Cycling cardiomyocytes along the E2F pathway for regeneration and preservation of myocardium.

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The E2F pathway governs a cell`s decision to proliferate, die or differentiate. The E2F pathway consists of nine family members together with pocket proteins (Rb, p107, p130) which critically regulate the cardiac cell cycle and development. The importance of the E2F pathway is exemplified by the fact that mutations in genes that constitute this pathway lead to 40% of all human proliferative disease. Since the heart continues to grow after birth, there has been much interest in harnessing the capacity of this pathway in preservation and regeneration of postnatal myocardium in aging and injury. However a major limiting issue has been the development of appropriate animal models to study the E2F pathway in postnatal myocardium as targeted gene deletion of molecular components have presented with either embryonic lethality or redundancy. We have developed a mouse model where we specifically expressed E2F6 in postnatal myocardium to modulate the E2F pathway via its unique repression properties. These mice live to develop a dilated cardiomyopathy in the absence of any hypertrophic growth or programmed cell death. Significant pressure on cardiac cell cycling is imposed by E2F6 such that adaptive cues for tissue preservation as well as differentiation are instigated with remarkable precision. Novel crosstalk of the E2F pathway with signaling mechanisms that impact major programs including metabolism, mechanical activity and cell death are revealed in the postnatal myocardium with E2F6 and E2F3 as the major directors.

Simple 3D direct laser writing for tissue engineering

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One of the main goals in tissue engineering research is to reproduce in vitro structures, mimicking the extracellular matrix (ECM), able to favorite the stem cell differentiation and the tissue growth. A 3D porous structure suitable to the scope is called scaffold. [1,2] The main concerns in 3D photolithographic applications is to restrict the polymerization to a well-defined volume in order to achieve the desired resolution. The vertical resolution is often achieved exploiting two photon absorption (2PA) due to the dependency from the square of the light beam intensity. That requires the use of ultrafast-pulsed lasers able to concentrate enough energy in a well-confined spatio-temporal volume.
We propose a simple and flexible process for 3D scaffold fabrication based on photolithography using one photon polymerization by a blue diode laser. The excitation wavelength falls on the tail of the absorption of the photopolymerizable PEGDA solution. Consequently, the efficiency of the photopolymerization activation is strongly reduced. The intensity of the light reduces by a factor 5 within a penetration depth equal to the depth of focus of the optical system. That sets the limit for the active volume to the minimal waist of the focused beam. The threshold for polymerization is not reached outside that volume where the intensity is not enough to trigger the process.
Evidence of cell differentiation using human Lin- Sca-1+ cardiac progenitor cells has been shown in absence of any concurring biochemical stimulus using woodpile scaffolds fabricated by this technique.[3]

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[2] P. Prosposito et al. (2017) Materials Science Forum, 879:1519-1523
[3] M.Ciocci et al, (2017) Stem Cell Dev. [Epub ahead of print, July 17th](2017)

Therapeutic potential of scleraxis in fibrosis and wound repair

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Repair of damaged tissue requires a carefully orchestrated interplay of inflammatory and reparative cells and processes. Impairment of these processes leads to wounds that fail to heal, or to excessive healing marked by tissue fibrosis as excessive extracellular matrix (ECM) is synthesized. Evidence from our lab and others has implicated the transcription factor scleraxis as a key player in controlling this balance during wound healing in the heart and other tissues. Our central hypothesis is that scleraxis expression and activity is induced in response to mechanical injury or stress, and in turn governs the activation of reparative cells such as fibroblasts while simultaneously regulating ECM gene expression.

Over-expression of scleraxis can accelerate and strengthen tendon wound healing, consistent with scleraxis' role regulating ECM expression. In contrast, our data has demonstrated that scleraxis plays an important role in cardiac fibrosis, and that this effect is also due to scleraxismediated
ECM gene up-regulation. We found that several key ECM genes are direct
transcriptional targets of scleraxis, including collagen Ia2, fibronectin, vimentin and matrix metalloproteinase 2. Significantly, scleraxis governs the conversion of fibroblasts to pro-fibrotic myofibroblasts and a-smooth muscle actin expression, and blockade of scleraxis attenuates this conversion. Scleraxis knockout results in the loss of 50% of both ECM and cardiac fibroblasts, confirming its critical role in ECM homeostasis. Scleraxis is sufficient to induce epithelial-tomesenchymal
transition of A549 epithelial cells – a key finding, since such transition is the developmental source of cardiac fibroblasts. Scleraxis expression is significantly elevated in both the cardiac infarct scar and following pressure overload, and we found that scleraxis gene deletion attenuates cardiac fibrosis in vivo.

Scleraxis thus appears to be an excellent candidate for therapeutic manipulation – however, the benefit of induction or blockade of scleraxis expression is highly dependent upon the specific tissue and pathology in question.

Micro-fractured Adipose Tissue for Cartilage Regeneration

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Cartilage is a very precious tissue and, till now, no effective treatment has been developed for arthritis-associated and injury-mediated cartilage loss. Researcher attention has recently been focused on adult mesenchymal stem cell therapy. In particular, the adipose tissue seems to be a very promising source to obtain adipose-derived stem cells (ASCs), pluripotent cells, with an extensive proliferative capacity and the ability to multidifferentiate. The procedure is easy and safely, not causing much patient discomfort, and yields significantly more stem cells than bone marrow. To meet the clinical need to work with minimally manipulated tissues, a new technology, based on mild mechanical tissue cluster size reduction in a completely closed system, has been developed. It has been demonstrated that the obtained clusters maintain the stromal/vascular tissue architecture and ASCs can be obtain by in vitro culturing. Even if clinically promising results have been obtained, some issues still need to be addressed, such as: cellular types present in the micro-fractured adipose tissue, and if cartilage regeneration is mediated by the cellular direct engraftment and differentiation or by the effect of their secreted factors or by a combination of the two. Our aim is to completely characterized the micro-fractured adipose tissue, as clusters and after centrifugation (to separate the lipid-derived part from the "serum" part), evaluating the cellular components and their properties and the secreted factors and their functions by in vitro and ex vivo analysis. Moreover a multitextured 3D scaffold-in-scaffold to favor cell-scaffold interactions and drive cell chondrogenic differentiation and flexible and biocompatible electrode arrays to analyze the dynamics of the electric/magnetic field self-generated by differentiating ASCs will be developed. The maps of the self-generated electric/magnetic field could represent a reliable non-destructive tool to be used in clinic to monitor cell differentiation during intensive tissue fabrication processes.

