

PW 27:
Cell therapies – Myoblasts

PW27-334	<p><u>IN VIVO BIOLUMINESCENCE IMAGING OF TRANSPLANTED HUMAN MYOBLASTS USING RENILLA-LUCIFERASE TRANSGENE EXPRESSION</u> LAUMONIER T¹, KONIG S², BADER C², BERNHEIM L², HOFFMEYER P¹, MENETREY J¹ (1) Department of Orthopaedic Surgery, University Hospital of Geneva, Geneva, SWITZERLAND. (2) Department of Basic Neurosciences, University Medical Center, Geneva, SWITZERLAND.</p>
To contact the author:: thomas.laumonier@medecine.unige.ch.	<p>Cellular therapies for Duchenne muscular dystrophy and other muscle diseases are limited by a massive early cell death following injections. The quantification of cell survival is an essential step to evaluate the efficiency of myoblast transplantation. In the present study, we describe an approach for in vivo follow-up of human myoblast grafts transplanted in mice using non-invasive imaging techniques (bioluminescence). Human myoblast clones, each derived from single post-natal human satellite cell, were transduced with a lentivirus containing the renilla-luciferase (Rluc) gene under a HSV-TK promoter. Reporter gene expression had no adverse effects on myoblast viability, proliferation, or differentiation in vitro. A robust correlation was found between Rluc signals and cell numbers by ex vivo imaging analysis ($R^2 = 0.98$) and by in vitro enzyme assay ($R^2 = 0.93$). Similar correlations were observed between Rluc signals and the number of infectious virus particles per cell (multiplicity of infection). Afterward, up to 1×10^6 Rluc or control myoblasts were injected into the tibialis anterior (TA) muscle of mice. Bioluminescence imaging 6 hours after transplantation showed a correlation between Rluc signals and the number of cells injected, while only background level of bioluminescence was observed within control cells. These results demonstrate that optical imaging of human myoblasts containing renilla-luciferase reporter gene can be used for the rapid and accurate evaluation of cell survival post transplantation. With further development, this approach will help to improve myoblast transplantation biology, notably by facilitating the screening for therapeutic agents acting on grafts.</p>

PW27-335	<p><u>HEAT SHOCK TREATMENT INCREASES ENGRAFTMENT OF HUMAN TRANSPLANTED MYOBLASTS INTO IMMUNODEFICIENT MICE</u> BENCZE M¹, RIEDERER I¹, NEGRONI E¹, BIGOT A¹, DI SANTO JP², BUTLER-BROWNE GS¹, MOULY V¹ (1) UMR S 787 - Groupe Myologie-Inserm / UPMC / Institut de Myologie-105, bld de l'hôpital., Paris, FRANCE. (2) Unité des Cytokines et Développement Lymphoïde, Département d'Immunologie, Institut Pasteur., Paris, FRANCE.</p>
To contact the author:: bencze@chups.jussieu.fr	<p>One of the phenomena known to limit the success of myoblast transfer therapy (MTT) is the massive and early cell death observed during the first few hours after cell injection. A major part of injected cells die by necrosis and apoptosis, thus decreasing the number of muscular progenitors that could be involved in fusion events during muscle regeneration. In the mouse it has already been shown that a short incubation of myoblasts at a high temperature (42°C/ ~107°F) increases their resistance to death in vivo and in vitro.</p> <p>Our objective was to determine if heat shock (HS) treatment can increase the survival of human myoblasts leading to a better participation of the injected cells in muscle regeneration, and to quantify this potential improvement. We show in vitro that HS treatment of human myoblasts doesn't change significantly their proliferation, but does increase their resistance to staurosporin induced apoptosis, as compared to untreated cells. Control or HS treated human myoblasts were also injected in vivo into Tibialis anterior muscles of immuno-deficient RAG^{-/-} γC^{-/-} C5^{-/-} mice. We have found that, 24 hours post-injection, the number of HS cells in proliferation was the same as that of control cells, while the number of apoptotic cells was significantly lower in muscles transplanted with heat shocked myoblasts. The contribution of human myoblasts to host regeneration was assessed using species specific antibodies, and a dramatically increase in the number of human fibres was observed in muscles injected with HS treated cells, as compared to untreated cells. These results suggest that heat shock treatment of human myoblasts promotes a significant protection against apoptotic cell death and improves muscle regeneration in vivo. This treatment could be applied to clinical trials involving intra-muscular cell therapy.</p>

