**Institutul de Biologie si Patologie Celulara**
**"Nicolae Simionescu"**

**va invita la lucrarile**

# Workshop-ului Exploratoriu

# Tendinte si Emergente in Biologia Celulelor Stem si Cercetarea Embriologica

**Bucuresti, 22-23 Septembrie 2010**

**Organizat in cadrul Conferintei**

## "Diaspora in Cercetarea Stiintifica si Invatamantul Superior din Romania"

**sub inaltul patronaj al**
**Presedintiei Romaniei**

**in parteneriat cu:**
**Ministerul Educatiei, Cercetarii, Tineretului si Sportului,**
**Autoritatea Nationala pentru Cercetare Stiintifica,**
**Consiliul National al Cercetarii Stiintifice din Invatamantul Superior,**
**Unitatea Executiva pentru Finantarea Invatamantului Superior si a Cercetarii Stiintifice Universitare, Camera de Comert si Industrie a Romaniei,**
**Romanian Association of Research Managers and Administrators**

**si Academia Romana**

## *CUVANT INAINTE*

*Dragi prieteni si colegi,*

*In numele echipei Institutului, sunt bucuroasa sa urez bun venit tuturor participantilor la acest Workshop exploratoriu. Este o placere pentru noi, sa va fim gazde si sa va spunem:* ***"bun venit acasa".***

*Va marturisesc ca ma bucur de aceasta inititiva care creeaza cadrul in care sa ne (re)cunoastem, sa ne impartasim experientele, bucuriile si esecurile cercetarii, sperantele si, sa dovedim inca o data, infailibilul optimism al cercetatorilor autentici.*

*Explorarea fenomenelor biologice este una dintre cele mai frumoase aventuri ale cunoasterii, aventura in care multi cercetatori din lumea intreaga, inclusiv din Romania, au avut cutezanta de a incerca si talentul de a reusi.*

*Tematica workshop-ului nostru este foarte actuala si captivanta. Folosirea celulelor stem/progenitoare, de diverse origini, ca pe o unealta terapeutica destinata sa inlocuiasca celule bolnave sau moarte si sa contribuie la refacerea tesuturilor afectate de diverse patologii este o provocare si in acelasi timp o mare speranta de viitor pentru tratamentul diverselor maladii.*

*Sunt sigura ca acum, aici, vom participa la un crampei de stiinta adevarata si vom audia rezultate noi prezentate de excelentii cercetatori, participanti la aceasta conferinta.*

*Pentru noi toti, doresc ca rezultatul acestei intalniri sa fie o mai buna cunoastere a fenomenelor biologice, noi colaborari inter- si trans-disciplinare si o mai mare coeziune a cercetatorilor romani de pretutindeni, prin stiinta si pentru stiinta.*

*Prin inclinatie nativa si multa munca, contributia oamenilor de stiinta romani la dezvoltarea cercetarii mondiale este bine reprezentata in toate domeniile, inclusiv in biomedicina. Din aceste motive, doresc sa* ***dedicam aceasta manifestare stiintifica cercetatorilor romani de pretutindeni, care au contribuit la fundamentarea biomedicinei moderne.***

*Dumneavoastra, tuturor si fiecaruia in parte, bun venit la simpozion, iar celor de departe, bine ati venit in Romania, bine ati venit acasa!*

*Acad. Maya Simionescu*
*Director*
*Institutul de Biologie si Patologie Celulara "Nicolae Simionescu"*

# PROGRAM

## Chair Persons

**Iacob Checiu**, Department of Biology, School of Chemistry-Biology-Geography, University of Western Timisoara, Timisoara, Romania

**Ioan Ovidiu Sirbu**, Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

**Horia Maniu**, Cell Therapy Laboratory, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

# Exploratory Workshop

## "Trends in Stem Cell Biology and Embryology"

***Wednesday, September 22nd, 2010***

9:30- 11:35 **Session 1: The promise of stem cells**

**Moderator: Iacob Checiu**
*University of Western Timisoara, Timisoara, Romania*

**Maya Simionescu:**
*Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
Workshop Opening: "The cell: the elementary patient and the therapeutic tool of molecular medicine of the XXI century"

**Mihnea Ioan Nicolescu / Catalin Gabriel Manole:**
*"Victor Babes" National Institute of Pathology, Bucharest, Romania and Department of Cellular and Molecular Medicine," Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
"Telocytes in non-cavitary organs"/ "The involvement of telocytes in cardiac repair/regeneration after cardiac myocardial infarction"

**Horia Maniu:**
*Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
"Challenges in cryopreservation of fetal and adult stem cells"

**Dimitry Spitkovsky** (funded from "RAMSES" FP7 project, No. 245691):
*Institute of Neurophysiology, University of Cologne, Cologne, Germany*
"Derivation of cardiomyocytes from cryopreserved embryoid bodies"

**Felix Mircea Brehar:**
*"Bagdasar Arseni" Clinical Hospital, Bucharest, Romania*
"Infiltrating growing pattern xenografts induced by glioblastoma and anaplastic astrocytoma derived tumor stem cells"

*11:35-12:00 Coffee Break*

12:00-13:15 **Session 2: Embryology I**

**Moderator: Octavian Voiculescu**
*University of Cambridge, Cambridge, United Kingdom*

**Ioan Ovidiu Sirbu:**
*Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany*
"Retinoic acid controls the balance between Wnt-canonical and PCP signaling in mouse embryo neural ectoderm"

**Tudor Fulga:**
*Harvard Medical School, Boston, USA*
"Understanding microRNA functions in intact multicellular organisms"

**Victor Luria:**
*Columbia University Medical Center, New York, USA*
"Variability and decision-making in the assembly of sensory-motor circuits"

*13:15-14:30 Lunch Break*

14:30-16:10 **Session 3: Insights into angiogenesis and vascular regeneration**

**Moderator: Kurt Pfannkuche**
*Institute for Neurophysiology, University of Cologne, Cologne, Germany*

**Nicanor Moldovan:**
*Davis Heart and Lung Research Institute, Ohio State University, Columbus, USA*
"Vascular stem/progenitor cells in peripheral circulation: Detection, characterization and functions"

**Mihaela Crisan:**
*Erasmus MC Stem Cell Institute, Medical Faculty Rotterdam, Netherlands*
"Blood vessel resident stem cells"

**Marilena Lupu:**
*Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
"Direct contact of umbilical cord blood endothelial progenitors with living cardiac tissue is a prerequisite for vascular tube-like structures formation"

**Mara Pitulescu:**
*Max–Planck–Institute for Molecular Biomedicine, Münster, Germany*
"The LIM domain protein Lmo2 regulates sprouting angiogenesis"

*16:10-16:30 Coffee Break*

16:30-18:10 **Session 4: Tissue Engineering and Regeneration using Mesenchymal Stem Cells**

**Moderator: Irinel Popescu**
*Center of Gastroenterology and Hepatology, "Fundeni" Clinical Institute, Bucharest, Romania*

**Razvan Iacob:**
*University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania*
"Sequential expression of liver enriched transcription factors for hepatic differentiation of adult liver derived progenitor cells"

**Mihaela Chivu:**
*"Stefan S. Nicolau" Institute of Virology, Bucharest, Romania*
"Directing human mesenchymal stem cells to express liver specific genes"

**Irina Titorencu:**
*"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania and*
*Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
"Mesenchymal stem cells in osteobiology and bone regeneration"

**Oana Gavriliuc:**
*"Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania* "Plasticity of human mesenchymal stem cells - *In vitro* assays"

*20:00 Networking Event (organized by the Institute of Cellular Biology and Pathology "Nicolae Simionescu" at the Scientists' House)*

## *Thursday, September 23rd, 2010*

9:30- 11:35 **Session 5: Prospects for cardiac regeneration I**

**Moderator: Nicanor Moldovan:**
*Davis Heart and Lung Research Institute, Ohio State University, Columbus, OH, USA*

**Catalin Toma:**
*University of Pittsburgh Medical Center, Pittsburgh, USA*
"Cardiovascular applications of mesenchymal stem cells"

**Roland Adelmann** (funded from "RAMSES" FP7 project, No. 245691):
*Pediatric Cardiology Department, University of Cologne, Cologne, Germany, and Institute for Neurophysiology, University of Cologne, Cologne, Germany*
"Fibroblasts support functional integration of purified embryonic stem cell-derived cardiomyocytes in avital myocardial tissue"

**Kurt Pfannkuche** (funded from "RAMSES" FP7 project, No. 245691):
*Institute for Neurophysiology, University of Cologne, Cologne, Germany*
"Fibroblasts facilitate the engraftment of cardiomyocytes on collagen type I matrices"

**Alexandrina Burlacu:**
*"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania and Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania*
"Stem cell sources for myocardial regeneration"