Improvement of iPSC-derived cardiomyocyte survival and integration into a pre-clinical model of MI using isolated microvessels significantly recovers cardiac function

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While human stem cell-derived cardiomyocytes (hSC-CMs) are a promising source for CMs for tissue regeneration post-myocardial infarction (MI) its poor survival post-transplantation hinders its application. Death of transplanted CMs occurs in the first few days post-transplantation due to ischemia. Thus, attempts have been made to promote blood perfusion (i.e. addition of endothelial cells and/or angiogenic factors). However, these approaches require weeks for new vessels to form and to carry blood compared to the rapid death of transplanted CMs (2-3 days). We developed an innovative strategy using ready-made microvessels from adipose tissue to form a vasculature and to carry blood within the first days post implantation. We co-implanted hiPSC-CMs and microvessels into the hearts of male immuno-compromised rats that underwent left anterior descending artery (LAD) ligation to mimic MI. Compared to hiPSC-CM transplantation alone, microvessels promoted a ~400% increase in hiPSC-CM survival. Echocardiography and pressure–volume (PV) loop analysis performed 4 weeks post-MI revealed that hiPSC-CM transplantation attenuated post-infarct ventricular dilation and enhanced left ventricular contractility by demonstrating a significant improvement in fractional shortening (FS), ejection fraction (EF) as well as other functional parameters (end-systolic volume, end-diastolic volume, dP/dt max and min, Tau). Remarkably, co-transplantation of hiPSC-CMs with microvessels showed significantly superior functional recovery compared to hiPSC-CMs alone in all the parameters assessed. Given the ability to use microvessels harvested from adipose tissue from patients, this study presents a new, personalized approach to cell-based therapy for heart failure post-MI whereby improving hiPSC-CM survival it is possible to recover heart function.

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Platforms for growth of tissues and bio-mimetic ceramics by the engineering of Carbon nanostructures

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The need for highly performing functional interfaces to produce platforms, grafts and scaffolds for regenerative medicine is rapidly increasing and requires advanced tools to improve and maintain tissue function . Most tissues are not able to regenerate, following a disease or injury especially if defects are large where the natural healing process cannot bridge the gap without the presence of such supporting scaffolds. In the last years it has been demonstrated that the engineering of Carbon nanostructures allows to produce 3D frameworks with multivalent architectures able not only to assist cell proliferation/differentiation and to organize the cells into ordered assemblies, but also to supply a rigid support for tissue growth. Carbon nanotubes , dendrimers, graphenes and nanodiamonds, characterized by good biocompatibility, high stability and long-time reliability, are now being proposed to solve the task of producing scaffolds/platforms suitable for tissue engineering and generation of artificial organs. As regards the nanodiamonds, this material has proved not only the ability to increase adhesion and growth of several cell lines, but also to catalyze nucleation and growth of calcium phosphates. This last property has been tested by growing crystalline synthetic carbonate-hydroxyapatite (CHA), a biomimetic material able to promote the osteointegration in bones and dental tissues, using a series of nanodiamond active platforms immersed in simulated body fluid solution (SBF).
The shaping of nanoC-based materials able to mimic the nanostructures of natural extracellular matrices is obtained in our labs by the integration of several CVD growth techniques, of MW-RF plasma sculpturing, and of wet chemistry processes.
The control of surface chemistry of the C nanostructures, that can also selectively bind various biological molecules, offers an added value for interactions with biological systems.
This presentation will illustrate some relevant synthetic strategies for the engineering of nanocarbon-based platforms and some examples of multivalent systems for bio-related applications.

Allogeneic mesenchymal stem cell therapy for cardiac regeneration

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INTRODUCTION: Bone marrow derived allogeneic mesenchymal stem cells (MSCs) from young and healthy donors are immunoprivileged. The outcome of animal studies and initial clinical trials suggested that transplanted MSCs were safe and improvement in the heart function was observed. However, long term fate of implanted cells in these trials was not determined. We recently reported that MSCs lost their immunoprivilege late after implantation in the ischemic heart and were rejected. Current study reveals the mechanisms responsible for post-transplantation rejection of allogeneic MSCs in the heart. METHODS/RESULTS: We found that immunoprivilege of allogeneic MSCs is mediated by a soluble factor prostaglandin E2 (PGE2), the levels of PGE2 decreased in rat MSCs after exposure to hypoxia/ischemic conditions which was associated with loss of immunoprivilege. We found that hypoxia exposed MSCs were prone to rejection by allogeneic T cells in the in vitro co-culture. MSCs immunoprivilege is established by the absence of MHC-II molecules. Our data suggest that MHC-II expression increased in hypoxia exposed MSCs. PGE2 treatment of hypoxic MSCs decreased MHC-II expression and preserved immunoprivilege. In a rat myocardial infarction model, allogeneic MSCs (3Å~10$_6$ cells/rat), with or without a biodegradable hydrogel that slowly released PGE2, were injected into the infarct region. Five weeks later, MSCs were rejected in the control group, but in PGE2-treated group, significant number of cells survived and heart function were significantly improved. CONCLUSIONS: The immunoprivilege of allogeneic MSCs is maintained by PGE2 mediated downregulation of MHC-II levels, exposure to hypoxia/ischemia decreased PGE2 and increased MHC-II levels that was associated with loss of immunoprivilege and rejection of MSCs. Maintaining PGE2 levels preserved immunoprivilege and restored cardiac function after an MI.

The clinical application of autologous bone marrow mononuclear cell therapy for ischemic cardiovascular disease.

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Direct stem cells application has the potential to stimulate tissue regeneration in a paracrine and/or autocrine manner; thus, they have been extensively studied as candidate cell sources for cardiovascular regeneration. Several preclinical and clinical studies addressing the therapeutic potential of autologous bone marrow mononuclear cell in limb ischemia and myocardial infarction. However, clinical studies using stem cell therapy approaches have produced mixed results.
In our clinical trials, we are analyse of factors associated with the therapeutic benefit of cell therapy in "no-option"patients with critical limb ischemia and validating intramyocardial bone marrow stem cell therapy in combination with coronary artery bypass grafting. We examined which properties of bone marrow mononuclear stromal cells are relevant for responding and non-responding patients. We suggest that the quality cells shown in the expression of cell surface markers and specific genes expression plays an important role in therapeutic outcome. We believe that paracrine mechanisms are main drivers in the induction of reparatory processes in ischaemic patients and differences in stem cell properties are relevant in relation to their involvement in the effectiveness of reparatory process.
In this way our trial, besides providing evidence regarding the impact of surgical stem cell therapy on patients' functional and clinical outcome, helps to further standardize the GMP produced, autologous cell product and to answer questions raised by the basic research.