PW27-336	<p><u>OPTIMIZATION OF HUMAN MYOBLAST TRANSPLANTATION USING THE RAG IMMUNODEFICIENT MOUSE MODEL</u> RIEDERER I¹, NEGRONI E², BENCZE M², WOLFF A², DI SANTO JP⁴, SAVINO W³, BUTLER-BROWNE GS², SILVA-BARBOSA S³, MOULY V² (1) UMR5787 – Groupe Myologie; Inserm / UPMC-ParisVI; Institut de Myologie - Oswaldo Cruz Foundation, Department of Immunology, Laboratory on Thymus Research, Paris/Rio de Janeiro, FRANCE. (2) UMR5787 – Groupe Myologie; Inserm / UPMC-ParisVI; Institut de Myologie, Paris, FRANCE. (3) Oswaldo Cruz Foundation, Department of Immunology, Laboratory on Thymus Research, Rio de Janeiro, BRAZIL. (4) Institut Pasteur – Département d' Immunologie, Unité des Cytokines et Développement Lymphoïde, Paris, FRANCE.</p>
To contact the author:: ingo@ext.jussieu.fr.	<p>Myoblast transplant therapy (MTT) can be envisioned as a clinical alternative in the treatment of certain diseases. The problems that still remain to be solved to improve the efficiency of MTT are the massive early cell death, the limited proliferation and the inefficient migration of these cells. Recently, we showed that immunodeficient mice such as RAG-/-gammaC-/-C5-/- and RAG-/-gammaC-/- are both attractive and efficient systems to support engraftment of human myoblasts. We have used this model to study early events which occur following human myoblast transplantation. 5x10⁵ myoblasts were injected into Tibialis Anterior (TA) muscles. Mice were sacrificed after 0h, 1h, 3h, 6h, 24h, 12h, 3 days, 5 days and TA muscles were analyzed by immunofluorescence. Human cells were detected with a human specific laminin A/C antibody. We observed a progressive decrease in the percentage of proliferating human myoblasts from 0h to 5 days pos-transplantation. A peak in cell death was observed at 12 and 24h whereas by 5 days there was virtually no cell death. After cell injection we observed cell dispersion, but this was only clearly detected after 24h. Cell dispersion following transplantation was correlated with a deposition of laminin within the pocket of human cells that peaked between 12h and 24h, a time at which there is a huge macrophage infiltration in the area of injected cells. However, despite a significant increase in the area occupied by the injected cells between 3 and 5 days, this area corresponded to only a portion of the total muscle. Interestingly, markers of differentiation were expressed by the human myoblasts after 24h, indicating an “early” differentiation of human cells following transplantation. A better knowledge of the mechanisms involved in survival, proliferation migration and differentiation of transplanted human myoblasts, will certainly be important to design new strategies for cell mediated therapy.</p>

PW27-337	<p><u>IN VITRO ARRESTED MUSCLE CELLS AS MODELS FOR THE IDENTIFICATION OF GENES INVOLVED IN THE QUIESCENCE OF SKELETAL MUSCLE SATELLITE CELLS</u></p> <p>KANDALLA P¹, JACQUEMIN V¹, REIDERER I¹, BUTLER-BROWNE G¹, MOULY V¹ (1) UMRS 787- Groupe Myologie; Inserm / UPMC-Paris VI; Institut de Myologie, Paris, FRANCE.</p>
To contact the author:: kandalla@chups.jussieu.fr.	<p>Satellite cells, the post-natal muscle progenitors, are responsible for the regenerative capacity of skeletal muscle. They remain quiescent on the edge of muscle fibers, and become activated when fibres degenerate, either after trauma or in genetic diseases. Once the regeneration process is terminated, satellite cells, which have not engaged into the myogenic process, return to quiescence on the edge of the newly formed fibres. Therefore, the asymmetric division that sends progenitors back to quiescence will define the number of cells available for regeneration, and thus the future regenerative capacity. Furthermore, the group of Partridge showed that transplantation of single fibres with their resident satellite cells is by far more efficient in intra-muscular transplantation than any other type or state of progenitor, thus raising the possibility that quiescence may confer this increased efficiency. Our aim is to try to identify genes involved in the state of quiescence in human muscle progenitors. In order to reach this goal, we use two in vitro human cell culture models - the Reserve cells model and the suspension culture model of muscle stem cells. We are currently characterizing these models in terms of viability, proliferation, cell cycle profiling, myogenic markers, cell cycle regulators and expression of specific genes. Our preliminary work on reserve cells model and the suspension cultures of muscle cells showed that the cells are desmin positive, Pax7 positive, myogenin negative, MyoD negative. 90 – 92 % of the Reserve cells are in G0/G1 phase of the cell cycle. In addition a micro-array experiment comparing reserve cells, myoblasts and myotubes has been carried out and we are screening and validating the genes specifically upregulated in the reserve cells. These two in vitro models will be used to further identify, validate and characterize quiescence-induced genes.</p>