**Elisa Liehn:**
*Institute for Molecular Cardiovascular Research, Aachen, Germany*
"Cell therapy in myocardial infarction: present and perspectives"

*11:35-12:00* *Coffee Break*

12:00-13:30 **Session 6: Embryology II**

**Moderator: Ioan Ovidiu Sirbu**
*Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany*

**Octavian Voiculescu:**
*University of Cambridge, Cambridge, United Kingdom*
"Embryo morphogenesis and patterning in higher vertebrates"

**Adrian Salic:**
*Harvard Medical School, Boston, USA*
"Mechanisms of signal transduction in the vertebrate Hedgehog pathway"

**Ioan Ovidiu Sirbu / Octavian Voiculescu:**
*Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany*
*University of Cambridge, Cambridge, United Kingdom*
Future collaboration trends: "Proposal for the establishment of the Romanian Society of Embryology and Stem Cell Biology"

*13:30-14:30* *Lunch Break*

*14:30-15:30* **Poster Session**

15:30-16:30 **Special Session – Part I**

"Criterii de evaluare a cercetarii in Romania pe domenii specifice" – Proiect FSE "Doctoratul in Scoli de Excelenta"

**Simona Dima**
*Center of Gastroenterology and Hepatology, "Fundeni" Clinical Institute, Bucharest, Romania*
"Integration of Romanian cell therapy research in the European area of research and development"

**Moderators:**
**Irinel Popescu**
*Center of Gastroenterology and Hepatology, "Fundeni" Clinical Institute, Bucharest, Romania*
**Octavian Popescu**
*"Babeş-Bolyai" University, Cluj-Napoca, Romania*

*16:30-17:00* *Coffee Break*

17:00-18:00 **Special Session – Part II**

"Criterii de evaluare a cercetarii in Romania pe domenii specifice" – Proiect FSE "Doctoratul in Scoli de Excelenta"

**Moderators:**
**Octavian Popescu**
*"Babeş-Bolyai" University, Cluj-Napoca, Romania*

*20:00* *Networking Event (organized under the auspices of the Romanian President)*

---

***** Due to the international participation, the workshop Program and Abstracts have been typed in English.

# Posters

**1. Microparticles and endothelial progenitor cells as markers of vascular dysfunction induced by combined hypertension and hypercholesterolemia**
**Adriana Georgescu**, Nicoleta Alexandru, Doina Popov, Eugen Andrei, Irina Titorencu, Emanuel Dragan, Maya Simionescu

**2. CAD/CAM fabrication of idealized tissue constructs for dermatological use**
S. Grigorescu, **Rodica Cristescu**, E. Axente, F. Sima, G. Dorcioman, I.N. Mihailescu, A.M. Forsea, O. Gallet, D.B. Chrisey

**3. Insulin-secreting mesenchymal stem cells for type-1 diabetes**
**Oana Gavriliuc**, Adriana Rosca, Valentin Ordodi, Alexandra Gruia, Felix Mic, Florina Bojin, Calin Tatu, Virgil Paunescu

**4. Characterization of human umbilical cord blood- and Wharton's jelly-derived endothelial progenitors for use in cellular therapy**
**Marilena Lupu**, Florin Iordache, Eugen Andrei, Cosmin Buzila, Horia Maniu

**5. ECG recording as a tool for validating myocardial ischemia-reperfusion procedure in mouse model**
**Mihai Bogdan Preda**, Alexandrina Burlacu

**6. Characterization of mesenchymal stem cells isolated from mouse bone marrow**
Ana-Maria Rosca, Ph.D. Student, Alexandrina Burlacu

***7. Calendula officinalis* extracts stimulate adhesion molecules expression and *in vitro* motility of human endothelial progenitor cells**
**Florin Iordache**, Marilena Lupu, Cosmin Buzila, Eugen Andrei, Andrei Constantinescu, Aneta Pop, Horia Maniu

**8. Paracrine properties of human endothelial progenitor cells under hypoxic conditions**
**Gabriela Grigorescu**, Alexandrina Burlacu

**9. Characterization of cryopreserved stem cells isolated from human umbilical cord blood**
**Cosmin Buzila**, Florin Iordache, Marilena Lupu, Eugen Andrei, Horia Maniu

**10. Fractalkine-CX3CR1 interaction on chemotaxis of monocytes towards smooth muscle cells activated with resistin ± high glucose; potential application for cell therapy with CX3CR1+ progenitor cells**
**Viorel Simion**, Ana-Maria Gan, Daniela Stan, Monica Parvulescu, Manuela Calin, Elena Dragomir, Ileana Manduteanu

# ABSTRACTS

# Platform Presentations

## Session 1: The promise of stem cells

### The cell: the elementary patient and the therapeutic tool of molecular medicine of the XXI century

**Maya Simionescu, Ph.D.**
Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
maya.simionescu@icbp.ro

Discoveries of the last 50 years led to the concept that "the cell" is the site of life, diseases and death. All disorders have an origin, a cause or result from dysfunctional cell organelles, molecules and mechanisms. Thus, it is widely accepted today that there is a "cell organelle pathology". Aggressive factors induce a pathology of plasma membrane (all diseases affect the plasmalemma) manifested as disorders of membrane receptors or permeability, defects in ion pumps, in junctional communication, blebbing, removal of carbohydrate coat, lipid peroxidation, expression of new proteins, and others. There is a pathology of ribosomes caused by toxic agents that triggers dispersion of polysomes, or their detachment from the endoplasmic reticulum and aggregation, leading to alteration or shutdown of protein synthesis and cell apoptosis. It was reported a pathology of the endoplasmic reticulum manifested by endoplasmic reticulum storage diseases or faulty protein folding. The pathology of the Golgi complex may lead to defects in the secretion of lysosomal enzymes (absence of the recognition markers for transport to destination). The pathology of mitochondria consists of biochemical defects in substrate utilization, the respiratory chain or in uncoupling of oxidation with phosphorylation, leading to mitochondriomas (benign tumor of mitochondria). The pathology of lysosomes due to absence and/or failure of lysosomal enzymes to digest substrates is known as lysosomal storage diseases (~ 40 diseases are known).

Since the clinical manifested diseases have the origin within the cell, one could consider that the cell is the "elementary patient" and the future therapeutic target. Nowadays, a new, but foreseeable role was ascribed to the cell, namely that of therapeutic tool. Commitment of stem / progenitor cells of various origins to generate specialized cells that are destined to replace the diseased cells is one of the major challenges of the biomedical research. Attempts to use endothelial progenitor cells (for angiogenesis), osteoprogenitors (in bone diseases), to replace diseased or dead cardiomyocytes with viable, functional cardiomyocytes (in myocardial infarction) are all new promising venue for future treatments of numerous diseases.

# Telocytes in non-cavitary organs

**Mihnea Ioan Nicolescu, M.D.**[1,2], M. Gherghiceanu[1], L.C. Suciu[1,2], L.M. Popescu[1,2]
[1] "Victor Babes" National Institute of Pathology, Bucharest, Romania
[2] Department of Cellular and Molecular Medicine,"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
mihnea.nicolescu@gmail.com

During the last five years, our group has shown that a distinct cell population - "telocytes" (previously named by us "Interstitial Cajal-like Cells" – ICLC) is present in the interstitium of several organs. We hereby present highlights of our latest results from the study of this new type of interstitial cell.

Transmission electron microscopy (EM) was performed on pancreas, mammary gland as well as placenta specimens. For placenta and mammary gland study we also used light microscopy of semithin sections, immunohistochemistry, and immunofluorescence of tissue sections.
EM images revealed characteristic telocyte features, which unequivocally distinguishes them from all other interstitial cells. Telocytes have a small cellular body and 2-5 prolongations (telopodes), very thin (most of them below 0.2mm, under the resolving power of light microscopy), extremely long (tens to hundreds of micrometers), with a moniliform aspect (many dilations along), as well as caveolae. Two-dimensional reconstruction from serial photos suggest a network-like spatial distribution of TC. Most of the telocytes are positive for c-kit, vimentin, as well as for caveolin-1.

Telocytes seem to be key players in the formation and arrangement of interstitial network(s). However, further studies are required in order to distinctly characterize the mechanisms that underlie the telocytes behavior and function.

For supplemental materials on telocytes, please visit www.telocytes.com.

# The involvement of telocytes in cardiac repair/regeneration after cardiac myocardial infarction

**Catalin G. Manole, M.D.**[1,2], Mihaela Gherghiceanu[1,2], Laurentiu M. Popescu[1,2]
[1] Department of Cellular and Molecular Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] "Victor Babes" National Institute of Pathology, Bucharest, Romania
catalin.manole@jcmm.org

Most people perceive the myocardial pump as only consisting in contractile units and fibroblasts. Although the structure and function of contractile cardiomyocytes are quite well known, those of heart interstitium are not. In the last years we also demonstrated the presence of a distinct cell population within the interstitial space of the heart. We named them telocytes (by using the Greek affix '*telos*'). The presumptive roles of these newly discovered cells are essential for myocardial life, renewing, repair or death. The questions we would like to answer are: what **telocytes** are?, what are their roles? and whether are they implicated in heart renewal/repair?

