Tissue Engineering Today

Wednesday, 05 June 2013
The Royal College of Pathologists, Carlton House Terrace, London, SW1Y 5AF, United Kingdom

This meeting sets out to bring researchers together to discuss the latest developments in the rapidly expanding field of Tissue Engineering and Regenerative Medicine

This event has CPD accreditation
This event is part of the 2013 Euroscicon Stem Cell Trilogy. To find out more see www.stemcells2013.com

Meeting chair: *Dr Paolo De Coppi,* MD, PhD, Senior Lecturer and Consultant Paediatric Surgeon, Head of the Surgery Unit, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK

9:00 – 9:30 **Registration**

9:30 – 10:00 **Introduction by the Chair:** *Dr Paolo De Coppi*, MD, PhD, Senior Lecturer and Consultant Paediatric Surgeon, Head of the Surgery Unit, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK

Congenital malformation and regenerative medicine: moving towards therapy
The number of neonates born with congenital malformations is increasing. The correction of congenital malformations has not significantly changed during the last 10-20 years and complex and often multiple operations still represent the only practical solution. The current approach to treatment of birth defects and various diseases is to modify the defective organs or to replace them with artificial substitutes or organ transplant. Stem cells can be derived from human amniotic fluid, which could be collected safely at prenatal diagnosis. Evidence provided in the last few years, suggests that they can harbour a therapeutic potential for human diseases, as amniotic fluid stem (AFS) cells have been isolated. AFS cells have intermediate characteristics between embryonic and adult stem cells. C-Kit(+)Lin(-) cells derived from amniotic fluid displayed a multilineage hematopoietic potential and they can be easily reprogrammed to a pluripotent status. With the aim of engineering functional organs, AFS cells have been seeded into decellularised organs and could represent in the future an alternative therapy for congenital malformations.

10:00 – 10:30 **Steps towards Physiological simulation of Dental Tissues**
Dr Reem El-Gendy, Leeds Dental Institute

10:30 – 10:40 **TISSUE ENGINEERED BONE GRAFTS BASED ON BIOMIMETIC NANOCOMPOSITE PLGA/AMORPHOUS CALCIUM PHOSPHATE SCAFFOLD AND HUMAN ADIPOSE-DERIVED STEM CELLS**
Dr. Johanna Buschmann, Division of Plastic and Hand Surgery, University Hospital Zurich, Sternwartstrasse 14, CH-8091 Zurich, Switzerland

10: 40 – 10: 50 **MesoRex PROCEDURE USING AN AUTOLOGOUS STEM CELL DERIVED VEIN**
VK.Kuna, Laboratory for Transplantation and Regenerative Medicine, Sahlgrenska Science Park, Medicinaregatan 8B, S41346, Gothenburg, Sweden.

10:50 – 11:10 **Speakers' photo then mid-morning break and trade show**
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11:10 – 11:20 **CLINICAL TRANSPLANTATION OF A TISSUE-ENGINEERED HUMAN TRACHEA WITH AUTOLOGOUS STEM CELLS: PREPARATION, TRANSPLANTATION AND HISTOPATHOLOGICAL REPORT**
Nikhil Nayakwad Laboratory for Transplantation and Regenerative Medicine, Sahlgrenska Science Park, Medicinaregatan 8A, 2nd floor, 413 46 Göteborg, Sweden.

11:20 – 11:30 **TISSUE ENGINEERING OF HUMAN SMALL INTESTINE USING BONE MARROW STEM CELLS**
PB Patil, Laboratory for Transplantation and Regenerative Medicine, Sahlgrenska Science Park, Medicinaregatan 8B, S41346, Gothenburg, Sweden.

11:30 – 12:00 **Immunocompetent models of human respiratory epithelium**

Dr Amir Ghaem-Maghami, Nottingham University

Despite enhanced patient care, the morbidity and mortality of patients with lung disease have remained high. This is partly due to lack of efficient therapeutic strategies and also that a large proportion of patients do not respond to treatments. There is a lack of predictive preclinical models of asthma and new treatments that enter clinical trials frequently fail, possibly because preclinical animal studies are often limited in their physiological relevance to the human lung. To address some of these issues we are developing a physiologically relevant immune responsive 3D model of human lung that can be used for drug assessment and disease modelling.

