini" jctrainini@hotmail.com

Stem cells implant in chronic ischemic cardiomyopathy

Implantation of myoblasts and bone marrow mononuclear stem cells (MNSC) including endothelial progenitors cells to repair fibrotic myocardium is shown to be safe and effective. We evaluated clinical and functional improvements induced by myoblasts and MNSC in non viable myocardium segments with transmural infarction (TMI) and non transmural infarction (NTMI) in patients (p) with chronic ischemic cardiomyopathy. Fifty p (17 with collagen matrix) were treated with cellular implants. Five p with myoblasts and 13 treated with MNSC were evaluated at one year follow-up. We implant the cells by epicardial approach with concomitant surgical revascularization in remote, viable and ischemics areas. The protocol implantation of myoblasts involves: two hundred million cells, density of fifty million cells per milliliters and upper to sixty per cent of concentration. Enriched suspension of MNSC: 5.9±2.2 ml containing 1.5±0.8x108 MNSC. Functional class (FC), left ventricular ejection fraction (LVEF) and Sestamibi Tc99m Gated-SPECT test at rest and exercise were compared before and after implant cells in each p. TMI and NTMI segments changes were assessed. In 4 p who survived after 33± 6.05 months treated with myoblasts the number of segments with TMI decrease from 15 to 3 ($p=0.0005$) and the NTMI segments decrease from 7 to 2. No mortality or complications were observed at a follow-up of one year. FC improved from 2.4±0.5 to 1.1±0.3 ($p<0.0001$), radionucleid left ventricular ejection fraction increased from 27±9 to 38±14% ($p<0.005$). The TMI segments showed an improved of 52% and the NTMI segments improved 69%. In these patients treated with myoblasts and MNSC in TMI and NTMI segments, myocardial perfusion improved. Radioisotopic evaluation seems to be an adequate alternative to measure stem cell therapy.

Vindigni Vincenzo
Barbara Zavan, Giovanni Abatangelo, Francesco Mazzoleni, Franco Bassetto, Roberta Cortivo, Vincenzo Vindigni (1). (1) Department of Histology, Microbiology and Medical Biotechnology, (2) Unit of Plastic and Reconstructive Surgery, University of Padova, Italy. E-mail: vincenzo.vindigni@unipd.it

New 3D hyaluronan-based scaffold for in vitro reconstruction of the rat sciatic nerve

For peripheral nerve regeneration, three-dimensional distribution and growth of cells within the porus scaffold are of clinical significance. The purpose of this study was to test in vitro a novel hyaluronic-acid-(HA)-based tubular conduit (HYAFF-11™ biomaterials – 1 x 10 mm) as a nerve guide. Human fibroblasts, RN22 Schwann cell lines, human umbilical vein endothelial cells (HUVEC), and primary nerve cells, obtained from neonatal rat sciatic nerve were harvested and seeded on HYAFF-11™ devices. Histological (Haematoxilin-Eosin), immunohistochemical (antibodies to S-100, CD31 and von Willebrand factor) and PCR analyses were performed after 7 and 14 days from cell seeding onto biomaterials. MITT-based (thyazolyl blue) and DELFIA cell proliferation kit tests were performed to observe the biocompatibility of the cells cultured within the biomaterial devices. We concluded that the conduits were not cytotoxic and demonstrated that cultured RN22 Schwann cells and rat Schwann cells grow in vitro on new artificial nerve conduits. We thus inferred that the HYAFF-11™ conduit was a suitable biomaterial able to support nerve cell growth in vitro and, after 14 days of cultivation, remained circular with round lumen, maintaining the size and shape of its original architecture. Finally, attachment and proliferation of endothelial cells attest the feasibility of developing a co-culture system to promote in vivo integration of a microvascularized nerve substitute.

Vitiello Libero
Sarah Pigozzo (1), Ian Mclachlan (2), Ellen Ambegia (2), Maurizio D Baroni (1), Libero Vitello (1)
(1) Department of Biology, University of Padova, Padova, Italy; (2) Protiva Biotherapeutic Inc., Burnaby, BC, Canada.
E-mail: libero.vitiello@unipd.it

Systemic administration of antisense oligonucleotides via encapsulated lipoplexes can lead to dystrophin restoration in cardiac and skeletal muscle of mdx mice
Antisense-mediated exon skipping holds great potential for the treatment of Duchenne muscular dystrophy patients. We have recently explored the potential of systemic (intra-arterial) delivery of therapeutic morpholino oligos in aged mdx mice, whose phenotype resembles more closely that of DMD. We now present the preliminary results obtained with a novel formulation of lipid-complexed oligos, stable nucleic acid lipid particles (SNALP), delivered intra-venously. This particular system was chosen because it had been designed to specifically deliver its genetic cargo to areas of tissue inflammation and therefore appeared well suited for reaching dystrophic muscles. Our data showed that delivering just 35 μg of encapsulated 2-Me-O phospho-thioate ribonucleotides per animal was sufficient to notice an increase in the amount of dystrophin-positive fibers (over the revertant background) in all analyzed muscles. Importantly, it appeared that SNALP could deliver the therapeutic oligos also in the heart, a target that so far has eluded all protocols based on naked oligos alone. The absolute number of dystrophin-positive fibers in the cardiac muscle was smaller than that found in its skeletal counterparts, but the virtual absence of revertant cardiomyocytes in mdx animals made their presence even more statistically significant. Compared to morpholino oligos, however, the ribo-oligos used here appeared to be much less stable in the cells, as the levels of skipped mRNA fell below detection threshold within few days from injection.