We used transmission and scanning electron microscopy (EM), cell cultures and immunocitochemistry of normal and pathological heart tissue samples, from various species (including humans). Experimental myocardial infarctions in rats were also performed.

EM and cell cultures revealed typical immunophenotype and ultrastructural features of telocytes, as they were previously described in heart and other cavitary and non-cavitary organs. The characteristics that unequivocally distinguish telocytes from all other interstitial cells are: a small cellular body with 2-5 abruptly emerging prolongations – **telopodes**; telopodes with a very thin (most of them below 0.2μm, under the resolving power of light microscopy) and extremely long (tens up to hundreds of micrometers), with a moniliform silhouette (with many dilations along), as well as caveolae. The distribution and relationships of telocytes within endocardium, myocardium, and epicardium suggest a interstital network-like spatial arrangement of them.

Cardiac regeneration was previously revealed at some animal species (*e.g. zebra fish or newts),* their heart containing a great number of telocytes. Epicardial telocytes, cardiac stem cells and cardiomyocyte progenitors sustain a continuous cardiac renewal process from the so-called cardiac stem cells niches (CSCN). Within CSCN, telocytes could be considered as "nursing cels". Beside cardiac resident stem cells, telocytes might be perceive as active players in cardiac remodeling/renewing, and should candidate for further therapeutic cardiac regeneration protocols based on local autologous cells.

# Challenges in cryopreservation of fetal and adult stem cells

**Horia Maniu, Ph.D.**
Cell Therapy Laboratory, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
horia.maniu@icbp.ro

The regenerative cellular therapy using fetal - umbilical cord blood and adult - bone marrow stem cells represent an innovative treatment strategy, less invasive and less expensive, addressing a large spectrum of chronicle degenerative conditions, which has severe socio-economic consequences and high premature death percentage, necessitating hospitalization and recovery procedures, forcing a desperate effort and extraordinary medical costs. A stem cell bank can be a source of cells for basic research, but at the same time, it may provide a rich, well characterized cryopreserved isolated biologic material with high clinical relevance. Moreover, in a stem cell bank are developed cell therapies strategies starting with cryopreserved units of standardized stem cells and finishing with clinical procedures of autologous transplantation applied in the regenerative medicine field. The purpose of a stem cell bank is to offer stable fetal or adult SC lines, obtained in ethical conditions, tested and thoroughly characterized by standard quality tests, meant to warrant their authenticity, purity, performance and quality for research, as well as their therapeutic value. The final objective of stem cell banking is to have the possibility to use umbilical cord blood and adult bone marrow stem cells stored units with research and clinical value as a stock of transplantable cells. New knowledge will be presented concerning isolation techniques, testing, characterization, cryopreservation and cellular differentiation, as well as the strategies for standardization of tissue engineering studies, gene therapy and tissue regeneration in degenerative diseases.

# Derivation of cardiomyocytes from cryopreserved embryoid bodies

V. Turchyn, J. Hescheler, **Dimitry Spitkovsky, Ph.D.**
Institute of Neurophysiology, University of Cologne, Cologne, Germany
dimitry.spitkovsky@uni-koeln.de

Embryonic stem (ES) cells are capable to differentiate into any somatic cell type and therefore they are of interest for future downstream applications in regenerative medicine. Derivation of cardiomyocytes (CMs) attracted particular attention due to the fact that cardiovascular diseases are the major death course in the Western hemisphere. In experimental animal models it was demonstrated that ES cell-derived CMs could lead to significant heart function improvement after their transplantation into infarcted heart. Additionally CMs could serve as a valuable cell source for toxicology screening in new drugs development. For potential applications it is important developing standardized and scalable clinically complied cardiomyocytes differentiation protocols. One of existing challenges for developing CMs doses is a relatively long duration of CMs generation starting from available ES stocks, and this may not be compatible with immediate clinical requirements. Direct cryopreservation of CMs could be one of possible solutions of the problem. Nevertheless CMs are particularly vulnerable to cryopreservation with only about 50% survival rate. Furthermore the survived CMs could potentially accumulate cryopreservation-associated damages compromising their function. Moreover current CMs cryopreservation procedures are relied on single cell CMs cryopreservation, while CMs engraftment is better supported after transplantation of cardiac clusters with tight cell to cell contacts (pseudo cardiac tissue) which could be isolated in the course of cardiac differentiation of ES cells. In order to reduce timing required for on demand CMs generation, we have evaluated a possibility to cryopreserve pre-differentiated ES cells. We have demonstrated that under defined conditions cryopreserved embryoid bodies are capable to survive cryopreservation and develop into functional CMs.

*This work was supported in part by the EU project "CRYSTAL"*

# Infiltrating growing pattern xenografts induced by glioblastoma and anaplastic astrocytoma derived tumor stem cells

**Felix Mircea Brehar, M.D.**, A.V. Ciurea, A. Tascu, O. Zarnescu, C. Bleotu, D. Dragu
"Bagdasar Arseni" Clinical Hospital, Bucharest, Romania
felixbrehar@yahoo.com

**Objective:** The number of evidences regarding the role of tumor stem cells (TSC) in the initiation and progression of high-grade astrocytomas became more and more numerous in the last years. This issue has been intensively tested in glioblastoma, but little attention has been paid for anaplastic astrocytoma. The main objective of this paper was to study the morphological characteristics of the xenografts developed from glioblastoma and anaplastic astrocytoma derived cancer stem cells. **Methods:** The authors of this study successfully isolated and partially characterized primary cultures of glioblastoma and anaplastic astrocytoma derived TSC. Tumors stem cells have been stereotactically inoculated in nude mice brains and the xenografts have been studied using morphological and imunohistochemistry techniques. **Results:** The tumor xenografts, which have been established in nude mice using TSC, had different characteristics when compared with U87 xenografts previously developed by our group, and depend on the origin type of the tumors (glioblastoma versus anaplastic astrocytoma). The diffuse growing pattern and cells infiltration have been more pronounced in both anaplastic astrocytoma and glioblastoma derived TSC xenografts compared with U87 line xenografts. **Conclusion:** Our results support the hypothesis regarding the role of TSC in the infiltration process of glioblastoma and anaplastic astrocytoma. The extensive infiltration growing patterns of these types of xenografts make them useful models for studying the invasion mechanisms in gliomas. **Key words:** glioblastoma, anaplastic astrocytoma, tumor stem cells, nestin, xenografts. **Abbreviations:** TSC - tumor stem cells

## Session 2: Embryology I

### Retinoic acid controls the balance between Wnt-canonical and PCP signaling in mouse embryo neural ectoderm

**Ioan Ovidiu Sirbu, M.D., Ph.D.**
Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany
ovidiu.sirbu@uni-ulm.de

Retinoic acid (RA) signaling has been shown to be essential for axial elongation and AP patterning of vertebrate embryos through a mechanism involving attenuation of FGF signaling. Here we show that RA produced in the paraxial mesoderm regulates the expression of Fgfr1 and Planar Cell Polarity (PCP) genes Vangl2 and Fzd3 in the neural ectoderm of mouse embryos. Using Xenopus laevis embryos and P19C6 embryocarcinoma cells as alternative experimental models, we show that Fgfr1 relays the action of the RA signal upon Vangl2, Fzd3 and Fgfr1, a mechanism that we suggest to be conserved in evolution. Furthermore, we found that disruption of PCP signaling in the neural ectoderm of Raldh2-/- embryos is accompanied by a strong up-regulation of β-catenin/TCF/LEF signaling. Our study identifies RA as the first long-range non-Wnt signaling molecule required for the maintenance of the balance between canonical Wnt-β-catenin/TCF/LEF signaling and non-canonical Wnt/PCP signaling in the neural ectoderm of vertebrate embryos.