12:00 – 12:30 **Bone regenerating ceramic materials**

Professor Joost de Bruijn, Professor of Biomaterials, School of Engineering and Materials Science, QMUL, UK

The use of growth factors or progenitor/stem cells for functional bone tissue regeneration have received much attention as potential alternatives to autologous bone grafting in the past decades. Some of the hurdles to overcome in these technologies include ensuring cell survival with the cell therapy approach and using potent but less supra-physiological concentrations of growth factors to minimize adverse reactions. To circumvent the necessity of cells or growth factors in bone tissue regeneration, we have developed a micro/nanostructured calcium phosphate ceramic that is capable of inducing bone formation without the necessity of adding cells or growth factors. These osteoinductive ceramics have shown excellent bone regeneration potential of large, critical sized bone defects. In this talk, an overview will be provided of the research performed in various pre-clinical and clinical case studies on this new group of bone regenerating ceramics.

12:30 – 13:30 **Lunch and trade show**

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13:30 – 14:30 **Question and Answer Session**

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:30 – 15:00 **Seeing around the corner at future strategies in corneal regeneration**

Dr Andrew Hopkinson, Queen's Medical Centre, Nottingham, United Kingdom

15:00– 15:30 **Afternoon Tea/Coffee and trade show**

15:30 - 16:00 **3-Dimensional Culture Models of Joint Tissues: Applications in Tissue Engineering and Arthritis Research**

Dr Ali Mobasheri, Associate Professor and Reader in Comparative Physiology, University of Nottingham, UK

This presentation will focus on some of the most popular in vitro models that have been developed for cartilage tissue engineering. Many in vitro models can be used as drug screening systems and as culture models for studying the biology of cartilage and the pathophysiology of joint disease. This presentation will also highlight the fact that regenerative medicine and tissue engineering have important consequences for animal research and can be exploited to develop powerful animal sparing in vitro models. Refining these models will advance tissue engineering and regenerative medicine and may significantly reduce our dependence on animals in research.

16: 00 - 16:30 **Novel Biomaterials for use in Regenerative Medicine**

Professor Sandra Downes, Manchester University, UK

The development of novel biomaterials for regenerative medicine will be examined. The opportunities and challenges will be discussed. The processes involved in translational research will be considered in the context of a number of applications including peripheral nerve repair and bone repair. The concept of cell therapy in two major clinical applications, namely: Ageing Macular Degeneration (AMD) and Diabetes Type 1. Recent scientific results will be presented, including Materials Chemistry, Biomechanics and in vitro cell Biology.

16:30 – 17:00 **Cadaver Bone Marrow Mesenchymal Stem Cells for the Treatment of Large Burns**

Professor Eduardo Mansilla, Director Tissue Engineering, Regenerative Medicine and Cell Therapies Laboratory CUCAIBA Province of Buenos Aires, Ministry of Health, La Plata, Argentina, Professor of Internal Medicine, School of Medical Sciences, National University of La Plata, Argentina

Cadaver Stem Cells (CSCs), will probably be generating soon a profound research activity in Regenerative Medicine and Transplantation. Maybe also, a real scientific revolution in these fields. As far as we know, we have been the first ones in the world, to use Cadaveric Bone Marrow MSCs to treat large severe burns, in a human clinical trial. We are really confident and see feasibility as well as a great potential for the routine salvage and therapeutic use of CSCs in the near future. If this comes to be true, it might surely significantly change the way we see today the possible sources of stem cells obtention. In this way, we might be finally considering CSCs as very important tools for many and different cell therapies including large burns.