PW27-338	<p><u>A SEMI-MANUAL DEVICE FOR MULTIPLE REPETITIVE INTRAMUSCULAR CELL INJECTIONS</u></p> <p>RICHARD P-L¹, GOSSELIN C¹, PARADIS M², GOULET M², TREMBLAY JP², SKUK D² (1) Laboratory of Robotics, Laval University, Quebec, CANADA. (2) Human Genetics Unit, CHUL Research Center, Quebec, CANADA.</p>
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To contact the author::
Daniel.Skuk@anm.ulaval
.ca.

Intramuscular transplantation of muscle-precursor cells in nonhuman primates and humans requires meticulous repetitive injections. Performed with single syringes operated manually throughout large regions, the procedure takes a lot of time, becoming tiring and thus imprecise. Trying to solve this difficulty, we decided to develop a semi-manual cell injector specially conceived for this task. Diverse mechanical principles and mechatronic components were examined to choose the optimal mechanical arrangement to create a prototype. Simulations were done to verify the concept before made-up a functional prototype. First tests were carried out focusing on control, precision, mechanical behavior and robustness. Thereafter, we tested the prototype by performing intramuscular myoblast transplantations in monkeys. The prototype was able to repeatedly inject a cell suspension simultaneously through six needles or less, delivering few microliters equally through the intramuscular trajectory of each needle. The success of the graft was comparable to that previously observed in monkeys by our standard methods, but the task is facilitated by several facts. (1) Injecting through several needles at the same time accelerates the procedure. (2) The operator needs to be concentrated only in positioning the needles for each cycle of injections, since the distance of needle penetration to reach the muscle and the distance of cell delivery into the muscle can be preset in the device. (3) The course of the needles into the muscle is done automatically after pushing a button. (4) Continuous refilling of the cell suspension for each round of injections is done automatically from a container in the device. This device could be useful not only for intramuscular cell transplantations, but also for intramuscular injections of gene vectors.

PW27-339

INTRA-ARTERIAL DELIVERY OF MYOBLASTS TO SKELETAL MUSCLES IN NONHUMAN PRIMATES.

SKUK D¹, PARADIS M¹, GOULET M¹, TREMBLAY J¹

(1) Human Genetics Unit, CHUL Research Center, Quebec, CANADA.

To contact the author::
Daniel.Skuk@anm.ulaval
.ca.

The main constraint of the therapeutic strategy of intramuscularly injecting myogenic cells is that the implanted cells fuse only with the myofibers reached by the injection trajectories. An intravascular delivery of myogenic cells may be obviously a better strategy of cell delivery, but so far this strategy seemed to work only with special cells such as the so-called "mesoangioblasts". Previous experiments of intravascular delivery of myoblasts in mice produced limited results, but this animal model is quite different from human in several aspects of the myoblast transplantation biology. According to our experience, nonhuman primates are more appropriate models for human extrapolations in this field: transplantation biology and myoblast culture are very similar. Thus, we performed intra-arterial injections of beta-galactosidase-labeled allogeneic myoblasts in tacrolimus-immunosuppressed cynomolgus monkeys. The myoblast suspension was injected in one femoral artery. Some muscles were damaged with a 27G needle 3 days before or at the time of the cell infusion. Several organs and muscles were biopsied 1 hour, 1 day and 1 month post-transplantation, and the biopsies were analyzed by histology. We observed that most intra-arterial delivered myoblasts were retained mainly in the capillaries of the skeletal muscles of the leg ipsilateral to the cell injection. Scarce beta-galactosidase-positive cells were observed in the lungs only at 1 hour post-transplantation, and no beta-galactosidase-positive cells were observed in other organs (liver, brain, spleen, heart, gut) or other muscles. One month post-transplantation, beta-galactosidase-positive myofibers were observed only in the skeletal muscles of the ipsilateral leg to the injection, when these muscles were damaged at the time of the cell injection.