# Understanding microRNA functions in intact multicellular organisms

**Tudor Fulga, Ph.D.**
Cell Biology Department, Harvard Medical School, Boston, USA
Tudor_Fulga@hms.harvard.edu

The discovery of microRNAs added an additional level of complexity to the landscape of gene expression regulation in metazoan and plant species. High-throughput sequencing led to the identification of hundreds of microRNAs in species from *C. elegans* and *Drosophila* to humans, and these numbers are predicted to increase. As the diversity of microRNA identity and expression patterns unfolds, biologists face an increasing challenge to discern microRNA contributions in highly dynamic or complex biological processes. Surmounting this obstacle requires tools capable of disrupting their function with precise spatial and temporal specificity. We developed a novel method (transgenic microRNA sponges or miR-SP) designed to allow for the first time efficient spatiotemporal inhibition of microRNA function from the entire living organism to a single tissue, organ or cell type, at any stage during development or adult life. We provide evidence indicating that this technology can provide a rapid mean to understand the full repertoire of microRNA functions across a complex spectrum of developmental, physiological and behavioral processes. In addition, by harnessing the miR-SP technology, we uncovered the cellular and molecular logic by which *Drosophila* miR-8 controls synaptic morphogenesis and acts to promote presynaptic growth by limiting postsynaptic expression of the actin-regulatory protein Enabled. We also demonstrate that the miR-SP technology can be used to genetically dissect and define the cellular logic governing microRNA target regulation. Combined with quantitative genome and proteome-wide expression analysis, miR-SPs can yield essential information regarding *in vivo* targets that are differentially regulated in a particular tissue, developmental stage or process. Given that miR-SPs rely on a bipartite modular expression system, they could be used to elucidate the endogenous function of microRNAs in any species where conditional expression can be achieved.

# Variability and decision-making in the assembly of sensory-motor circuits

**Victor Luria, Ph.D.**
Department of Genetics and Development, Columbia University Medical Center,
New York, USA
avl@columbia.edu

I am interested in how gene expression variability may influence cellular decision-making during the ASSEMBLY and DIS-ASSEMBLY of neural circuits, at the level of trajectory selection by axonal growth cones. To address this question I study sensory-motor circuits whose output is spinal motor neuron-elicited muscle contraction.

First, I found the cellular rules (1) and the guidance molecules (EphBs, ephrin-Bs) that control the innervation of ventral limbs in vertebrates (2). Second, I showed that mutations in Ephs and ephrins result in motor axon growth along inappropriate dorsoventral trajectories in the limb and ultimately incorrect sensory-motor circuit topology. Interestingly, in animals carrying multiple Eph mutations these phenotypes are inordinately variable between mutant individuals, implying that large numbers of motor axons select the same dorsoventral limb trajectory (2). However, the molecular mechanisms underlying such variability (2, 3) have not been elucidated.

To gain insight into this question, first, I started carrying an experimental (genetic, cellular) and computational analysis of stochastic decision-making in motor axon pathfinding during circuit ASSEMBLY. The analysis is based on our computational model of axon decision-making as controlled by genes. The genetic experiments involve the analysis of compound EphA & EphB mutants. Using our validated methods (4), I have started measuring the level and noise of guidance gene expression in at single-cell level, examining cellular decisions taken by these cells and observing the phenotypic output. In EphA & EphB compound mutants, we have also started to evaluate the obvious gait defects, which are progressively more severe as the mutational load increases - thus linking changes in molecular levels and variability to cellular decision-making and ultimately to behavioral phenotypes. I have also started developing a cell-based guidance assay aimed at quantifying the strength of surface-bound guidance cues, alone or in combination, using fluorescent motor neurons derived from embryonic stem cells.

Second, to understand decision-making during the DIS-ASSEMBLY of sensory-motor circuits, I developed a genetic model of motor loss. Whichever way humans and other animals lose their motor neurons, whether to age or disease, they end with mismatched populations of sensory and motor neurons. We hypothesized this imbalance results in compromised sensory-motor connectivity in the spinal cord, explaining the striking loss of movement precision in elderly or diseased individuals who have lost most of their motor neurons.
We started to quantify the extent of neuronal connectivity changes and of motor behavior defects in mice with various levels of genetic ablation of motor neurons.

This integrated computational and experimental work suggests that molecular variability at gene expression level is translated into decision-making defects at cellular level, and ultimately into movement behavior defects, thus linking molecular variability to circuit emergence and function.

## Session 3: Insights into angiogenesis and vascular regeneration

### Vascular stem/progenitor cells in peripheral circulation: detection, characterization and functions

**Nicanor I. Moldovan, Ph.D.**
Davis Heart and Lung Research Institute, Ohio State University, Columbus, USA
Nicanor.Moldovan@osumc.edu

Evidence for the existence of a small number of non-terminally differentiated cells in peripheral circulation is mounting. However, there is little consensus about their phenotype and roles. Over the past decade, my laboratory contributed with a number of observations, methods and concepts to the progress in this field. Here I will illustrate this activity with the analysis of colonization of the subcutaneous Matrigel plug in the mouse, leading to formation of 'cell columns' and 'fibro-vascular bundles' as precursors of neovascularization in this hydrogel. Then I will propose that the cell colonies obtained *in vitro* from the mononuclear fraction of blood represent in fact the two-dimensional equivalent of these cell columns, a hypothesis in process of being experimentally demonstrated in our group. In this regard, I will also describe *CellTrap*, a novel solid phase platform we are developing for capture and characterization of circulating cells with colony-forming capacity. Finally, I will address the biochemical and biomechanical constrains experienced by circuiting progenitor cells during recruitment and engraftment in tissue areas in need of repairing, and suggest means to overcome them in the benefit of improved therapies with cardiovascular applications.

# Blood vessel resident stem cells

**Mihaela Crisan, Ph.D.**
Erasmus MC Stem Cell Institute, Medical Faculty Rotterdam, Netherlands
m.crisan@erasmusmc.nl

Mesenchymal stem cells (MSCs), the archetypal multipotent progenitor cells derived in cultures of multiple organs, are of unknown identity and native distribution. We have prospectively identified perivascular cells, principally pericytes, in multiple human organs including skeletal muscle, pancreas, adipose tissue, and placenta, on CD146, NG2, and PDGF-Rb expression and absence of hematopoietic, endothelial, and myogenic cell markers. Perivascular cells purified from skeletal muscle or non muscle tissues were myogenic in culture and *in vivo*. Irrespective of their tissue origin, long-term cultured perivascular cells retained myogenicity; exhibited at the clonal level osteogenic, chondrogenic, and adipogenic potentials; expressed MSC markers; and migrated in a culture model of chemotaxis. Expression of MSC markers was also detected at the surface of native, non cultured perivascular cells. Thus, blood vessel walls harbor a reserve of progenitor cells that may be integral to the origin of the elusive MSCs and other related adult stem cells. Blood vessels are also at the origin of the hematopoietic stem and progenitor cells. Cells from human placenta are able to regenerate the blood cell lineages when injected into hematopoietic-ablated recipient mice. Stromal cell lines generated from human placenta at several developmental time points are pericyte-like cells and support human hematopoiesis. Immunostaining of placenta sections during development localizes hematopoietic cells in close contact with pericytes/perivascular cells. Thus, the human placenta is a potent hematopoietic niche throughout development. Importantly, stromal pericyte-like cells isolated from human cancer prostate support tumor formation *in vivo.*

# Direct contact of umbilical cord blood endothelial progenitors with living cardiac tissue is a prerequisite for vascular tube-like structures formation

**Marilena Lupu, Ph.D.**
Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
marilena.lupu@icbp.ro

**Introduction:** The umbilical cord blood derived endothelial progenitor cells (EPCs) contribute to vascular regeneration in experimental models of ischemia. However, their ability participate to cardiovascular tissue restoration has not been elucidated, yet. **Objective:** We employed a novel coculture system to investigate whether human EPCs have the capacity to integrate into living and ischemic cardiac tissue, and participate to neovascularization. **Materials and Methods:** EPCs were cocultured with either living or ischemic murine embryonic ventricular slices, in the presence or absence of a pro-angiogenic growth factor cocktail consisting of VEGF, IGF1, EGF, and bFGF. Tracking of EPCs within the cocultures was done by cell transfection with green fluorescence protein or by immunostaining using anti-human vWF, CD31, nuclei, and mitochondria antibodies. **Results:** EPCs generated vascular tube-like structures in direct contact with the living ventricular slices. Furthermore, the pro-angiogenic growth factor cocktail reduced significantly tubes formation. Coculture of EPCs with the living ventricular slices in a transwell system did not lead to vascular tube-like structures formation, demonstrating that the direct contact is necessary and that the soluble factors secreted by the living slices were not sufficient for their induction. No vascular tubes were formed when EPCs were cocultured with ischemic ventricular slices, even in the presence of the pro-angiogenic cocktail. **Conclusion:** EPCs form vascular tube-like structures in contact with living cardiac tissue and the direct cell-to-cell interaction is a prerequisite for their induction. Understanding the cardiac niche and microenvironmental interactions that regulate EPCs integration and neovascularization are essential for applying these cells to cardiovascular regeneration.

**Acknowledgements:** This work was supported by the EU Framework Programme 7 (FP7) - Marie Curie international reintegration grant No. 224888/2008-2010; Romanian Ministry of Education and Research reintegration and cooperation PNCDI-II grants No. 2/2008-2010 and No. 31042/2007-2010, respectively; EU FP7 - Capacity grant No. 245691/2010-2013; Romanian Ministry of Education and Research/German Federal Ministry of Education and Research (BMBF) bilateral cooperation grant No. 347/2009-2010, and BMBF grant No. 01GN0947.