17:00 **Chairman's summing up**

Key words: arthritis, stem cell, tissue engineering, periodontal ligament, in situ models, Articular cartilage; osteoarthritis; tissue engineering; regenerative medicine; 3-dimensional culture, Bone graft, biomaterial, surface structure, osteoinduction, lung, epithelia cells, dendritic cells, respiratory diseases, Polymers, Regenerative Medicine, Cell Therapy, Nerve, Bone, Regeneration. Burns. Cadaver Mesenchymal Stem Cells, Corneal regeneration, amniotic membrane, tissue engineering, mesehnchymal stem cells

About the Chair

Paolo De Coppi is the Head of the Surgery Unit and Consultant Paediatric Surgeon at the Great Ormond Street Hospital for Children and the UCL Institute of Child Health (since 2006). Concomitantly he is an Adjunct Assistant Professor at the Wake Forest Institute for Regenerative Medicine, Wake Forest University, Wiston-Salem, NC, US (since 2009) and Honorary Assistant Professor Paediatric Surgery, University of Padua, Italy (since 2005).

He has a special interest in congenital malformation and their treatment using minimally invasive techniques. He has focused his research interests on stem cells and tissue engineering, trying to find new modalities for the treatment of complex congenital anomalies. While working with Dr A. Atala at the Childrens' Hospital in Boston-US, he had the opportunity of identifying a new source of cells for therapeutic applications showing the possibility of using stem cells from amniotic fluid. This finding generated an international patent, the cover of the January 2007 issue of Nature Biotechnology, and it has opened the development of new ways for the correction of congenital malformations. More recently, his team has demonstrated that these cells are able to differentiate into various tissues and to replace functional activity in animal model of diseases. He is now focused on developing reliable methods for stem cell isolation, expansion and differentiation at clinical level (GMP-grade). Finally, in 2010 he was part of the team, which performed the first successful transplantation of a tissue-engineered trachea on a child at the Great Ormond Street Hospital.

He has published more than 100 peer-reviewed articles in high-impact factor journals such as The Lancet, Nature Biotechnology, Blood and FasebJ; supervised more than 25 research fellow and PhD students and holds various National and International grants. Since 2009 he has joined the Editorial Board of Pediatric Surgery International, Stem Cell Development and Fetal and Maternal Medicine Review; since 2011 he has also become Associate Editor for Stem Cell Translational Medicine.

About the Speakers

Ali Mobasheri is an Associate Professor and Reader at the University of Nottingham. He currently serves on the University of Nottingham's Ethical Review Committee (ERC) and the EU strategy group which will develop the University's strategic approach to European Funding and in particular Horizon 2020. He has a strong background in cartilage biology in the context of ageing, inflammation and disease. He is the co-ordinator of the EU FP7 funded D-BOARD consortium, which brings together leading European academic institutions and SMEs to focus on the identification, validation and qualification of new biomarkers for degenerative and inflammatory diseases of joints.

Eduardo Mansilla is director Tissue Engineering, Regenerative Medicine and Cell Therapies Laboratory CUCAIBA Province of Buenos Aires, Ministry of Health, La Plata, Argentina
Professor of Internal Medicine, School of Medical Sciences, National University of La Plata, Argentina

Sandra Downes (The University of Manchester) has worked in the field of Biomaterials for more than 20 years in both the Academic and the Industrial environment. Her contributions to the field include publications and studies of cellular interactions with biomaterials, biopolymers, tissue repair, biocompatibility, novel polymers and composite materials. She has a strong interest in designing biomaterials that mimic the structures of tissues. Her expertise and experience span the fields of Biochemistry, Physiology, Cell/Molecular Biology, Chemistry, Biomechanics and in vivo Biology. She holds patents for novel medical devices in tendon, peripheral nerve and bone repair.

Andrew Hopkinson attained his PhD at the University of Nottingham, studying the healing properties of amniotic membrane as an ophthalmic biological bandage. His post doctorate studies involved investigating amniotic membrane as a tissue engineering substrate, and limbal and mesenchyme stem cells with the aim of developing corneal mimetic constructs. Since then he has established himself as a principal investigator in ophthalmic regenerative medicine, in the Division of Ophthalmology and Visual, Nottingham, researching innovative stem cell and tissue engineering technologies for the development of future therapies for corneal regeneration. His currently directs pre-clinical evaluation and clinical translation of innovate regenerative therapies

Amir Ghaem Maghami obtained his MD prior to studying for a PhD in Immunology at the University of Nottingham. Prior to his appointment as a lecturer in Immunology he worked as a research fellow in Leicester and Nottingham Universities, investigating the role of antigen presenting cells in infectious and allergic diseases. He is currently an Associate Professor (Faculty of Medicine, University of Nottingham) and leads the Allergy and Tissue Modelling Research Group. For many years he has been investigating the innate properties of allergens and early events at the interface of allergens and the immune system that lead to T cell polarisation. More recently his research has focused on using tissue engineering approaches for developing immunocompetent human tissue models as platforms for testing new drugs and disease modelling.