Autologous Bone Marrow-Derived Cell Therapy Combined With Physical Therapy Induces Functional Improvement in Chronic Spinal Cord Injury Patients

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Spinal cord injuries (SCI) cause sensory loss and motor paralysis and are treated with physical therapy, but most patients fail to recover due to limited neural regeneration. Here we describe a strategy in which treatment with autologous adherent bone marrow cells is combined with physical therapy to improve motor and sensory functions in early-stage chronic SCI patients. In a phase I/II controlled single-blind clinical trial (clinicaltrials.gov identifier: NCT00816803), 70 chronic cervical and thoracic SCI patients with injury durations of at least 6 months were treated with either intrathecal injection(s) of autologous adherent bone marrow cells combined with physical therapy, or with physical therapy alone. Patients were evaluated with clinical examinations, electrophysiological somatosensory evoked potential, MRI imaging, and functional independence measurements. Chronic cervical and thoracic SCI patients treated with autologous adherent bone marrow cells combined with physical therapy showed functional improvements over patients in the control group treated with physical therapy alone, and there were no cell therapy-related side effects. At 18 months posttreatment, 23 of the 50 cell therapytreated
cases (46 percent) showed sustained improvement using the American Spinal Injury Association (ASIA) Impairment Scale (AIS). Compared to those patients with cervical injuries, a higher rate of functional improvement was achieved in thoracic SCI patients with shorter durations of injury and smaller cord lesions. Therefore, when combined with physical therapy, autologous adherent bone marrow cell therapy appears to be a safe and promising therapy for patients with chronic spinal cord injuries. Randomized controlled multicenter trials are warranted.

Role of Bone-Marrow Derived Stem Cells in Liver Regeneration: a Multicentre Clinical Trial

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Background and study aims: Liver transplantation represents the only definitive treatment for cases of endstage liver failure. However, this procedure is hindered by a number of obstacles, namely, the marked shortage of liver donors, major operative procedures and lifelong immune suppression, in addition to the high expenses. Regenerative medicine, based on cellular approach for repairing and replacing damaged tissues and organs, is a rapidly growing field of medicine. Due to the heavy burden of liver diseases in Egypt, this study was designed to evaluate the efficacy of cellular therapy in the form of hepatocytes derived from patients' own haematopoietic stem cells (HSCs) transplanted directly through intra-splenic injections in patients with liver cirrhosis grade B and C Child-Turcotte-Pugh score (CTP B).

Participants and methods: 100 patients with liver cirrhosis CTP B score were divided into two groups according to the principle of treatment. Group (A) consisted of 50 patients (25 Child B and 25 Child C), who received hepatocytesderived from patients own (HSCs) in addition to conventional treatment. Group (B) received regular conventional treatment. Both groups of patients were followed up for six months after transplantation for assessment of liver functions.

Results: There was a significant improvement in the degrees of ascites, lower limb edema, HE, CTP scores and MELD scores in patients treated with hepatocytes derived from HSC. Also, we observed a slight improvement in serum albumin, prothrombin concentration and international normalized ratio in stem cell treated group. No procedure related complications were encountered

Conclusion: We demonstrated the safety and short term efficacy of autologous bone marrow derived hepatocyte transplantation for the support of cirrhotic liver.

Key words: Haematopoeitic stem cells; transplantation; liver cirrhosis.

Stem cell therapy for diabetic heart

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Repair and regenerative capability of bone marrow mesenchymal stem cells (BM-MSCs) is influenced by ability of homing and engraftment. Stromal cell-derived factor-(SDF-)1 / C-X-C motif chemokine receptor type 4 (CXCR4) axis is one of the commonest axes regulating stem cells homing as well metastatic behavior and survival of cancer stem cells. Metformin could interfere with CXCR4 expression thus regulating growth and viability of some cancer stem cells, which prompted us to investigate the efficacy of BM-MSCs administered in diabetic patients receiving metformin. Diabetes was induced by intraperitoneal (IP) injection of 52.5 mg/kg streptozotocin (STZ). Rats were divided to 4 groups: group 1; control; group 2, diabetic (D); group 3, diabetic treated with BM-MSCs (D-BM-MSCs) and group 4, diabetic treated with metformin and BM-MSCs (D-Met-BM-MSCs). At baseline, 10 weeks after DM and 4weeks after BM-MSCs treatment echocardiography was done for all groups. At the end of the study, heart tissues were collected for pathological examination. Result: metformin did not improve cardiac functions neither EF% nor FS% were significantly different from D group. After BM-MSCs transplantation cardiac function in D-Met-BM-MSCs remained reduced while BM-MSCs transplantation in D group that didn't receive metformin significantly improved. Histological examination for hearts showed significant decrease in area % of fibrosis in D-BM-MSCs versus D-Met-BM-MSCs, a significant decrease in area % of both SDF-1 and CXCR4 was observed in D-Met-BM-MSCs that was associated with a significant increase in area % of AMPK in D-Met-BM-MSCs versus D-BM-MSCs group which did not correlate with SDF1. Conclusion: therapeutic efficacy of BM-MSCs in cardiac regeneration is attenuated by chronic administration of metformin in diabetics by a mechanism that is not dependent on AMPK.
Key words: metformin, diabetic cardiomyopathy, BM-MSCs, homing, SDF-1, CXCR4.

Developmental expression of the cardiac BK_{Ca} channel and its role in cardioprotection.

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Our previous immunochemical and transcript analyses indicate that the large conductance calcium-activated potassium channel (BK_{Ca}) is present in cardiac mitochondria and encoded by the Kcnma1 gene. In addition, studies using a BK_{Ca} opener, NS1619, point to the view that opening BK_{Ca} expressed in mitochondria (mitoBK_{Ca}) contributes to cardioprotection after ischemic insult by modulating mitochondrial function. We have discovered that mitochondria from hearts preconditioned with NS1619 showed a significant increase in CRC from 140+11 to 280+23 nmoles/mg (n=3) in wt mice but no significant effect was observed in Kcnma1$^{-/-}$ mice (120+23 and 100+11 nmoles/mg, n=3) showing that after ischemic insult the protective effect of NS1619 requires expression of Kcnma1 gene. Likewise, mitochondrial ROS production stimulating complex I with 3 mM glutamate/malate was reduced by NS1619 preconditioning in the wt mice from 159+13 to 113+7 pmoles/min/mg but not in the Kcnma1$^{-/-}$ mice (n=3). In cardiomyocytes, BK_{Ca} is believed to be exclusively present in the inner membrane of mitochondria and combination of electrophysiology and immunochemical approaches could not detect BK_{Ca}-mediated currents. For the first time, we have established that in neonatal cardiomyocytes isolated from P3 pups, exhibit BK_{Ca}-mediated currents. These currents are reversibly sensitive to iberiotoxin (BK_{Ca} antagonist), and immune cytochemical approaches corroborate the presence of BK_{Ca} in the plasma membrane. Our results confirm that cardiac BK_{Ca} is encoded by Kcnma1 gene and its localization varies during the development of the heart.