# The LIM domain protein Lmo2 regulates sprouting angiogenesis

**Mara E. Pitulescu, Ph.D.**, Ralf H. Adams
Department of Tissue Morphogenesis, Max–Planck–Institute for Molecular Biomedicine, and Faculty of Medicine, University of Münster, Münster, Germany
mpitule@gwdg.de

During physiological growth and regeneration as well as in pathological processes such as tumor growth, metastasis, ischemia and inflammation, angiogenesis leads to the formation of new vessels from pre–existing vascular beds. This fundamental process involves endothelial cell proliferation, sprouting, migration and formation of new vascular connections. The vascular endothelial growth factors (VEGFs) stimulate sprouting of endothelial tip cells by activating their tyrosine kinase receptors. Notch signaling acts opposite and inhibits this process. However, the crosstalk of these two pathways remains poorly understood. Here we show an important role of LIM domain only 2 (Lmo2), a critical transcriptional regulator of hematopoietic stem cell development and leukemia, in vascular growth. Lmo2 is prominently expressed in the growing retinal endothelium including tip and stalk cells at the angiogenic front. In the absence of Lmo2 in postnatal endothelium, sprouting angiogenesis is compromised in the mouse retina. Sprouts, branching points and endothelial cell proliferation were reduced. Hinting at an important link to key angiogenic pathways, VEGF and Notch signaling components were down–regulated in cultured endothelial cells when Lmo2 expression was reduced by siRNA knock down. Our results indicate that Lmo2 is an important regulator of angiogenesis that might link two main signaling pathways.

## Session 4: Tissue Engineering and Regeneration using Mesenchymal Stem Cells

# Sequential expression of liver enriched transcription factors for hepatic differentiation of adult liver derived progenitor cells

**Razvan Iacob, M.D.**[1,2], U. Ruedrich[1], M. Rothe[1], S. Kirsch[1], M. Iken[1], I. Popescu[2], M. Manns[1], M. Ott[1], M. Bock[1]

[1] Hannover Medical School, Hannover, Germany

[2] University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

razvan.iacob@icfundeni.ro

**Introduction:** Adult liver derived progenitor cells (ALDPC) can be clonally selected from hepatocyte cultures and massively expanded. The hepatic differentiation potential by standard protocols is limited to an immature hepatocyte-like phenotype. **The aim** of our study was to induce hepatocyte differentiation in ALDPCs, by sequential expression of liver enriched transcription factors. **Methods:** ALDPCs were obtained by "plate and wait" technique from adult mouse hepatocyte cultures. The genetic sequences encoding murine FoxA2, Hnf4α and C/EBPα were cloned into lentivirus vectors and sequentially expressed in target cells, in a two step protocol. Liver specific gene expression was assessed by qRT-PCR for 12 liver specific genes. Albumin and AAT secretion were assessed by ELISA, glycogen storage ability and ureagenesys were also investigated and compared to adult mouse hepatocyte 24 hours cultures. **Results:** A FoxA2 transgenic ALDPC population was generated under puromycin selection. Furthermore, the cells were co-transduced with Hnf4α and C/EBPα lentiviruses and hepatic differentiation was assessed at day 7 post-transduction. By morphology, double (FoxA2, HNF4$\alpha$) and triple transduced cells (FoxA2, Hnf4α and C/EBPα) closely resembled binucleated adult hepatocytes. Twelve mRNA´s coding for apoliproteins, cytochrom p450 isoenzymes, liver metabolic enzymes, secreted proteins were detected by qRT-PCR at levels close to controls. Albumin secretion increased incrementally in single (Foxa2), double (Foxa2, Hnf4α) and triple transduced cells (Foxa2, Hnf4α, C/EBPα) reaching control levels**.** AAT secretion reached control levels after FoxA2 transduction and was not further up-regulated by the other transcription factors. Glycogen storage, as determined by PAS staining, was present in double and triple transduced cells. Ureagenesis was also induced in triple transduced cells, but at lower levels compared to the primary hepatocytes. **Conclusion:** Sequential expression of FoxA2, Hnf4α and C/EBPα induces a mature hepatocyte-like phenotype in an expandable liver derived progenitor cell line.

# Directing human mesenchymal stem cells to express liver specific gene

**Mihaela Chivu, Ph.D.**[1], Simona O. Dima[2], Cosmin I. Stancu[1], Camelia Dobrea[3], Valentina Uscatescu[2], Laura G. Necula[1], Coralia Bleotu[1], Cristiana Tanase[3], Carmen Ardeleanu[3], Irinel Popescu[2]

[1] "Stefan S. Nicolau" Institute of Virology, Bucharest, Romania

[2] "Fundeni" Clinical Institute of Digestive Disease and Liver Transplantation, Bucharest, Romania

[3] "Victor Babes" National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania

mihaela.chivu@virology.ro

**Introduction:** Obtaining unlimited number of human hepatocytes that can be used for clinical purposes in patients with liver failure is one of the major aims of current translational research. Insulin-transferrin-selenium (ITS), nicotinamide (NTA), dexamethasone (Dexa), and growth factors like hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF), are important factors in the hepatocyte differentiation pathway. **Objective:** The present study intended to determine the efficacy of these compounds in inducing hepatic differentiation. **Materials and Methods:** We designed a two-step protocol in which human mesenchymal stem cell (hMSC) cultures were pre-conditioned in low-serum media with ITS, FGF, and EGF for 3 days, and thereafter exposed to different serum-free media containing the differentiation compounds added individually or in various combinations. At different time points, cell cultures were investigated to determine the ability of each protocol in inducing the transcription of specific genes that are essential for hepatocyte differentiation: albumin (ALB), cytokeratin 19 (CK19), alpha-fetoprotein (AFP), and Nestin. **Results:** AFP, Nestin, CK19, and ALB were expressed in a time-dependent manner during differentiation. The mRNA levels of immature hepatocyte markers in hMSCs (AFP, Nestin, and CK19) decreased as differentiation progressed, and were higher in cultures induced with FGF+EGF, HGF, NTA, or Dexa alone. The expression profiles of ALB, HepPar-1, CK19, and AFP demonstrated that when HGF, NTA, or Dexa were added individually, an incomplete hepatocyte differentiation was achieved and the obtained cell populations contained progenitors that expressed both hepatic (ALB) and biliary (CK19) markers, together with AFP. Several functional tests were also performed on *in vitro* differentiated hepatocyte-like cells. The results demonstrated that the obtained cells acquired in time functions assign to liver cells. They were able to produce and accumulate glycogen and to synthesize and secrete urea, glucose and albumin at different levels depending on the culture condition. **Conclusion:** Considering the results obtained from the functionality tests and gene expression assays, we may argue that HGF and NTA were the factors with the most hepatogenic-induction potential. When all factors were added together, the cells became more committed to the hepatic lineage, showing increased levels of ALB and HepPar-1 expression, but not CK19. Inducing the differentiation of hMSC by *in vitro* manipulation may become a valuable tool to provide a cell source for liver transplant procedures, liver development studies, and pharmacological research.

# Mesenchymal stem cells in osteobiology and bone regeneration

**Irina Titorencu, Ph.D.**[1,2] ; V.V. Jinga[2]; E. Constantinescu[2]; A.V. Gafencu[2]; C. Zaharia[3]; M. Albu[4]; A. Vladescu[5]; M. Simionescu[2]
[1] "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania
[2] Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology, "Nicolae Simionescu", Bucharest, Romania
[3] Clinical Institute "Colentina", Bucharest, Romania
[4] Leather and Footwear Research Institute, Bucharest, Romania
[5] National Institute for Optoelectronics, Bucharest, Romania
irina.titorencu@icbp.ro