PW27-340

MUSCLE REGENERATION POTENTIAL OF CANINE MUSCLE-DERIVED STEM CELLS IN GRMD DOG MODEL

ROUGER K¹, DUBREIL L¹, FORNASARI B¹, DELORME B², JOUVION G¹, LEROUX-GOUBAULT I¹, LEDEVIN M¹, DESCHAMPS JY¹, CHEREL Y¹

(1) INRA, UMR 703, Ecole Nationale Vétérinaire de Nantes, Nantes, FRANCE. (2) INSERM, ESPRI EA3855, Faculté de Médecine de Tours, Tours, FRANCE.

<p>To contact the author:: rouger@vet-nantes.fr.</p>	<p>The last decade, multi-lineage stem cells encountered in various adult tissues have been described as exhibiting myogenic potential in experimental conditions. These stem cells include Side Population cells, mesoangioblasts, CD133⁺ progenitors and Muscle-Derived Stem Cells (MDSC). The last ones have been initially selected in mice as late-adherent cells, using preplating technique.</p> <p>Here, we focused on the canine MDSC isolation and their <i>in vivo</i> myogenic potential. Muscle-derived cells (MDC) were extracted from healthy dogs and submitted to classical preplating technique. MDSC were analyzed in primary and clonal cultures, and investigated for their cell cycle status, multi-lineage differentiation potential and phenotype. MDSC and myoblasts were transduced with nls <i>LacZ</i> retrovirus, then intramuscularly injected in 4-month old GRMD dogs, whom muscles show massive fiber necrosis with endomysial and perimysial connective tissue proliferation. After 5 weeks, the injected muscles were dissected and histologically analyzed for the presence of β-gal⁺ nuclei.</p> <p>We demonstrated that MDSC could be isolated in large animal model: MDSC represented 2.9% of all MDC. In addition to their proliferation/fusion behavior <i>in vitro</i>, MDSC displayed other stem cell features, including differentiation into adipogenic and osteogenic cells, G₀-cell cycle arrest and reduced expression of myogenic regulatory factors. Many thousand of β-gal⁺ nuclei are observed in MDSC-injected muscles while anyone is identified in myoblasts-injected ones: 55-64% are observed into muscle myofibers, 25-35% in satellite cell niche and ~10% in interstitial tissue. Some of MDSC in satellite cell localization display Pax7 expression, revealing that they participate to maintain the satellite cell pool of dystrophic muscle. β-gal⁺ nuclei presence is regularly associated with dystrophin expression. Also, β-dystroglycan and sarcoglycans (β and γ) are present throughout the sarcolemma of many hybrid fibers while utrophin is down-expressed.</p> <p>The outcome of our project provides novel insights into the myogenic potential of MDSC in clinically relevant context.</p>
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<p>PW27-341</p>	<p>MACROPHAGES IMPROVE CELL SURVIVAL AND MIGRATION DURING MYOBLAST TRANSFER THERAPY LESAULT PF¹, GHERARDI K¹, TREMBLAY P², CHAZAUD B¹ (1) INSERM U841, Creteil, FRANCE. (2) CRCHUL, Quebec, CANADA.</p>
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To contact the author::
benedicte.chazaud@ins
erm.fr.

Main limitations to efficient cell therapy in skeletal muscle include massive cell death, limited diffusion and poor fusion of the transplanted cells. Numerous attempts of myogenic cell transplantation in skeletal muscle have been performed in both animals and humans. Massive death of transplanted cells is observed even in autologous grafts or immunosuppressed recipients and is distinct from rejection. Acute deprivation in survival cues likely participates to massive death of myogenic cells that are mass-injected. They therefore have lost their privileged relationship with their stromal environment.