Joost D de Bruijn (1966) holds the Chair of Biomaterials at Queen Mary University of London, United Kingdom and is Professor of Regenerative Medicine and Entrepreneurship at Twente University, The Netherlands. His current research focuses on tissue instructive materials (bone, cartilage) and mesenchymal stem cells. Prof de Bruijn is also founder and CEO of the Dutch biotech start-ups Xpand Biotechnology BV and Progentix Orthobiology BV that focus on stem cell expansion bioreactor technology and bone inducing ceramic materials. To date, he has published 135 papers in peer-reviewed journals and is inventor of 28 international patents

Event Web Site: www.regonline.co.uk/Regen2013

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- You will be issued with a FULL delegate list within 14 days of the event, which will include the email addresses of the delegates (we are sorry that there is this delay in emailing the list, but we need to make sure that it takes into account any late arrivals). You will not be included in this list if you have opted out and you can do this by logging into your registration details. This list will not be sold or ever give out to third parties. Only people attending or sponsoring the event have access to the list
- There may be an independent meeting report published within a few months of this event. If this is published we will send you an email to let you know the reference details
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POSTER PRESENTATIONS

TISSUE ENGINEERED BONE GRAFTS BASED ON BIOMIMETIC NANOCOMPOSITE PLGA/AMORPHOUS CALCIUM PHOSPHATE SCAFFOLD AND HUMAN ADIPOSE-DERIVED STEM CELLS

J. Buschmann, Ph.D., Härter, L., Ph.D., Gao, S., Ph.D., Hemmi, S., Welti, M., Hild, N., Schneider, O. D., Ph.D., Stark, W. J., Ph.D., Lin¬denblatt, N., M.D., Werner, C. M. L., M.D. , Wanner, G. A., M.D. , Calcagni, M., M.D.
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For tissue engineering of critical size bone grafts, nanocomposites are getting more and more attractive due to their controllable physical and biological properties. We report in vitro and in vivo behaviour of an electrospun nanocomposite based on poly-lactic-co-glycolic acid and amorphous calcium phosphate nanoparticles (PLGA/a-CaP) seeded with human Adipose-Derived Stem Cells (ASC) compared to PLGA. Major findings were that cell attachment, three-dimensional ingrowth and proliferation were very good on both materials. Cell morphology changed from a spindle-shaped fibroblast-like form to a more roundish type when ASC were seeded on PLGA, while they retained their morphology on PLGA/a-CaP. Moreover, we found ASC differentiation to a phenotype commited towards osteogenesis when a-CaP nanoparticles were suspended in normal culture medium without any osteogenic supplements, which renders a-CaP nanoparticles an interesting osteoinductive component for the synthesis of other nanocomposites than PLGA/a-CaP. Finally, electrospun PLGA/a-CaP scaffold architecture is suitable for a rapid and homogenous vascularisation confirmed by a complete penetration by avian vessels from the chick chorioallantoic membrane (CAM) within one week.