Vrbova Gerta
Autonomic Neuroscience Institute, UCL and RF Medical School, Rowland Hill Str. London NW3 2PF, UK. E-mail: ucgagev@ucl.ac.uk

Possible use of cholinergic neurones from the myenteric plexus for reinnervation of skeletal muscles
The assumption that axons of CNS neurones are unable to regenerate has been overruled by experimental evidence collected at the end of the last century. Axons of CNS neurones can regenerate if they are given a conduit that provides them with the appropriate environment such as Schwann cells from peripheral nerves (David S., Aguayo AJ. Science 1981 :214, 931-933). Moreover our results show that grafted embryonic motoneurones can survive and mature in the spinal cord of adult rats provided their axons are lead to a skeletal muscle via a peripheral nerve conduit. The grafted motoneurones were able to form functional connections with the denervated muscles (David S., Aguayo AJ. Science 1981 :214, 931-933). However, the practical use of embryonic motoneurones for reinnervation of denervated muscles is fraught with many problems, the most important being that the neurones are obtained from a different individual and may be immunologically incompatible with the host. Therefore a source of cholinergic cells that could be derived from the same individual would be desirable. A possible source of such neurones may be the myenteric plexus of the gut. It has indeed been shown that grafts of the myenteric plexus survive in the striatum for up to one year (Tew E.M.M., Anderson P.N., Saffrey J.M and Burnstock G. Exp.Neurol. 1994; 129, 120-129). Whether such cholinergic neurones grafted into the spinal cord would be able to extend their axons along peripheral nerve conduits to denervated skeletal muscles will be studied. Possible other alternatives of reinnervation of skeletal muscles by these neurones will also be explored.

Vrbova Gerta
Autonomic Neuroscience Institute, UCL and RF Medical School, Rowland Hill Str. London NW3 2PF, UK. E-mail: ucgagev@ucl.ac.uk

Differences between exercise and muscle activity induced by electrical stimulation
Muscle activity induced by electrical stimulation has often been viewed with some reservation. Two fundamental differences exist between electrical stimulation and voluntary contractions where a strict hierarchical order of recruitment of motor units activating the smallest motor units first, followed by larger units is always maintained . Due to the biophysical properties of the axons that innervate the muscle electrical stimulation excites preferentially the largest motor units thus reversing the natural order of recruitment and muscle fibres that are usually used rarely are active most frequently. The consequences of this difference of recruitment on the muscle fibre changes after exercise or electrical stimulation are: i) electrical stimulation will affect most muscle fibres that are rarely used (IIB) and convert them into IIA or type I fibres; while ii) exercise will affect muscle fibres that are used most frequently, i.e. type I and type IIA, enhancing their phenotypic specialisation. Electrical stimulation exploits more fully the adaptive potential of

muscle then exercise. High amounts of activity can be imposed on a muscle from the beginning of training since the central nervous, cardiovascular and other systems will not limit the amount of activity carried out by the muscle, as during exercise. However, during exercise induced activity coordinated movement is carried out and the individual's skills improve, the flexibility of joints and of the cardiovascular system improve by exercise better then by electrical stimulation. Nevertheless, muscles that are less fatiguable then usual, due to electrical stimulation enables the individual to exercise more efficiently.

Zampieri Sandra
Sandra Zampieri (1,2), Donatella Biral (3), Nicoletta Adami (1), Anna Ghirardello (2), Marta Tonello (2), Andrea Doria (2). (1) Laboratory of Translational Myology of the University of Padova, Interdepartmental Research Institute of Myology, c/o Department of Biomedical Sciences, Padova, Italy; (2) Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy; (3) Italian C.N.R. Institute of Neuroscience, c/o Department of Biomedical Sciences, Padova, Italy. E-mail: sanzamp@unipd.it

Expression of myositis specific autoantigens during post-natal myogenesis and muscle regeneration
Idiopathic inflammatory myopathies such as polymyositis (PM) and dermatomyositis (DM) are a group of rare autoimmune diseases, characterized by an inflammatory infiltrate within the skeletal muscle and high titer of circulating autoantibodies in the patient's serum. In muscle biopsies from PM and DM patients regenerative features induced by muscle damage are typically observed. The aetiopathogenesis of these diseases is not known and the relationship between the specific muscle involvement and the ubiquitary presence of the targeted antigens is still unclear. In order to understand whether candidate autoantigens in myositis are expressed during postnatal myogenesis and muscle regeneration, we performed immunolocalization studies of myositis specific autoantigens in normal skeletal muscle from newborn rats and in marcaine-induced muscle regeneration from sciatectomized rats using monospecific sera from autoimmune patients affected with PM and DM. Our observations indicate the presence of myositis candidate autoantigens during post-natal myogenesis and muscle regeneration. Preliminary results from immunoblotting analyses of newborn, regenerating and adult normal muscle homogenates show that myositis autoantigens are expressed during post-natal myogenesis, while they cannot be detected in adult normal muscles. Our observations contribute to a better understanding of the process leading to the induction and/or amplification of the immune-inflammatory response in patients affected with autoimmune myositis. Moreover, the expression of candidate autoantigens during muscle regeneration may be also of interest for gene therapy in clinical settings.