Co-Transplantation of Liver Sinusoidal Endothelial Cells, Kupffer Cells and Hepatocytes Improved Cell Engraftment and Repopulation in the Mouse Liver

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Maintenance of hepatic homeostasis requires tightly regulated interactions among liver cell types. For instance, liver sinusoidal endothelial cells(LSECs) with Kupffer cells(KCs) serve major roles via paracrine signaling to influence other cells, including hepatocytes(Hep), which helps in induction and completion of liver regeneration. After cell transplantation, multiple cell types are activated to regulate engraftment and proliferation of transplanted Hep. Moreover, disruption and activation of LSECs, or modulation of KCs may facilitate cell engraftment. Therefore, to develop further insights into cell-cell interactions in the cell transplantation setting, we transplanted liver cells in combination and examined the potential of this "niche" in liver repopulation. Recipient DPPIV- mice were preconditioned with monocrotaline(MCT)±gadolinium(GdCl3) before cell transplantation. Hep were isolated from C57Bl/6 mice, KCs and LSECs from DPPIV- mice. Preconditioning-induced hepatic changes were analyzed by gene expression. Liver injury was evaluated by ALT/AST. Engraftment was evaluated by immunohistochemistry 7-30days after transplantation. Transplanted cell proliferation was studied in mice preconditioned with MCT plus Rifampicin and Phenytoin and livers were analyzed after 6wks. In preconditioned mouse liver, qPCR showed greater IL6 and IL1β expression vs controls with 5-and 4-fold increases, respectively, 30h after MCT+GdCl3 injection followed by decreases at 48h. Similarly TGF-β expression was upregulated at 30h and remained high even at 72h. AST levels increased at 48h and decreased at 72h. With MCT+GdCl3 preconditioning, Hep engraftment was superior. However, when Hep, KCs and LSECs were transplanted together, Hep engraftment increased significantly vs controls up to 2-fold after 1wk and 1 mo. This superior engraftment was maintained in mice preconditioned with MCT, rifampicin and phenytoin. Cotransplantation of LSECs, KC and Hep was superior to transplantation of Hep alone. This ability to better reconstitute the liver by cotransplantation of LSECs, KCs and Hep will be helpful for basic studies of cell-cell interactions and advancing protocols for liver cell therapy.

The physiopathologic role of tissue niche in muscle regeneration

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The prolongation of skeletal muscle strength in neuromuscular disease has been the objective of numerous studies employing a variety of approaches. Stem cell therapy represents a promising tool to cure genetic diseases. However, this approach is not definitive yet and several hurdles limit the immediate translation of this strategy into clinic.
One of the crucial parameters of tissue regeneration is the microenvironment in which the stem cell populations should operate. Stem cell microenvironment, or niche, provides essential cues that regulates stem cell proliferation and that directs cell fate decisions and survival. It is therefore plausible that loss of control over these cell fate decisions might lead to a pathological transdifferentiation and contribute to the exacerbation of a pathologic condition, such as muscular dystrophy.
Among critical parameters, the activation and persistence of inflammatory and fibrotic pathways may render the dystrophic muscle incapable to sustain and complete an efficient muscle regeneration, leading to a progressive loss of muscle tissue due to chronic degeneration of muscle and to the exhaustion of satellite cells that replace damaged fibers. Indeed, the progressive loss of tissue function and integrity observed in dystrophic muscles are the eventual consequences of a history of continuous rounds of degeneration and regeneration.
Specific factors are required to trigger stem cells toward a specific lineage, to improve their survival, and to render them effective in contributing to tissue repair. Studies on stem cell niche leaded to the identification of critical players and physiological conditions that improve tissue regeneration and repair.
Preliminary evidences demonstrated that the local form of Insulin-like Growth Factor–1 (mIGF-1) sustains muscle hypertrophy and regeneration in senescent skeletal muscle, enhances the recruitment of circulating stem cells in injured muscle and counteracts muscle wasting in mdx dystrophic mice, reducing the inflammatory response and improving muscle mass and strength and elevating pathways associated with muscle survival and regeneration. Among the factors modulated by mIGF-1, we observed a specific down-regulation of the inflammatory cytokines IL-6, which has been associated with the switch from acute to a chronic inflammatory response that therefore can exacerbate the dystrophic phenotype. We will discuss the role of mIGF-1 and IL-6 in the modulation of muscle regeneration under physiological and pathologic conditions.

Biomaterials for tissue engineering at the Research Consortium Hypatia

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Hypatia is a research consortium established in 2008 by the encounter between universities, research institutes and enterprises with the aim to promote, develop and enhance scientific, technological and human resources of the territory. Biomedical research activities are mainly focused on biomaterials science and on the development of scaffolds for tissue engineering and regenerative medicine applications. In this framework, one of the main issues to be addressed is to deal with suitable microarchitectures, mimicking the natural extra-cellular matrix (ECM) of the tissue to be healed and capable to effectively contribute to its regeneration and host integration. For this aim, electrospinning can be regarded as a valuable technique, allowing a wide range of polymers to be processed into micro- and nanofibres with targeted properties for the intended use. Structural properties of the collected scaffolds can furnish specific cues to the seeded cells, dictating their response. However, it should be underlined that this characteristic, even if pivotal, is just a partial approach to the problem as ECM is also a complex mixture of biochemical signals which defines a unique tissue-specific microenvironment.
The research activity is aimed at fabricating and evaluating electrospun scaffolds including selected single polymers (natural or synthetic ones), blends or composites in order to provide substrates with tailored mechanical properties. With the aim to design active devices, mats are also collected as delivery systems loaded with drugs or growth factors to be topically released. The potential of the electrospinning technique to realize complex and functional threedimensional structures is investigated as well. In this regard, tubular scaffolds for vascular graft applications and heart valve prototypes can be readily produced and tested into cardiovascular simulators.
As a next step, the additive manufacturing approach will be combined with electrospinning in order to develop innovative engineered devices.