**Introduction and Aim:** Bone marrow-derived mesenchymal stromal cells (MSC) represent a potential material for cellular therapy used in a variety of disorders, including bone healing deficiencies. To this aim, osteoprogenitor cells (OPC) from human bone marrow were isolated and their capacity to proliferate and differentiate in vitro to osteoblasts was estimated. Also we study in vitro colonization of different biocompatible materials and alloys with osteoprogenitor cells (OPC) as supports for autografting. **Methods:** Human MSC were separated on Histopaque and cultured in DMEM supplemented with 15% human serum AB Rh negative. To induce differentiation towards osteoblasts, the cells in culture were exposed to 10-7M dexamethasone (dexa) or/and 10-3M sodium β-glycerophosphate (β-GlyP). In other experiments, after this treatment, the cells were incubated for 48h with 1, 25-dihydroxyvitamin D3 (calcitriol) or 9-cis-retinoic acid (9-RA). At 7, 14, 21 days, alkaline phosphatase (AP) activity, calcium deposits, the expressions of osteocalcin, bone sialoglycoproteins (BSP), osteonectin and cellular ultrastructure were assessed. The colonization capacity of the cells on different collagen matrices and alloys coated with different (Zr,Ti)CN substrates was monitored by fluorescence microscopy and by transmission electron microscopy and the viability by MTT assay. **Results:** The AP activity was detected in cells with or without dexa and / or β-GlyP treatment. After 14 days of dexa and β-GlyP treatment, the initiation of extracellular calcium deposition was observed. Gene and protein expression of osteonectin and BSP increased under the combined dexa and β-GlyP treatment. Osteocalcin gene expression was induced only after the additional treatment with calcitriol or 9-RA. BSPI gene expression was induced progressively having its maximal after 3 weeks of combined treatment. Ultrastructural analysis revealed the secretory phenotype of OPC, a feature that was maintained during the dexa/β-GlyP treatment, and the presence of large vesicles containing electron-dense structures – representing most likely calcium deposits. In vitro biological tests demonstrated that OPC developed on those collagen matrices and alloys with different substrates. Ultrastructural aspects of the cells integrated within matrix pores were unmodified compare to the control (OPC grown on culture plates). **Conclusion:** Under appropriate treatment, MSC can be induced to give rise to OPC that have the capacity to differentiate into osteoblasts characterised by the express of a variety of osteogenic markers and osteoblastic properties. These cells may represent a promising material to be utilised in orthopedic cellular therapy.

# Plasticity of human mesenchymal stem cells – *In vitro* assays

Gabriela Tanasie, **Oana Gavriliuc, M.D., Ph.D.,** Florina Bojin, Simona Anghel,
Calin Adrian Tatu, Carmen Bunu, Virgil Paunescu
"Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania
gtanasie@umft.ro

**Introduction:** The mesenchymal stem cells (MSCs) role in the adult human body is the generation of mesenchymal cell lines and they are involved in development, maintaining and restoration of connective tissues. The concept of plasticity means the property of stem cells to differentiate in a distinct cell line apart from the originated tissue. The *in vitro* differentiation techniques are based on using a differentiation agent, coculture with specific cells or structures and modification in some gene expression. **Materials and Methods:** Bone marrow samples (n=8) were harvested by drilling the femoral bone of patients suffering joint replacement. The writing informed consent from the patients was obtained before the experiments. The MSCs were isolated using plastic adherence procedure and the cell growth characteristics were evaluated by morphological study and surface markers analysis. MSCs at third passage were used in plasticity assays. The protocols used for *in vitro* MSCs differentiation were based on biochemical induction, using various combinations of growth factors, cytokines and other supplements. The presence of specific markers for osteoblastic, adipocytic, chondrogenic, myogenic, epithelial and neuronal lineage was evaluated by immunecitochemistry, immune fluorescence; RNA extraction and RT-PCR analysis of gene expression have also been performed. **Results:** Lineage characteristic markers were indentified for osteoblastic (cbfa1, alkaline phosphatase, osteonectin, osteopontin) adipocytic (FABP4, lipoprotein lipase, PPARgamma), chondrogenic (aggrecan, collagen II, collagen X), myogenic (myogenin, MYF5), epithelial (E-cadherin, Cytokeratin) and neuronal lineage (beta III tubulin, GFAP, oligodendrocyte marker 4). **Conclusions:** Human adult MSCs are able to differentiate *in vitro* in the mesodermal cell types but also they can be induced in the cell lineages belonging to other embryonic layers. The differentiated cells expressed specific lineage markers but they seem to be incompletely maturated. MSCs represent the best alternative for cell therapy applications: they have self-renewal, great plasticity with differentiation potential in functional cell lines.

## Session 5: Prospects for cardiac regeneration I

# Vascular delivery of Mesenchymal Stem Cells for therapeutic applications: current barriers and potential solutions

**Catalin Toma, M.D.**
University of Pittsburgh Medical Center, Pittsburgh, USA
tomacx@UPMC.EDU

Therapeutic vascular delivery of mesenchymal stem cells (MSC) is being explored in clinical trials for a variety of conditions, however little is know about the fate of MSCs in the microcirculation and their mode if tissue integration. Systemic delivery of MSCs leads to entrapment of the majority of the cells in the lungs during first pass. We are hereby reporting on our research on the fate of MSCs in the microvasculature, and our efforts at bioengineering an MSC with a favorable rheologic profile. Intravital microscopy revealed that the 92 ±7% of the arterially delivered MSCs arrest during the first pass at precapillary level, with interruption of flow. This translated to decreased flow at the level of feeding resistance arteries (velocity pre-injection 6.3±1.0 mm/s, postinjection 4.6±1.3 mm/s, $p<0.001$, $n=6$). MSCs deformability was evaluated using filtration through polycarbonate membranes, revealing that the cortical tension of MSCs (0.49±0.07 dynes/cm, $n=9$) was not different from that of blood mononuclear cells (0.5±0.05 dynes/cm, $n=7$). When intravital microscopy was performed days following the injection, the number of MSCs in the cremaster decreased dramatically by 72 hrs, due to cell death *in situ* as indicated by nuclear condensation and cell fragmentation. *In vivo* labeling of the basement membrane revealed that at 24 hrs, the surviving cells were spread out on the luminal side of the microvessel, while at 72 hrs they integrated in the microvascular wall, resembling pericytes. These data clearly indicate that despite their relative deformability, MSCs entrap at the pre-capillary level during the first pass due to their large size (23 μm diameter), with a small proportion of surviving cells integrating in a perivascular niche. The large size of these cells is a consequence of culture expansion, as the MSCs are smaller in their native state. To improve on the vascular deliverability of MSCs, we have developed a suspension culture system for expanding these cells on microcarrier beads, resulting in significantly smaller cells (14 μm diameter), capable of transiting the microvasculature in greater numbers compared to standard MSCs. We are currently investigating the potential advantage of suspension-expanded MSCs in terms of tissue integration and survival compared to standard MSCs.

# Fibroblasts support functional integration of purified embryonic stem cell-derived cardiomyocytes in avital myocardial tissue

**Roland Adelmann, M.D.**
Pediatric Cardiology Department, University of Cologne, Cologne, Germany, and
Institute for Neurophysiology, University of Cologne, Cologne, Germany
roland.adelmann@uk-koeln.de

**Introduction:** Transplantation of pluripotent stem cell-derived cardiomyocytes (ES-CMs) into damaged myocardium might become a therapy to improve contractile function after myocardial infarction. However, current knowledge on the mechanisms of cell integration and processes of physiological reconstitution as well as mechanical and electrical coupling after transplantation into the host tissue is still fragmentary. Undifferentiated cells remain after *in vitro* differentiation and bare the risk of tumorgenicity when transplanted. This could be overcome by lineage-selection of differentiated cells. Puromycin-selection of ES-CMs gives rise to highly purified populations without tumor-forming progenitors. Previous own experiments suggested that purified murine ES-CMs did not integrate as well as the non-purified human ES-CMs. There is cumulating evidence reporting beneficial effects of cell transplantation strategies combining a source for CMs with other cell types. From these observations we hypothesized that non-myocytes might be necessary for integration. **The aim** of this study was to investigate whether murine embryonic fibroblasts (MEF) support the functional integration of purified embryonic stem cell-derived cardiomyocytes ES-CMs. **Methods:** Neonatal murine ventricular tissue slices were subjected to irreversible simulated ischemia using oxygen and glucose deprivation. Vital tissue slices served as control. Vital and avital tissue slices were co-cultured with puromycin-selected ES-CM clusters with or without MEF. Integration of ES-CM clusters was assessed morphologically by immunohistochemistry and electromicroscopy, functional integration by isometric force measurements. Migratory activity of ES-CMs with and without MEF was analyzed by time lapse microscopy. **Results:** ES-CMs without MEF showed no motility at all whereas ES-CMs with MEF demonstrated a high motility with a velocity of 10.30 ± 1.41µm/h. The mobility of the ES-CMs was caused by the attachment to the moving MEF. We observed that ES-CM clusters integrated morphologically into vital but poorly into avital slices. Adding MEF to the co-cultures improved morphological integration into irreversibly damaged slices and enabled purified ES-CMs to confer force. **Conclusion:** We conclude that non-cardiac cells like MEF support morphological integration and force transmission. The adhesion of ES-CMs to fibroblasts and the subsequent enablement of migration could be an additional mechanism of improved engraftment.