MesoRex PROCEDURE USING AN AUTOLOGOUS STEM CELL DERIVED VEIN

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Background Extra-Hepatic Portal Vein Obstruction (EHPVO) can have severe health consequences. Variceal bleeding associated with this disorder causes upper gastrointestinal bleeding, leading to substantial. We report the clinical transplantation of a deceased donor iliac vein graft repopulated with recipient autologous stem cells in a patient with extrahepatic portal vein obstruction.
Methods A 10 year old girl with EPHVO was admitted to the Shalgrenska University Hospital in Gothenburg, Sweden, for a bypass procedure between the superior mesenteric vein and the intrahepatic left portal vein (meso Rex bypass). A 9cm segment of allogeneic donor iliac vein was decellularized and subsequently recellularized with endothelial and smooth muscle cells differentiated from stem cells obtained from the bone-marrow of the recipient. This graft was used because the patient´s umbilical vein was not suitable and other strategies (eg, liver transplantation) require lifelong immunosuppression.
Findings The graft immediately provided the recipient with a functional blood supply (25-30 cm/s in the portal vein and 40 ml/s in the artery was measured intraoperatively and confirmed with ultrasound. The patient had normal laboratory values for 9 months. However, at 1 year the blood flow was low and, on exploration, the shunt was patent but too narrow due to mechanical obstruction of tissue in the mesocolon. Once the tissue causing the compression was removed the graft dilated. We therefore used a second stem-cell populated vein graft to lengthen the previous graft. After this second operation, the portal pressure was reduced from 20 mm Hg to 13 mm Hg and blood flow was 25-40 cm/s in the portal vein. With restored portal circulation the patient has substancially improved physical and mental function and growth. The patient has no anti-endothelial cell antibodies and is receiving no immunosuppressive drugs.
Interpretation An acellularized deceased donor vein graft recellularized with autologous stem cells can be considered for patients in need of vascular vein without the need for immunosuppression.

CLINICAL TRANSPLANTATION OF A TISSUE-ENGINEERED HUMAN TRACHEA WITH AUTOLOGOUS STEM CELLS: PREPARATION, TRANSPLANTATION AND HISTOPATHOLOGICAL REPORT

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Innovative cell-based therapies, involving tissue engineering represent interesting and potentially important strategies for treatment of patients with various disorders. Here, using a detergent-enzymatic method we successfully prepared an intact 3-dimensional scaffold of an extracellular matrix (ECM) derived from a human cadaver donor trachea, which we repopulated with autologous stem cells and implanted into a 76-year old patient with tracheal stenosis. The graft at once provided the patient with a functional airway. However, twenty-three days later the patient died due to cardiac arrest but with a patent and stable tracheal transplant and intact anastomoses. Histopathological results of the transplanted tracheal graft post mortem showed a squamous epithelium, neovascularization, bundles of -sma positive muscle cells, serous glands and nerve fibres with S-100 positive nerve cells in the submucosa and intact chondrocytes in the cartilage. Our findings suggest that autologous stem cells- engineered tracheal matrices represent a promising tool for clinical tracheal replacement.

TISSUE ENGINEERING OF HUMAN SMALL INTESTINE USING BONE MARROW STEM CELLS

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We aimed to produce an acellular human tissue scaffold with a view to test the possibility of recellularization with bone-marrow stem cells to produce a tissue-engineered small intestine (TESI). Human small bowel specimens (n=5) were obtained from cadaveric organ donors and treated sequentially with 6% DMSO in hypotonic buffer, 1% Triton X and DNAse. Each SI piece (6cm) was recellularized with EPCAM+ and CD133+ allogeneic bone-marrow stem cells. Histological and molecular analysis demonstrated that after decellularization, all cellular components and nuclear material were removed. Our analysis also showed that the decellularized human SI tissue retained its histioarchitecture with intact villi and major structural proteins. Protein films of common extracellular matrix constituents (collagen I, laminin, and fibronectin) were found in abundance. Furthermore, several residual angiogenic factors were found in the decellularized SI. Following recellularization, we found viable mucin positive goblet cells, CK18+ epithelial cells in villi adjacent to an innervated muscularis mucosa with alpha actin+ smooth muscle cells and a high repopulation of blood vessels with CD31+endothelial cells. Our results show that in the future, such a TESI would be ideal for clinical purposes, since it can be derived from the recipient's own immunocompatible BM cells, thus avoiding the use of immunosuppression.