EVALUATION OF THE HDAC INHIBITOR SULPORAHANE AS A POTENTIAL MOLECULE FOR GENERATING INDUCED PLURIPOTENT STEM CELLS

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Cell reprogramming is done by somatic cell nuclear transfer techniques; establishment of immortal pluripotent cell lines by fusion of embryonal carcinoma cells (ECCs) with somatic cells and most popular methods for generating induced pluripotent stem cells by a variety of techniques e.g.ectopic expression of a number of molecules including genes (e.g. OCT-4, Sox-2, NANOG, Klf-4, c-Myc etc) & small molecules (e.g.HDAC inhibitors). The IPSC generation, evaluation and application methodologies are highly developed recently. Histone deacetylase inhibitors (HDACi) play crucial pleotropic roles on cellular homeostawsis and may control/ promote the self renewal /pluripotency and differentiation of emvryonic stem cells. HDACs are also defined to effect the various IPSCs generating strategies when used in combination to OCT-4/ SOX-2 and other important IPSC generating molecules. DNA methylation and Histone modification are the major regulators of epigenetic gene expression regulation factory during developmental and differentiation events. HDAC inhibitors can in fact be very helpful tools to increase the efficiency of nuclear transfer experiments. For instance, Trichostatin A (TSA), m-carboxycinnamic acid bishydroxamide, (CBHA), suberoylanilide hydroxamic acid (SAHA) and oxamflatin are important HDAC inhibitors reported for cellular reprogramming, apopotosis regulation and differentiation. These reports indicates opportunities for full reprogramming with chemical reagents alone and thus offer a procedure both safe and practical to be used in human therapies. Sulforaphane is anticancer/anti-oxident compound found in cruciferous vegetables (e.g.Broccoli. Its role in vastly described in various disease progression. Apart from it widely spread effects in various disease, it may have HDAC inhibition activity. It inhibits the activity of class 1 and class 2 HDAC. As discussed earlier HDAC inhibitors are potent regulators of cellular reprogramming and variety of HDACIs are already explored for their IPSC generating capabilities. This proposal aims to evaluate the potential IPSCs generating use/effect of sulforafane in combination to other well known HDAC inhibitors. The objectives of the proosed work would be to define the significance of sulforafane as apotent IPSCs generating molecules and outline the molecular mechanistic underlying these actions by using state of the art tissue cellular molecular biology techniques. As evident from previous studies that small molecules alone may be sufficient to generate IPSCs the outcome from these studies would be important in developing more important small molecule cocktails for possible therapeutic/clinical uses along with cellular reprogramming. The sulforafane and other small molecules may be implied in various combination to assess the HDAC inhibition assays, effects on ROS production, DNA modification assessment, etc and pluripotancy specific assays to evaluate their specific role in these processes.

Targeting microRNA to enhance stem cell-mediated cardiac repair

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Cardiovascular disease (CVD) remains to be the leading cause of disability and death in humans around the world. Stem cell therapy, more specifically endothelial progenitor cell (EPC) transplantation has been shown to enhance neovascularization and improve myocardial infarction (MI)-induced ventricular dysfunctions. However, persistent inflammation in the ischemic myocardium, adversely affect EPC survival and function, thereby compromising full benefits of EPC-mediated vascular repair. MicroRNAs (miRNA/miR) are small non-coding RNAs that have been shown to modulate a wide range of biological functions under various pathophysiological conditions. MiRNA act as molecular switches of gene expression and are thought to regulate complex cardiac signaling and transcriptional circuits in physiology and disease. However, its effects on endothelial progenitor cells (EPC) biology and function, particularly in the context of cell-based therapy for cardiac repair is not fully understood. The purpose of this study was to determine the miRNA profile of EPCs in response to inflammatory stimuli and to explore the role of miRNA in human CD34+ cell (hCD34+) dysfunction in vivo after transplantation into the myocardium under ischemiareperfusion (I-R) conditions. miRNA array data from EPCs in response to inflammatory stimuli has indicated modulation of number of miRNAs related to angiogenesis with a robust decrease in miR-377. miR-377 treatment inhibits EPCs migration and vascular tube formation ability. The proteome profile of hCD34+ cells transfected with miR-377 mimics showed significant decrease in the levels of proangiogenic proteins vs control-transfected cells. Computational analysis and dual luciferase reporter assay confirmed that serine/threonine kinase 35 is a target of miR-377. In a relevant mouse model of myocardial infarction (MI), intramyocardial transplantation of miR-377-silenced (anti-miR-377 transfected, GFP-labeled) EPCs promote neovascularization, attenuate LV dysfunction and therefore improves myocardial repair (reduced fibrosis and infarct size). Taken together, these data suggest that inhibiting miR-377 in EPCs enhances their angiogenic ability and therapeutic efficiency.

Correcting the bleeding phenotype in hemophilia A using lentivirally FVIIIcorrected endothelial cells differentiated from hemophilic induced Pluripotent Stem Cells (iPSCs)

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Background: Hemophilia A (HA) is caused by factor VIII (FVIII) gene mutations. Somatic cells can be reprogrammed to generate autologous, disease-free iPSCs and differentiated into cells relevant for gene and cell therapy. Aim: To generate patient-specific iPSCs after cell reprogramming of CD34+ cells from peripheral blood and differentiation into FVIII-secreting endothelial cells (EC) after genetic correction by lentiviral vectors (LV). Methods: CD34+ cells isolated from healthy and HA donors and reprogrammed with a Cre-Lox LV carrying OCT4-SOX2-KLF4 and miRNA302/367. iPSCs were characterized for stem cell
markers, telomeres length and karyotype analysis. Germ layers markers expression and differentiation potential assessed on embryoid bodies (EB). iPSCs were differentiated in EC and characterized by FACS and RT-PCR. HA EC were transduced with LV carrying GFP and/or FVIII under the control of EC-specific promoter (VEC) and transplanted in NOD/SCID-γNull (NSG) HA mice. Cell engraftment and proliferation analyzed by immunofluorescence. Results: iPSCs were differentiated into endothelial cells (EC) with an optimized protocol, acquired endothelial-like morphology, expressed ECs markers and were able to form tubules when cultured in matrigel. EC transplanted intraportally in NSG mice, engrafted and proliferated in the livers up to 12 weeks and confirmed by FACS analysis to be GFP and CD31+ representing the 30% of liver non-parenchymal cells. Moreover, transplanted cells formed vessels-like structure in the host liver. Finally, we transplanted HA-IPSC-derived ECs corrected by LV-VEC-FVIII in NSG-HA mice that showed a reduced bleeding time and a stable 5% FVIII activity after 12 weeks. Conclusion: These data will be instrumental to assess engraftment, proliferation and the FVIII expression from differentiated EC, gene corrected and reprogramming factor-free iPSCs to confirm the suitability of this approach for HA gene-cell-therapy

Applying the concepts of regenerative medicine to reprogram cancer cells?