# Fibroblasts facilitate the engraftment of cardiomyocytes on collagen type I matrices

**Kurt Pfannkuche, Ph.D.**
Institute for Neurophysiology, University of Cologne, Cologne, Germany
kurt.pfannkuche@uni-koeln.de

There is growing interest in purified cardiomyocytes derived from embryonic stem cells for *in vitro* or *in vivo* tissue engineering. Up to date, the role of non-cardiac cells in cardiomyoplasty remains elusive. We established a three-dimensional tissue culture model based on a freezed-dryed collagen matrix with tubular structure (Matricel Incorporation, Germany), which was seeded either with highly purified cardiomyocytes, which have been purified by puromycin selection, alone or in combination with fibroblasts. The collagen sponges that were transplanted with embryonic stem (ES) cell derived cardiomyocytes alone did neither show morphological nor functional integration of the cells. However, when cocultured with embryonic fibroblasts (mouse strain HIMOF1, embryonic day 14.5) cardiomyocytes formed fibre-like structures of rod-shaped cells with organized sarcomeric structure. Electrical coupling between cardiomyocytes was demonstrated by strong expression of connexin43. We conclude that fibroblasts are needed for morphological and functional engraftment of purified cardiomyocytes on collagen matrices.

# Stem cell sources for myocardial regeneration

**Alexandrina Burlacu, Ph.D.**[1,2]

[1] "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania

[2] Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu" Bucharest, Romania

sanda.burlacu@icbp.ro

Understanding of the mechanisms by which stem cells interact with host cardiac tissue will facilitate their use in the cellular therapy in the near future. One of the main questions is how pluripotent stem cells respond to the cardiac environment and differentiate towards several differentiated cells, including cardiomyocytes, endothelial cells and fibroblasts. In order to study the interaction of stem cells with ischemic cardiac tissue, as well as the succession of stages until stem cells become differentiated cardiomyocytes, we derived several embryonic and adult stem cell lines. These stem cells lines are being used to evaluate the effect of the cardiac inflammatory factors on their proliferation, commitment and differentiation into cardiomyocytes. Studies will be done with emphasis on those factors appeared in ischemic myocardium which are reported to modulate the stem cell differentiation toward cardiomyocytes (TGFβ, FGF, VEGF, IGF-1, HGF). We are also evaluating the capacity of endothelial progenitors cells isolated from peripheral blood to contribute to neovasculation in ischemia.

# Cell therapy in myocardial infarction: present and perspectives

**Elisa Liehn, M.D., Ph.D.**
Institute for Molecular Cardiovascular Research, Aachen, Germany
eliehn@ukaachen.de

Myocardial infarction represents the most common cause of death in the western country. The current existing therapies try to improve the function of surviving myocardium, considering dead the scar tissues resulted after an ischemic injury. Based on our recently results, we propose a new approach to the treatment myocardial infarction, by manipulating the myocardial scar and adapting it to the myocardial necessities. This should have an additionally role to the classical known therapies in improving the heart function. After myocardial infarction, the healing and scar formation depend on the leukocytes infiltration. Changing leukocytes recruitment by inhibiting or enhancing their number and function, we are able to influence and predict the consistence and the final structure of the future scar. Moreover, after the scar is formed, we could be able to change the scar conformation by inducing leukocyte recruitment and remodelling, improving the structure and consecutive heart function. Cell-therapy was and is considered a revolutionary procedure, but the present results are disappointed. Clinical studies were controversial and the molecular effects of such a treatment are far to be understood. Based on our results, we think that cell-therapy induces an inflammatory reaction, which remodels the myocardial scar, and therefore improves heart function.

## Session 6: Embryology II

### Embryo morphogenesis and patterning in higher vertebrates

**Octavian Voiculescu, Ph.D.**
Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, United Kingdom
ogv20@cam.ac.uk

The basic germ layers of the early embyo are laid down and patterned during gastrulation, which also sets the stage for the early neural development. In the first part, I will present my work describing the cellular mechanisms of gastrulation in higher amniotes (chick embryo) and its associated movements. Against this background, I will present our current work focusing on the generation and morphogenesis of the early central nervous system.

# Mechanisms of signal transduction in the vertebrate Hedgehog pathway

**Adrian Salic, Ph.D.**
Department of Cell Biology, Harvard Medical School, Boston, USA
adrian_salic@hms.harvard.edu

Communication between cells is fundamental to almost every event in the life of multicellular organisms. The Hedgehog pathway is a conserved cell-cell signaling system, which plays critical roles in embryonic development, in the maintenance of adult stem cells, and in many human cancers. Our lab uses biochemistry, cell and chemical biology to investigate the molecular mechanisms involved in Hedgehog signal transduction in vertebrates. My talk will focus on two aspects of the vertebrate Hedgehog pathway: 1) the role of cholesterol in regulating Hedgehog signaling; and 2) the biochemical mechanism by which Hedgehog signaling activates its specific transcriptional output.

# Posters

## Microparticles and endothelial progenitor cells as markers of vascular dysfunction induced by combined hypertension and hypercholesterolemia

**Adriana Georgescu, Ph.D.**[1,2], Nicoleta Alexandru [1,2], Doina Popov[2], Eugen Andrei[2], Irina Titorencu[1,2], Emanuel Dragan[2], Maya Simionescu[2]

[1] "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania

[2] Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

adriana.georgescu@icbp.ro

This study aimed to (a) employ our newly designed model, the hypertensive–hypercholesterolemic hamster (HH) to find whether a correlation exist between circulating microparticles (MPs), endothelial progenitor cells (EPCs) and their contribution to vascular dysfunction and (b) to assess the effect of irbersartan treatment on HH animals (HHI). Aortic arch and mesenteric resistance arteries were explanted from HH, HHI and control (C) hamsters. The results showed that compared to C group, HH displayed: **(i)** a significantly increase in plasma cholesterol and triglyceride concentration, and an enhanced systolic and diastolic arterial blood pressure, and heart rate; **(ii)** a marked elevation of MPs and a significant decrease in EPCs; **(iii)** structural changes of arterial wall correlated with modified protein expression of MMP2, MMP9, MMP12, TIMP1, TIMP2 and collagen type I and III; **(iv)** a considerably altered reactivity of the arterial wall closely correlated with MPs and EPCs adherence; **(v)** an inflammatory process characterised by augmented expression of P-Selectin, E-Selectin, vWF, TF, IL-6, MCP-1, RANTES, eNOS, VEGF and SDF-1. Additionally, the experiments showed the potential of irbersartan to correct all altered parameters in HH. In conclusion, hypercholesterolemia associated with hypertension is accompanied by structural modifications and expression of pro-inflammatory molecules by the vessel wall, alteration of the vascular tone, enhanced release of MPs and reduced EPCs; the ratio between the latter two may be considered a marker of vascular dysfunction. Irbersartan that exhibit a pharmacological control on the levels of MPs and EPCs has the potential to restore homeostasis of the arterial wall.

This work was supported by CNCSIS –UEFISCSU, project number 1159, PNII – IDEI code 1043/2008, by CNMP project number 42138, PNII – Parteneriat code 3334 /2008 and by European Social Fund –„Cristofor I. Simionescu" Postdoctoral Fellowship Programme (ID POSDRU/89/1.5/S/55216), Sectoral Operational Programme Human Resources Development 2007 – 2013.

# CAD/CAM fabrication of idealized tissue constructs for dermatological use

S. Grigorescu[1], **Rodica Cristescu, Ph.D.[1]**, E. Axente[1], F. Sima[1], G. Dorcioman[1], I.N. Mihailescu[1], A.M. Forsea[2], O. Gallet[3], D.B. Chrisey[4]

[1] National Institute for Lasers, Plasma & Radiation Physics, Lasers Department, P.O. Box MG-36, Bucharest-Magurele, Romania

[2] "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[3] ERRMECe - EA 1391, Université de Cergy-Pontoise, France

[4] Rensselaer Polytechnic Institute, School of Engineering, Department of Materials Science & Engineering, Troy, 12180-3590, NY, USA

rodica.cristescu@inflpr.ro

Matrix Assisted Pulsed Laser Evaporation Direct Write (MAPLE DW) has recently arisen as a novel technique suitable for biologic material transfer. Due to CAD/CAM compatibility, biomolecules or individual cells can be accurately placed by MAPLE DW in appropriated sites without influencing their integrity, viability and functionality, offering therefore the possibility to create artificial scaffolds precisely controlled by the computer software. This technique opens new perspectives in the fabrication of artificial skin constructs for *in vitro* studies as well as for emerging therapy models. We attempted to extend the use of this emergent technique for the transfer of fibronectin patterns onto uniform substrates in order to induce the local functionalization and hence the preferential growth of cells onto the precise sites, considering that the fibronectin is the ECM glycoprotein that offers adhesion towards cellular membrane receptors - the integrins. The starting elements, known as ribbons, were obtained by depositing an uniform layer of fibronectin onto quartz substrates by spin coating. Each laser pulse of forward-directed irradiation of the ribbons results in the transfer of a small volume of fibronectin from the film. The patterns were obtained by collecting the spallated spots onto a substrate placed close and parallel with the ribbon. The patterns obtained were observed by conventional inverted optical fluorescent microscopy, while the fibronectin presence after the transfer was evidenced by specific antibody staining using anti-human fibronectin rabbit polyclonal serum and FITC-conjugated anti-rabbit IgG. The results demonstrate the successful obtaining of fibronectin patterns, as well as their functionality. Our preliminary results are promising and further study is warranted in order to optimize this technique for the creation of idealized skin scaffolds.