POLYMER COATING ENHANCES AFFINITY OF BONE MARROW-DERIVED MSCs TO BONE

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Bone remodeling is a process orchestrated by two types of cells: osteoblasts and osteoclasts. The reduction in osteoblastic activity results in the progressive bone loss, which could be amended by introduction of osteoblast progenitor cells (OPCs). Bone targeted OPCs delivery can potentially increase the efficiency of cell therapy and result in alleviation of such conditions as osteoporosis.
We have synthesized and tested in vitro a novel polymer which shows high affinity to bone tissue and could be used to deliver OPCs to the site of bone injury or lesion.
The primary active sites of the polymer are bisphosphonate functional groups that target hydroxyapatite molecules (HA) on the bone surface. NHS groups on the other end of the molecule allow polymer to bind to the cell surface components. As a result, polymer acts as a high affinity coat linking cells to the bone surface. In this study we have used mesenchymal stem cells (MSCs) as a model for OPCs.
The polymer was mixed with the MSCs at various concentrations for varying times to determine binding rates, number of binding sites per cell, stability of the binding, and cell viability. The polymer was not shown to be cytotoxic by cell viability assay (MTT) and does not affect further differentiation into osteocytes.

Polymer functionalized cells were incubated with bone fragments for varying times to determine the stability of cell-bone binding. The polymer coated cells were shown to be stably attached to bone fragments for at least 2 hours, confirming the bone targeting potential of the polymer. Our next step will be to test this approach in rat model of osteoporosis to determine its actual performance in vivo.

ASSESSMENT OF THE EFFECTS OF CERIUM OXIDE NANOPARTICLES ON RAT MESENCHYMAL STEM CELLS (MSCs) STIMULATION AND DIFFERENTIATION

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Tissue repair and regeneration is made possible by the presence of mesenchymal stem cells (MSCs) which are solicited during the entire lifespan by different factors influencing cell proliferation and differentiation. However, MSCs suffer from some important limits to their in vitro manipulation, like proliferation and maintenance of their stemness along passaging, and controlling their differentiation along a specific lineage. The increased intracellular oxidative stress could be the basis for the progressive functional decline of MSCs during long-term in vitro culture. Oxidative stress causes intracellular accumulation of reactive oxygen species (ROS) leading to DNA and protein damage, which in turn might activates p53 signaling increasing the amount of senescent and dysfunctional cells. These negative effects seem to occur independently of the donor age during in vitro expansion, and sustain the idea that chronological and in vitro aging are distinct processes. Nanotechnology holds great promise for the study of stem cell biology and for the development of new approaches for their expansion, differentiation and transplantation. The use of cerium oxide nanoparticles (NC) could be of great interest in this field due to the redox activity of this nanomaterial. Recently, it has been shown that NC could scavenge ROS mimicking the natural enzyme that catalyze the mutation of the superoxide radical anion in living cells and also possess a catalase-like activity decomposing H2O2 in O2 and H2O. NC could be used in vitro with MSCs as regulators of ROS level in order to protect cells from oxidative stress in culture, thus permitting their expansion and the maintenance of their stem potential. The present study is aimed at developing a nanoparticle system for MSCs stimulation and differentiation. Our preliminary biocompatibility findings on the interactions of NC with MSCs demonstrated that the proliferation of MSCs, evaluated up to ten days of culture increases with NC treatment. Moreover, our investigation enabled the establishment of a non-toxic NC concentration that preserves growth and function in MSCs. The analysis of the organization of f-actin microfilament network and of focal adhesion evaluated by immunofluorescent staining, confirmed that NC does not modify the cytoskeleton conformation. These results lead us to assess antioxidant effects and the differentiation potential of the nanoceria on MSCs.