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The complex signal network of hierarchical interaction between different levels of gene expression regulation is beginning to give a coherent picture, and to be applied in biotechnological and biomedical approaches. These mechanisms are exploited as the basis for the explosive applicative research field of "stem cell reprogramming", the novel strategy aiming at obtaining functional stem cells from adult (rather than embryo) tissues, to be then re-differentiated in vitro, and used in regenerative medicine for transplantation into damaged/injured organs. These studies show that transfection with as low as 4 transcription factors acting as master switches is sufficient to put in motion intracellular signals that reprogram differentiated into stem cells (iPS). This allowed breaking several supposed rules in biology, showing that cell differentiation can be reverted by reprogramming the pattern of gene expression of differentiated cells with specific transcriptional and epigenetic modulators, thereby reversing the unidirectional occurrence of the process; in fact, it is only the multiple locks in chromatin accessibility that obliges the direction of the differentiation process. Surprisingly, this knowledge is only marginally exploited in oncology, in spite of the fact that it would be theoretically easier to efficiently reprogram cancer-to-normal, rather than differentiated-to-stem cells: in the former case, cancer cells would be required to undergo short-term, specific changes, sufficient to promote oncogene-induced apoptosis or terminal differentiation; in the latter instead, the differentiated cells would require long-term and widefield reprogramming to become stem cells and take part to the tissue regeneration process. Interestingly, it was shown that cancer cells treated with the iPS cocktail could be reprogrammed to healthy stem cells reexpressing
the silenced tumor suppressor gene p16. It seems therefore an obliged step that an effective anticancer therapeutic intervention should consider the simultaneous or consequential use of multiple drugs synergistically acting on different mechanisms regulating gene expression, and that reprogramming cocktails conceptually similar to those used for iPS, but with different (opposite?) effects may be setup for research purposes and eventually clinical use. Overwhelming evidence that we'll discuss, pointing to a recessiveness of the cancer features, are for some reason disregarded in clinics, although they would place gene expression reprogramming therapy at a privileged position in terms of clinical significance and perspectives.

Human adipose stem cell differentiation is highly affected by cancer cells both in vitro and in vivo: implication for autologous fat grafting.

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Recent studies showed that mesenchymal stem cells derived from adipose tissue can promote tumour progression, raising some concerns regarding their use in regenerative medicine. In this context, we co-cultured either SAOS2 osteosarcoma or MCF7 breast cancer cells with human adipose stem cells (hASCs), in order to evaluate potential effects of cancer cells on hASCs differentiation, in vitro and in vivo. In this study we observed that both SAOS2 and MCF7 cell lines induced an increase in hASCs proliferation, compared to hASCs alone, but, surprisingly, neither changes in the expression of CD90, CD29, CD324 and vimentin, nor variations in the Twist and Slug mRNAs were detectable. Noteworthy, SAOS2 and MCF7 cells induced in hASCs an upregulation of CD34 expression and Stemness genes, including OCT3/4, Nanog, Sox2 and leptin, and a decrease in angiogenic factors, including CD31, PDGFα, PDGFRα, PDGFRβ and VEGF. SMAD and pSMAD2/3 increased only in hASCs alone. After 21 days of co-culture, hASCs differentiated both in adipocytes and endothelial cells. Moreover, co-injection of MCF7 cells with hASCs led to the formation of a highly vascularized tumour. Taken together our findings suggest that mesenchymal stem cells, under tumour cell induction, do not differentiate in vitro or facilitate the angiogenesis of the tumour in vivo, thus opening interesting new scenarios in the relationship between cancer and stem cells. These findings may also lead to greater caution, when managing autologous fat grafts in cancer patients.
Key words
Adipose stem cells, cancer cells, breast cancer, co-culture, pSMAD2/3

The control of cortex anatomy via miRNA target mimicry

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Through secondary growth of the cortex, roots control air quantity in plants growing on wet soils (aerenchyma), and store nutrients in plants growing under adverse weather and nutritive conditions (storage parenchyma). Root cortex anatomy, basically the number of cortex layers, thus varies between different plant species.
Comparing the development of Arabidopsis thaliana (one cortex layer) and Cardamine hirsuta (two cortex layers), very recently we identified a novel mechanism where the spatial distribution of a miRNA (miR165/6) in the root ground tissue controls the localization of a transcription factor (PHABULOSA, PHB) that in turn controls the number of cortex layers (Di Ruocco et al, final revision). This mechanism is most likely conserved in (related) crop species such as turnip (Brassica rapa).
We will reduce the activity of miR165/6 in the turnip cortex by miRNA target mimicry, i.e. by tissue-specific expression of MIM165/6, a DNA fragment that mimicks the miR165/6 target sequence: this will increase the number of cortex layers, thus increasing turnip storage capability and performance.

3D BIO-PRINTING AND MUSCLE DERIVED PERICYTES FOR ARTIFICIAL SKELETAL MUSCLE HUMAN-LIKE SIZE.

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The skeletal muscle tissue exhibits good regenerative capabilities, which are however limited by injury size. As a matter of fact, large muscle lesions are characterized by poor recovery accompanied by scar formation and functional detriment, condition common to people suffering from volumetric muscle loss and needing reconstructive therapeutic approaches. Even if surgical autologous transplantation is a standardized procedure, the outcomes are often unsatisfactory. Hence, the pressing need to develop engineered artificial tissues to replace wasted muscle. Tissue engineering (TE), exploiting stem cells embedded in biomimetic scaffolds, aims to mimic organogenesis by building artificial tissues to replace the damaged ones. Skeletal muscle TE is an up-and-coming biotechnology with great potential for muscle repair, but no conclusive strategy has been demonstrated yet. Reconstructing the skeletal muscle architecture and function is still a challenge requiring the parallel alignment of myofibrils arranged into organized sarcomeres. Recently we demonstrated the great potential of a hybrid biomimetic matrix, namely PEG-Fibrinogen, for enhancing the engraftment of myogenic cell progenitors by providing a suitable 3D environment for mouse muscle reconstruction. Starting from these observations, we developed a novel approach for the regeneration and/or reconstruction of skeletal muscle tissue segments of human-like size by exploiting a population of adult myogenic stem cells, namely pericytes, in combination with 3D bio-printing technology to guarantee a functional architecture. In vitro characterization of cell-laden constructs showed enhanced myogenesis and positive myostructure alignment. Thanks to the enhanced control over cell deposition and alignment, the presented technology has the potential to support skeletal muscle repair and regeneration.