We have previously shown that macrophages are important for skeletal muscle regeneration and may exert beneficial effects on myogenic cell growth through mitogenic and anti-apoptotic activities. Our aim was to evaluate the potential beneficial effect of co-injection of macrophages on myogenic precursor cell (mpc) survival and migration after transplantation into mdx skeletal muscle.

Co-injection of macrophages with mpcs increased in a dose-dependent way the signal associated with mpcs, indicating a beneficial effect of macrophages on myogenic cell survival. This has been evaluated by using [¹⁴C]thymidine loaded mpcs and mpcs isolated from Tg:CAG-GFP mouse. After injection of GFP-mpcs with macrophages, the GFP signal even increased with time, suggesting a beneficial effect of macrophages on proliferation of myogenic cells, as we have previously demonstrated in vitro.

We have also shown that coinjection of macrophages with mpcs stimulated their migration within the muscle tissue, in a dose-dependent manner.

These data show that co-injection of macrophages improve myogenic precursor cell transplantation by several effects: 1) limitation of mpc post-transplantation mortality; 2) stimulation of mpc proliferation; 3) stimulation of mpc migration within the muscle tissue. Macrophages may be therefore used as a stromal adjuvant for cell therapies.

PW27-342

RHOE CONTROLS MYOBLAST ALIGNMENT PRIOR FUSION THROUGH RHOA AND ROCK

FORTIER M¹, COMUNALE F¹, KUCHARCZAK J², BLANGY A¹, CHARRASSE S¹, GAUTHIER ROUVIERE C¹

(1) Universités Montpellier 2 et 1, CRBM, CNRS, UMR 5237, IFR 122, Montpellier, FRANCE. (2) IBCP UMR 5086 CNRS Université de Lyon, Lyon, FRANCE.

To contact the author::
mathieu.fortier@crbm.cn
rs.fr.

Differentiation of skeletal myoblasts into multinucleated myotubes is a multi-step process orchestrated by several signaling pathways. The Rho small G protein family plays critical roles both during myogenesis induction and myoblast fusion. We report here that, in C2C12 myoblasts, expression of RhoE, an atypical member of this family, increases until the onset of myoblast fusion before resuming its basal level once fusion has occurred. We show that RhoE accumulates in elongated, aligned myoblasts prior fusion and that its expression is also increased during injury-induced skeletal muscle regeneration. Moreover, although RhoE is not required for myogenesis induction, it is essential for myoblast elongation and alignment before fusion and for M-cadherin expression and accumulation at the cell-cell contact sites. Myoblasts lacking RhoE present defective p190RhoGAP activation and RhoA inhibition at the onset of myoblast fusion. RhoE interacts also with the RhoA effector ROCK1, whose activity must be down-regulated to allow myoblast fusion. Consistently, we show that pharmacological inactivation of RhoA or ROCK restores myoblast fusion in RhoE deficient myoblasts. RhoE physiological up-regulation before myoblast fusion is responsible for the decrease in RhoA and ROCK1 activities, which are required for the fusion process. Therefore, we conclude that RhoE is an essential regulator of myoblast fusion.

PW27-343

SATELLITE CELL HETEROGENEITY BOTH BETWEEN, AND WITHIN, MUSCLES

ONO Y¹, ZAMMIT P¹

(1) King's College London, London, UNITED-KINGDOM.