CAPABILITIES OF SUSPENSION PERIPHERAL BLOOD STEM CELLS IN DEVELOPING BONE CELLS

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Peripheral blood is one of the stem cells sources which contained two types of cells, i.e., adherent and suspension (non-adherent) cells. However, study on suspension peripheral blood cells is not as extensive as bone marrow cells. Our aim in this study was to identify the capabilities of murine suspension peripheral blood cells to differentiate into osteoblast, osteoclast and chondrocyte cells. Mononucleated cells were isolated from murine peripheral blood using Ficoll-PaqueTM Plus and in vitro cultured for 15 days. The stemness analysis showed that murine suspension mononucleated cells were positive for hematopoietic stem cell (HSC) markers. The suspension mononucleated cells were subjected for osteoblast, osteoclast and chondrocyte cells differentiations. Differentiation analyses comprise of viability (trypan blue staining), biochemistry (enzyme markers assays), morphology (cells stainings) and molecular biology (reverse transcription polymerase chain reaction; RT-PCR) analyses then were done. The viability analysis of differentiated cells showed that murine suspension cells were able to survive until 10 (osteoclast), 14 (osteoblast) and 21 (chondrocyte) days in the presence of respective differentiation factors without significant increased in the medium. Biochemical analyses for suspension mononucleated cells showed the significant increment ($p<0.05$) of enzyme activities when cultured in osteoblast (Alkaline Phosphatase; ALP), osteoclast (Tartrate Resistant Acid Phosphatase; TRAP) and chondrocyte (ALP) differentiation mediums. When cultured in their respective differentiation mediums, the suspension mononucleated cells showed morphologies like osteoblast, osteoclast and chondrocyte cells after stained by von Kossa, May-Grunwald-Giemsa and toluidine blue, respectively. In addition, the molecular biology analyses towards suspension mononucleated cells differentiation also showed activation of molecular markers for osteoblast (Alp+, Opn+, Ocn+), osteoclast (Trap+, Catk+) and chondrocyte (ColII+, Agcn+) cells. These indicate that murine suspension mononucleated cells able to differentiate into osteoblast, osteoclast and chondrocyte cells. Osteoblasts and osteoclasts are bone cells originated from two

different lineages, i.e., mesenchymal stem cells and hematopoietic stem cells, respectively. While, chondrocyte which is also important in bone development derived from mesenchymal stem cells lineage. In conclusion, the murine suspension peripheral blood cell population has capabilities in multilineage differentiation therefore, can be categorized as multipotent stem cells.

Keywords: suspension mononucleated cells; osteoblast; osteoclast; chondrocyte; bone development.

CLINICAL TRANSLATION OF A TISSUE ENGINEERED URINARY DIVERSION USING A BIODEGRADABLE SCAFFOLD SEEDED WITH ADIPOSE DERIVED SMOOTH MUSCLE CELLS

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Therapeutic cystectomy removes a cancerous or malformed bladder and requires reconstructing a channel to divert urine outside the body. The most common method for creating an incontinent urinary diversion involves harvesting a segment of bowel. However, in ~70% of patients, the use of gastrointestinal tissue in the urinary tract results in long-term co-morbidities that are linked to absorption of excreted metabolites and altered metabolic conditions including acid-base and electrolyte imbalances. Additionally, these patients can suffer from recurrent and chronic urinary tract infections. As an alternative to the use of intestinal tissue, Tengion is developing a urinary diversion which regenerates non-absorptive urinary tissue mucosa from a product known as the Neo-Urinary Conduit™ (NUC). The NUC is produced by seeding an autologous population of adipose-sourced smooth muscle cells (SMC) onto a biodegradable PLGA scaffold. In this presentation, we provide a macroscopic and histological assessment of results from animal studies showing the regenerative outcomes of the NUC. We compare these results to the results from conduits explanted 7 weeks and 7 months post-implantation from two patients enrolled in a Phase 1 clinical study. NUC implantation in a porcine model of cystectomy resulted in the regeneration of an incontinent urinary-tissue-lined diversion within 3 months following implantation. Analysis of the regenerated tissue using immunohistochemical markers specific for urothelium (cytokeratin 7), epithelium, (cytokeratin AE1/AE3) and smooth muscle cell (calponin 1) demonstrated the formation of urinary tissue containing all layers of a native urethra. The luminal surface was covered by urothelium that extended from the ureteral-conduit junction (UCJ) to the mid segment of the NUC, followed by the presence of squamous epithelium extending into the distal end of the conduit to form a native-like mucocutaneus junction (similar in structure and function to native anterior urethra), and ending at the stoma-skin interface. The presence of smooth muscle layers/bundles were visualized by calponin and predominantly observed in the proximal and mid segments of the NUC with less organization towards the distal end and adjacent to the stoma. At 7-weeks post implantation, the human explant showed evidence of early stages of urinary tissue formation consistent with that observed in the porcine model. The presence of urothelium (CK7+) was predominantly observed in the UCJ and extended into the proximal segment of the NUC, followed by squamous epithelium (AE1/AE3+) which extended throughout the remaining luminal surface, including the distal end. Smooth muscle bundles stained positive for calponin and were primarily observed at the UCJ, followed by non-layered smooth muscle cells (calponin +) in the remaining body of the NUC. Additional stromal components observed were described as fibrovascular rich matrix with focal elements of blastema-like structures, proliferating and branching out from the outermost layer (in direct contact with the omentum) towards the mucosal surface. At 7-months post-implantation a mature organ composed of urinary tissue was obtained from a patient. The presence of urothelium was observed in the UCJ and extended towards the proximal, mid and distal segments of the NUC, followed by squamous epithelium near the stomal-end. The tunica muscularis layer was prominently developed in the proximal and mid segments of the NUC, characterized by the presence of layered smooth muscle bundles surrounded by a fibrovascular stroma, followed by a non-layared/bundle (calponin + staining) region involving the distal end of the regenerated conduit's wall. Collectively, the findings in human explants are consistent with the outcomes of a translational porcine model, and support the use of adipose-derived SMC's seeded onto a biodegrade scaffold as an alternative approach to an ileal conduit diversion. The data also suggest that NUC implantation triggered an innate regenerative response. Thus, translation of technology from a porcine model is demonstrated through a native-like tissue regeneration in humans enrolled in a Phase 1 FIH clinical study.