Gold coated silicon nanowires as multifunction agents for combined near infrared photothermal treatment of cancer cells and Raman monitoring of the process evolution

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We report on the exploitation of strong absorption of near infrared light by a Au thin film covering a dense and disordered Si nanowire (Si NW) array both to induce the photothermal death of cancer cells and to monitor their metabolic evolution during the treatment. To show the great potential of this strategy, 1-2 μm long Si NWs with diameter of few nanometers were grown by plasma enhanced chemical vapor deposition and covered by an evaporated Au film, 150 nm thick. Hence a monolayer of human colon carcinoma cells (CaCo-2) was cultured on Au covered Si NWs (Au/SiNWs) and then irradiated by continuous infrared laser at 780 nm with a spot size of 1-2 μm. We found the laser irradiation to induce the cell death over controlled areas ranging from few tens up to hundrends of microns and to allow the continuous recording of the Raman spectra from the irradiated zone. The ultimate attractive feature of the proposed nanostructure is the ease and scalable fabrication methodology involving relatively low temperature procedures, compatible with polymeric or glasses supports which provide an effective method of direct incorporation of the Au/Si NWs in optical fiber laser devices for endoscopic photothermal treatments.

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A Novel Platform for Immune Tolerance Induction in Hemophilia A Mice

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Hemophilia A (HA) is an X-linked bleeding disease caused by factor VIII (FVIII) deficiency. We previously demonstrated that FVIII is produced specifically in liver sinusoid endothelial cells (LSECs) and to some degree in myeloid cells, and thus, in the present work, we seek to restrict the expression of FVIII transgene to these cells using cell-specific promoters. With this approach, we aim to limit immune response in a mouse model by lentiviral vector (LV)-mediated gene therapy encoding FVIII. To increase the target specificity of FVIII expression, we included miRNA target sequences (miRTs) (i.e., miRT-142.3p, miRT-126, and miRT-122) to silence expression in hematopoietic cells, endothelial cells, and hepatocytes, respectively. Notably, we report, for the first time, therapeutic levels of FVIII transgene expression at its natural site of production, which occurred without the formation of neutralizing antibodies (inhibitors). Moreover, inhibitors were eradicated in FVIII pre-immune mice through a regulatory T cell-dependent mechanism. In conclusion, targeting FVIII expression to LSECs and myeloid cells by using LVs with cell-specific promoter minimized off-target expression and immune responses. Therefore, at least for some transgenes, expression at the physiologic site of synthesis can enhance efficacy and safety, resulting in long-term correction of genetic diseases such as HA.

Microfluidic impedance flow cytometry for label-free single-cell characterization

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The aim of this talk is to give an overview of the present status, challenges and future prospects of microfluidic impedance flow cytometry, which is a label-free technique for the electrical characterization of single particles as they flow through a microchannel with integrated electrodes. It has applications in different biological assays including particle sizing and counting, cell phenotyping and disease diagnostics (see, e.g., the reviews [1,2]). In particular, microfluidic impedance flow cytometry enables non-invasive identification of stem cells and the state of stem cell differentiation, a task that is vital to the development of clinically feasible regenerative therapies [3]. In the last decade, novel concepts and ideas, coupled with the development of micro and nano technologies, have enhanced the sensitivity and specificity of the technique (see, e.g., the reviews [3,4]). However, some interdisciplinary challenges have to be addressed in order to allow a full exploitation of the research results at the industry level.

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Biomaterials

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CIMER

Innovative reporter gene imaging technique to probe therapeutic cardiac repair

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The infarcted heart undergoes profound changes to repair damaged tissues and compensate for lost function. The process must strike a delicate balance among complex cellular and system-level signalling activities in an orchestrated fashion to rid the myocardium of debris and support the infarct scar. Much remains to be learned in the pathophysiology of the post-infarct heart in order to achieve better therapeutic intervention with improved long-term outcome. Emerging non-invasive strategies, in particular mechanistic measurements on a molecular level, hold promise in characterizing the spatiotemporal dynamics of cardiac repair/remodel. Innovative reporter gene imaging techniques are deployed to probe for the activities of molecular pathways potentially involved in cardiac repair. Using non-invasive imaging tool based on magnetic resonance imaging and gene therapy approach, we have observed increased canonical Wnt/β-catenin pathway activation in the peri-infarct border zone, and that this response is abolished in the presence of a small-molecule inhibitor for the Wnt/β-catenin signalling pathway. Our molecular imaging application will have a big impact in modern biomedical research in order to optimize clinical applications of new therapeutic approaches involving molecular pathways activation with small molecules or stem cell-derived factors.

Tumor Targeting by Magnetic Nanoparticles associated with Lentiviral Vectors or functionalized with monoclonal antibody in Mice

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Nanomaterials conjugated or complexed with biological moieties such as antibodies, polymers or peptides appear to be suitable not only for drug delivery but also for specific cancer treatment. Here, biocompatible iron oxide magnetic nanoparticles (MNPs) with or without a silica shell coupled with lentiviral vectors (LVs) or with monoclonal antibodies (mAbs) directed against the Met/HGF-R tumor associated marker are proposed as combined therapeutic approaches to specifically target gene expression and tumor cells in a cancer mouse model. Initially, four different MNPs were synthesized and their physical properties were characterized to establish and discriminate their behaviors. MNPs and LVs strictly interacted and transduced cells in vitro as well as in vivo, with no toxicity or inflammatory responses. By injecting LV-MNPs complexes intravenously, green fluorescent protein resulted in a sustained long-term expression. Furthermore, by applying a magnetic field on the abdomen of intravenous injected mice, GFP positive cells increased in livers and spleens. In a mouse model with a grafted tumor, intra-tumor LV-MNPs injection and magnetic plaque application next to the tumor demonstrated the efficient uptake of LV-MNPs complexes with high number of transduced cells and iron accumulation in the tumor site. More important, LV-MNPs with the application of the magnetic plaque spread in all the tumor parenchyma and dissemination through the body was prevented confirming the efficient uptake of LV-MNPs complexes in the tumor. In vitro mAb-functionalized MNPs displayed a significantly better interaction with cells expressing the targeted Met receptor than unfunctionalized MNPs, and also in this case in vivo intra-tumor mAb-MNPs injection and magnetic plaque application onto the Met-expressing tumor prevented MNPs dissemination through the body and allowed a higher dispersion of MNPs within the tumor.
These studies can significantly improve cancer therapy effectiveness with a selective and localized therapeutic transgenes delivery or through specific mAb-mediated targeting of MNPs in a mouse model.