<p>To contact the author:: peter.zammit@kcl.ac.uk.</p>	<p>Homeostasis, hypertrophy and repair of adult skeletal muscle are carried out by resident stem cells called satellite cells, located on the surface of the myofibre, below the ensheathing basal lamina. Normally mitotically quiescent, satellite cells must first be activated to undergo extensive proliferation to generate myoblasts that eventually differentiate to repair/replace myofibres. Satellite cell self-renewal is the primary mechanism responsible for maintaining a viable satellite cell pool. This process can be modelled in culture where satellite cell progeny adopt divergent fates. Quiescent satellite cells express Pax7, and when activated, co-express Pax7 with MyoD. After proliferation, most then down-regulate Pax7 and differentiate. In contrast, other satellite cell progeny maintain Pax7 but lose MyoD and withdraw from both cell cycle and immediate myogenic differentiation, returning to a quiescent-like state.</p> <p>Here we have explored functional heterogeneity both between, and within, the satellite cell pool of specific muscles; namely the extensor digitorum longus (EDL) and soleus in the hindlimb, the extensor carpi radialis longus (ECRL) in the forelimb, and the masseter in the head. Satellite cell numbers and differentiation potential are higher in the Soleus than the EDL, which in turn is higher than the masseter. Even within a specific muscle, there is a large range of proliferative potential and self-renewal capacity. In summary, the work to be presented demonstrates marked functional heterogeneity amongst satellite cells.</p>
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<p>PW27-344</p>	<p><u>TRACKING OF A LIVE POPULATION OF IMMORTALIZED MYOBLASTS BY CONFOCAL MICROSCOPY.</u> DUGUEZ S¹, RAJA B², PARTRIDGE T¹ (1) Center for Genetic Medicine, Children's Research Institute, Children's National Medical Center,, Washington DC, USA. (2) Flow Cytometry Facility, Children's Research Institute, Children's National Medical Center, Washington DC, USA.</p>
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<p>To contact the author:: sduguez@cnmcresearch.org.</p>	<p>Cell proliferation is an important parameter in the behavior of many cell types. Fluorescence activated cell sorting (FACS) in combination with cell tracker dyes allows tracking of cell divisions over time but is restricted to large numbers of dead (fixed) cells. We sought to develop a method by which cell division in a single small population of live cells could be followed. Current cell tracker dyes such as carboxyfluorescein diacetate succinimidyl ester (CFSE) label cell volume uniformly and are fully retained over time, segregating between daughter cells in proportion to cytosolic volume. Equal distribution of cytosolic volume between daughter cells results in halving of fluorescence intensity per cell per generation. Use of FACS to distinguish cells by size, complexity, and fluorescence intensity enables determination of generation time, proliferation index, and division index. Confocal microscopy allows accurate measurement of fluorescence intensity but intensity varies with cell volume. We used two cell trackers, CFSE and CMRA orange, to measure both dilution of labeling and final cell volume. This facilitated measurement of number of divisions. The confocal method was validated by a complementary FACS analysis. We applied this method to the H2K immortalized myoblast cell line and determined a highly synchronous generation time of 22.45 ± 1.21h. We were able to follow cell division out to four days. This method facilitates the tracking of cell division in a small population of live cells over time.</p>
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<p>PW27-345</p>	<p><u>EVALUATION OF THE AMPLIFICATION OF MYOBLASTS ON MICROCARRIERS</u> JENNY C¹, ALBERT V¹, VOVARD F¹, DENEFFLE P¹, MERTEN OW¹ (1) GENETHON, Evry, FRANCE.</p>
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<p>To contact the author:: jenny@genethon.fr.</p>	<p>Myogenic progenitor cells are promising tools for cell therapy to treat neuromuscular disorders. But a challenging question is their amplification to a satisfying scale for clinical applications.</p> <p>Until now, most of myogenic progenitor cells have been cultivated on plastic surfaces as they are naturally adherent cells. The scaling up of such procedures results in the multiplication of culture surface (eg. Cell Factories), which will be rapidly limited. As myogenic progenitor cells are not able to grow in suspension, we have decided to test an alternative strategy to grow adherent cells in suspension: use of microcarriers.</p> <p>Human myoblasts (CHQ5B) have been chosen as a study model. Different microcarriers (Cytodex, Cultispheres, ...) have been tested in order to find the surface best adapted for cell growth. The selected microcarriers (Cultispheres) are entirely made from gelatine and thus can be degraded by an enzymatic means (trypsin, collagenase...) and cells are collected by a simple centrifugation step. Preliminary results indicate that by using a 50ml spinner flask, an amplification of 1 week allows the production of $15 \cdot 10^6$ cells (starting with an inoculum of $2.5 \cdot 10^6$ cells). Further studies will address scale-up issues for getting 10^8 cells / spinner run. These amplified cells are characterized for their myogenic potential.</p>
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<p>PW27-346</p>	<p><u>CELL AUTONOMOUS ROLE FOR ATRX IN MYOBLAST SURVIVAL AND EXPANSION</u> HUH M¹, GRENIER G², RUDNICKI M¹, PARKS R¹, PICKETTS D¹ (1) Ottawa Health Research Institute, Ottawa, CANADA. (2) University of Sherbrooke, Sherbrooke, CANADA.</p>
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To contact the author::
dpicketts@ohri.ca.