DIRECT LASER WRITING OF 2D/3D NANOSTRUCTURED SCAFFOLDS FOR THE INVESTIGATION OF NEURON/SUBSTRATE INTERACTIONS IN TISSUE ENGINEERING.

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Direct Laser Writing (DLW) is a recent lithographic technique that takes advantage of multiphoton polymerization for the fabrication of 2D/3D nanostructured scaffolds, also suitable for cell culturing. In particular, this tool allows the fabrication of structures exploitable for the study of physical effects of the scaffold surface features on the cell behaviour, and also for the measurement of the forces that cells exert on the substrates.

We designed and fabricated scaffolds for the axonal guidance and for the promotion of the axonal outgrowth, since these two goals play a key role when aiming at obtaining efficient nerve guidance conduits for tissue engineering. Thanks to the photopolymerization of a biocompatible resist (Ormocomp®), we obtained aligned ridges of about 500 nm both in width and in height, at a distance of 2.5 μm from each other. We adopted two different in vitro complimentary neural models, namely rat PC12 cells and human SH-SY5Y cells. We observed that, for the both investigated lines, cells cultured on the nanostructured substrates were characterized by strongly aligned and significantly longer neurites compared to those differentiated on flat control substrates.

As previously mentioned, the scaffold can not only mechanically stimulate the cells, but, viceversa, cells can also produce forces that deform the substrate. Thanks to scanning electron microscopy (SEM), we detected and measured the deformations of the ridges produced by the PC12 axonal branches. We observed that filopodia of about 500 nm in diameter are able to contact the ridges and bend them of about 200 nm. In order to estimate the order of magnitude of the force required to accomplish such a deformation, we applied hydrostatic pressure at a short distance from the ridge via scanning ion conductance microscopy (SICM). In particular, we used a 500 nm diameter pipette, recording the pressure necessary to deform the structures of 200 nm, thus resembling a situation similar to that of cell-induced deformation. Being known the pressure applied by the pipette and its diameter, it was possible to calculate the force required to bend the ridge of 200 nm, that resulted to be of about 3 nN.

In conclusion, we have demonstrated that the multiphoton lithography technique allows the preparation of nanostructured substrates able to improve the neural regeneration and to guide the axons towards a desired target. Furthermore, thanks to scanning probe techniques, we could gain an estimation of the forces exerted by the developing neurites on the nanostructured substrates. We therefore successfully exploited DLW for an in vitro study of neuron/substrate interactions, demonstrating as this technology can be a promising tool for neuronal tissue engineering.

In order to develop artificial nerve conduits for in vivo applications, however, it will be necessary a translation to completely 3D architectures. Because of this, our next step will be the design, the fabrication, and the characterization of complex 3D structures for neuronal cell culturing and differentiation.