Computational Morphogenesis applied to regenerative medicine

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Morphogenesis is the biological process that defines how a system of cells is organised and shaped. The word originates from the Greek words "morf" which means shape and "gènesic" which means birth.
Before the beginning of the 20th century there were only descriptive theories and none of these could be verified experimentally. At around 1960s new ideas arised and with the aid of newly developed computers, scientists were able to shed new light into this subject.
The idea of chemical substances reacting and diffusing was first proposed by the mathematician and computer scientist Alan Turing. He published the mathematical theory in his seminal paper "The Chemical Basis of Morphogenesis" in 1952.
Lewis Wolpert introduces the concept of Positional Information (PI) in order to explain how complex patterns could arise from initial asymmetries in the tissue. The main idea of Wolpert is that the position of a cell in the tissue specifies the information about the molecular changes the cell will undergo.
On the contrary Murray and Oster 1983 considered, in addition to their chemical and usable appearance, the role they play the mechanical forces in pattern formation, what it was for many years ignored by embryologists despite being obvious that genetics and chemistry, having essential roles in embryology, should nevertheless be subject to the laws of mechanics. Their approach, missing in Turing's theory, is that the patterns that derive from it are formulated in terms of measurable quantities such as cell density, the forces, the tissue deformation.
At the beginning of the 80s, Skalak and coworkers gave the first analytical description of definite volumetric and surface growth in a continuum mechanics framework. Skalak's formulation of volumetric growth prepared the ground for the paper of Rodriguez and colleagues in which they formalized, in an elegant mathematical formulation, the relation between growth and remodeling on one side and residual stresses on the other side. Another modern approach to morphogenesis comes from the work of Lev Beloussov.
During the 70s he performed several experiments on embryos in order to investigate the effects of mechanical stresses on early morphogenetic events.
Aim of this paper is to define a mathematical model for volumetric growth and remodeling, based on the most modern models, to be used in the frame of regenerative medicine, taking into account the scaffold structure too.

Integrated Organs-on-Chip approach to immuno-oncology: image analysis and microfluidic assays for anti-cancer and immunomodulatory drug evaluation

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CNR - Tech4Bio

The immune system is a striking example of an integrated information system, engaged in coordinated host-protective activities. Organs-on-chip approach (OOC) models allow the direct simultaneous observation of hundreds of different cells, moving, interacting and responding to signals coming from the microenvironment nearby, that give access to a number of parameters describing the system that must be properly measured and elaborated. Combining microfluidics with the ability of cellular imaging enable to collect quantitative data from complex biological systems at a single-cell level.
Reconstitution of the immune-cancer system on chip opens a new window to live observation of the host immune response with or without drug treatments, making OOC a cornerstone for dissecting complex biological phenomena and pre-clinical testing of drugs. Smart implementation of image processing algorithms enable to quantify the simultaneous long-time interactions of huge number of cells and accurately solve the practical problems encountered in multi-cell type context.

Ex vivo evaluation of the regenerative response in human primary cells

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Osteoporosis (OP) and osteoarthritis (OA) are two major health burdens in our modern societies. OP is characterized by low bone mass due to an unbalance in favor of bone remodeling. These diseases are proposed to be mutually exclusive, however pathophysiological mechanisms leading to them may overlap. Bone degenerative pathologies are characterized by a reduced bone mass and microarchitectural deterioration of bone tissue, and it often correlates with sarcopenia. Bone and muscle tissues are in close relationship and, from a biological point of view, these degenerative disorders are characterized by unbalanced metabolism. A general age-related decrease in muscle and bone oxidative capacity and mitochondrial functions have been already reported in literature however the molecular basis for this pathology remains substantially unexplored. In our study, human cell (i.e.: osteoblasts, satellites cell and myocites) were isolated from bioptic tissues (trabecular bone or lateral vastum muscle) from osteoporotic and osteoarthritic patients. Preliminary results allow to speculate that the degenerative process is induced by biochemical or bio-mechanical signals in the cross-talk between the two tissues, dumping the regenerative cell capacity. To investigate this hypothesis we have studied the cellular behavior of human primary cells, from biopsies of osteoporotic patients and related aged-matched controls. In vitro evaluation of the regenerative capacity of primary cells (and of molecular processes underlying it) were measured by following their ability to adhere, to survive, to proliferate and to migrate on different substrates. Our findings revealed a number of disease-associated proteins which are candidates as key players in the cytoskeletal organization and in nuclear morphology of human cells. As exposure to microgravity has been associated with several pathological changes in astronauts, including an osteoporosis-like excessive bone resorption, further studies will focus on molecular mechanisms underlying weightless-induced bone loss.

How to empower SC engrafting aimed at tissue repair

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Mesenchymal stem cells (MSCs) and cardiac progenitor cells (CPCs) isolated from human heart biopsies are a promising tool to improve tissue repair (Monsel et al., 2014), but low survival and partial engraftment and migration are still practical problems due to the hostile necrotic and oxidative microenvironment. We have found that spheroid aggregates prepared by incubating overnight cells in methylcellulose hydrogel-coated microwellas maintained the expression of stemness/mesenchymal and ECM markers, growth factors and their cognate receptors, cardiac commitment factors, and metalloproteases and expressed a higher, but regulated, telomerase activity. Cells within spheroids quickly migrated, displaying an increased wound healing ability with or without pharmacological modulation, and reached confluence at a higher rate than cells from conventional monolayers. When spheroids were injected in the wall of healthy or cardiotoxin-injured myocardium, some cells migrated from the spheroids, engrafted, and remained detectable for at least 1 week after transplantation, while, when the same amount of cells was injected as suspension, no cells were detectable three days after injection.

In another study, we found that 24h pre- or post-treatment with clovamide, a polyphenolic compound derived from cocoa, could protect murine MSCs and CPCs, when they were exposed to H_2O_2-induced oxidative stress, by decreasing the number of apoptotic cells and re-establishing the expression of genes affected by oxidative stress.

These studies show the possibility to make more resistant to hostile microenvironments MSCs and CPCs by economic, easy and fast treatments, opening the pathway to their exploitation in view of a better cell engraftment in the context of regenerative medicine.

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Electron microscopic characterization of collagen-based

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Wound healing is a complex process resulting from a highly elaborate interplay between different cell types, various growth factors and several components of the extracellular matrix. In recent years, much research has been dedicated to the development of new biomaterials to unravel the intricasy of this process. As a result, wound healing has been greatly improved through the in vivo use of several equine collagen-based medical devices (gel, powder, foils and 3D scaffolds).
In this study, we analysed the bio-nanostructures of these devices by using Scanning and Transmission Electron Microscopy. Our purpose was: (1) to characterize the overall morphology and ultrastructure of different collagen-based substrates; (2) verify the stability of their native organization following fabrication of the 3D scaffold; (3) study the extent by which these prosthetic collagens affect cell behaviour in vitro and (4) verify how the collagen matrix is structurally modified when combined with other substances. This latter includes sodium hyaluronate, as a constituent of the extracellular matrix, silver nanoparticles for their antiinflammatory
properties and Biosecure and Honey for their antimicrobial properties.
Finally, we attempted to upload a number of lipid nanoparticles (Lipidots™) onto the collagenbased scaffolds (a) to verify whether they could stably bind to the collagen matrix and (b) whether they could be used as drug delivery carriers, potentially capable of modulating cell responses under varying in vivo conditions.