Boys born with the ATR-X syndrome have severe skeletal muscle weakness and hypotonia at birth. The resulting muscle weakness delays developmental milestones such as sitting, standing, and walking and many never become ambulatory during their life. To investigate the role of ATRX in muscle development a Cre-loxP approach was used to inactivate the ATRX gene specifically in skeletal muscle.

ATRX^{f/y}:Myf5Cre^{+/-} mice were born with a reduced muscle mass, smaller fibres, and a normal number of satellite cells. Primary myoblasts from *ATRX^{f/y}:Myf5Cre^{+/-}* muscle failed to proliferate in culture suggesting a defect in satellite cell activation or proliferation. To further characterize ATRX function, we infected adult derived *ATRX^{f/y}* primary myoblasts with Cre recombinase expressing adenovirus (*ATRX^{f/y}:Ad-Cre*). *ATRX^{f/y}:Ad-Cre* myoblasts display a lower proliferative capacity than *ATRX^{f/y}:Lac-Z* controls in high mitogen growth media. Despite their inability to expand in culture, ATRX deficient primary myoblasts were capable of sequentially expressing the early and terminal markers myogenin and Myosin Heavy Chain. The activation and proliferative potential of ATRX deficient myoblasts were tested *in vivo* by cardiotoxin (CTX) muscle injury experiments. CTX treated *ATRX^{f/y}:Myf5Cre^{+/-}* mice demonstrated a poor ability to regenerate, highlighted by fewer numbers of centrally located nuclei and the presence of improperly regenerated fibrotic tissue. Taken together, ATRX is required for the expansion of satellite cells that is necessary for the growth and regeneration of skeletal muscle.

PW27-347	<p><u>RGD-COUPLING TO COLLAGEN SCAFFOLD IMPROVES HUMAN MYOBLAST MIGRATION AND DIFFERENTIATION: NEW POSSIBILITIES FOR HUMAN SKELETAL MUSCLE ENGINEERING</u> COIRAULT C¹, MAMCHAOUI K², ALLAMAND V¹, MOULY V², BONNE G¹ (1) INSERM U582, Paris, FRANCE. (2) Inserm UMRS 787, Paris, FRANCE.</p>
<p>To contact the author:: c.coirault@institut-myologie.org.</p>	<p>There is increasing agreement that 3D culture of skeletal muscle satellite cells and myoblasts holds great promise to investigate pathophysiological mechanisms and to test therapeutic strategies for neuromuscular disorders. However, the construction of functional, tissue-engineered skeletal muscles (TESM) containing more than a few layers of mature muscle cells remains challenging. Obvious concerns relate to incomplete myotube differentiation, impaired nutrient diffusion and limited cell viability in bioartificial constructs. Because the Arg-Gly-Asp (RGD) motif of fibronectin plays a critical role in cell-extracellular matrix interactions through a specific interaction with integrins $\alpha 5\beta 1$, we hypothesized that TESM development could be improved by coupling RGD peptides to collagen scaffold. The method used for the RGD-collagen scaffold coupling allows i) high coupling yields and complete washout of excess reagent and by-products with no need for chromatography; ii) spectroscopic quantification of RGD coupling; iii) a spacer arm of 36 angstroms, a length reported as optimal for RGD peptide presentation and favorable for integrin receptor clustering and subsequent activation. A nonfunctional RGE peptide was used as control. Collagen-cross-linked RGD or RGE scaffolds were seeded with immortalized human myoblasts (hMS, clone 9) and incubated in proliferative and then differentiation medium. Morphological analysis demonstrated that both collagen scaffolds have the ability to support hMS cell proliferation and differentiation. However, the presence of RGD improved cell attachment by activating $\alpha 5$ integrin signalling pathway, enhanced proliferation and cell migration within the matrix, and enhanced differentiation of myotubes. In conclusion, we report a novel method of engineering a highly effective and stable mature tissue-engineered human skeletal muscle based on collagen matrix cross-linked to RGD peptides. It should provide a useful tool for basic research and for identifying new therapeutic strategies in human myopathies.</p>