# Insulin-secreting mesenchymal stem cells for type-1 diabetes

**Oana Gavriliuc, M.D., Ph.D.,** Adriana Rosca, Valentin Ordodi, Alexandra Gruia, Felix Mic, Florina Bojin, Calin Tatu, Virgil Paunescu
Department of Physiology and Immunology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania
oana_gav@yahoo.com

Multipotent bone-marrow derived mesenchymal stem cells (MSCs ) have shown promising immunomodulatory properties *in vivo* which include inhibition of proliferation and function of auto-reactive T cells in type 1 diabetes. While the beneficial effect of MSC co-infusion on lowering autoimmune-mediated destruction of transplanted β cells has been consistently documented, difficulties in differentiating MSCs to regulated insulin-secreting cells are still an issue. We report the cloning of proinsulin transgene into viral and nonviral expression vectors and the subsequent transfection of MSCs, in order to assess the capacity of these vectors to induce insulin secretion *in vitro* and *in vivo*. Insulin transcription in the genetically-modified MSCs coincided with the intracellular presence of the protein, as shown by the specific binding of anti-insulin antibodies, and with the detection of insulin in the cell culture supernatant. Preliminary data showed regulation of glycemia following the infusion of insulin-secreting MSCs into recipient diabetic rats.

# Characterization of human umbilical cord blood- and Wharton's jelly-derived endothelial progenitors for use in cellular therapy

**Marilena Lupu, Ph.D.**, Florin Iordache, Eugen Andrei, Cosmin Buzila, Horia Maniu
Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
marilena.lupu@icbp.ro

**Introduction:** The encouraging but limited success of the studies aiming to differentiation of placental tissues-derived stem cells into endothelial progenitor cells (EPCs) for use in cell therapy suggests the need for identification and validation of the angiogenic potential of more versatile populations of EPCs. **Objectives:** Isolation and characterization of umbilical cord blood (UCB)- and Wharton's jelly (WJ)-derived EPCs with high angiogenic potential *in vitro* upon exposure to endothelial proliferation and differentiation signals. **Materials and Methods:** UCB and WJ cell suspensions, as well as WJ explants were isolated after informed-consent collection of the umbilical cords. UCB mononuclear cells were isolated by Ficoll gradient centrifugation; WJ was enzymatically digested with collagenase-II and hyaluronidase. Cell suspensions and explants were cultured in endothelial cell growth medium (MV-2, Promocell, Heidelberg, Germany), supplemented with 15% FBS and 40 ng/ml VEGF. Cell morphology was assessed by light and electron microscopy. The expression of surface markers (CD13, CD31, CD34, CD45, CD54, CD90, CD105, CD117, CD133, CD144, CD146, CD151, VEGFR1, and VEGFR2) was done by flow-cytometry (MoFlo, Dako, Denmark). Gene and protein (CD31, CD34, CD133, CD144, Tie-2, VEGFR1, VEGFR2, VWF, GATA2, GATA3, GATA4, RAGE, and CXCR4) expression profiles were assessed by reverse transcriptase-PCR and western blot analyses, respectively. The *in vitro* angiogenic potential of EPCs was evaluated by vascular tube formation assay on Matrigel. **Results:** In specific long-term culture conditions, upon stimulation with growth factors and high serum concentrations, UCB- and WJ-derived cells showed a comparable ability to differentiate into adherent cells with epithelial-like morphologies. Expression of genes and proteins involved in EPCs proliferation, differentiation, chemotaxis, and survival, correlated with the angiogenic potential of the differentiated EPCs to form vascular tubes *in vitro*. **Conclusion:** These cells may be valuable for use in cellular therapy for vascular regeneration relying on angiogenesis and neovascularization.

**Acknowledgements:** This work was supported by the EU Framework Programme 7 (FP7) - Marie Curie international reintegration grant No. 224888/2008-2010; Romanian Ministry of Education and Research reintegration and cooperation PNCDI-II grants No. 2/2008-2010 and No. 31042/2007-2010, respectively, and Romanian Ministry of Education and Research/German Federal Ministry of Education and Research (BMBF) bilateral cooperation grant No. 347/2009-2010.

# ECG recording as a tool for validating myocardial ischemia-reperfusion procedure in mouse model

**Mihai Bogdan Preda, Ph.D. Student**[1], Alexandrina Burlacu[1,2]
[1] Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[2] „Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania
bogdan.preda@icbp.ro

Animal models are important tools to uncover the molecular mechanisms associated to heart pathology. This paper evaluates the modifications induced by ischemia and ischemia-reperfusion on mouse model by permanent or transient ligation of the left coronary artery (LCA) and establishes a correlation among the extent of ischemia, electrocardiograph (ECG) registration and infarct size. Under orotracheal intubation, ligation was performed 1 mm distal from the tip of the left auricle. Histological analysis revealed that 30-minute ischemia (n=9) induced infarct on 9.7±0.5% of the left ventricle, while one-hour ischemia (n=9) resulted in transmural infarct affecting 16.1±4.6% of the left ventricle. Twenty-hour ischemia (n=8) and permanent ischemia (n=8) resulted in similar infarct sizes (33±2% and 31.8±0.7% of the left ventricle), suggesting ineffective reperfusion following 24-hour ischemia. ECG recording revealed that LCA ligation induced significant ST height elevation (204 *vs.* 14 μV) and QTc prolongation (136 *vs.* 76 ms). Both parameters were rapidly normalized on reperfusion, demonstrating ECG was important for validating the proper ligation and reperfusion. Besides, ECG predicted the severity of the myocardial damage induced by ischemia. Our results showed that ECG changes observed after 30-minute ischemia were reversed on reperfusion; however, extended periods of ischemia developed pathologic ECG patterns that maintained even after reperfusion. By concluding, the experimental model of myocardial ischemia-reperfusion can be improved by using ECG recording to validate the ligation and reperfusion during the surgery and to predict the severity of infarct.

The work was funded by a grant from the Romanian Ministry of Education and Research, IDEA Program, 250/2007.

# Characterization of mesenchymal stem cells isolated from mouse bone marrow

**Ana-Maria Rosca**, **Ph.D. Student**[1], Alexandrina Burlacu[1,2]
[1] Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[2] „Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania
ana-maria.rosca@icbp.ro

**Introduction:** Bone marrow represents the main source of stem/progenitor cells in adult individuals. Due to their high plasticity, these cells are being highly investigated in tissue engineering for restoring the damaged organs. **Purpose:** Isolation and characterization of mouse mesenchymal stem cells for further use in differentiation and transplantation studies. **Materials and Methods:** Bone marrow isolated from mouse posterior limb bones (6-8 week old) was resuspended to obtain a single cell suspension and plated in DMEM supplemented with 10% fetal calf serum ($10^6$cells/$cm^2$). After 48 hours, non-adhered cells were removed by changing the medium and the remaining cells were maintained for two months with weekly passages, until a stable mesenchymal stem cell line was derived. The expression of adult stem cells markers was evaluated by fluorescence microscopy, FACS and RT-PCR. The pluripotency of these cells was checked by induction of differentiation towards osteoblasts, chondrocytes and adipocytes. For these purposes, cells were incubated in the presence of specific cocktails ($10^{-7}$ M dexamethasone, 10 mM β-glycerophosphate and 0.2 mM ascorbic acid for osteogenic induction, $10^{-6}$ M dexamethasone, 100 μM indomethacin, 1% ITS for adipogenic induction, and high glucose DMEM supplemented with 10 ng/ml TGF-β3, $10^{-7}$ M dexamethasone, 50 ug/ml ascorbate–2 phosphate, 40 ug/ml proline, 100 ug/ml pyruvate, 50 mg/ml ITS for chondrogenic differentiation). Their differentiation was assessed by specific histological stainings and RT-PCR assay. **Results:** Although the initial culture was highly heterogeneous, a homogenous culture of fibroblast-like cells was generated after repeated passages over a two month period. In agreement with the rules established by the International Society for Cellular Transplant, these cells expressed specific markers (Sca-1, Stro-1 and CD105) while are negative for hematopoietic stem cells markers e.g., c-Kit and CD45. The differentiation assays showed that these cells were able to generate osteoblasts (increased osteocalcin expression and calcium deposits in the extracelullar matrix as shown by von Kossa method), adipocytes (expression of adipsin and accumulation of lipid droplets stained by Oil Red O) and chondrocytes (expression of aggrecan and confirmation of acid mucopolisaccarides by Alcian Blue staining). **Conclusion:** Mesenchymal stem cells isolated from mouse bone marrow are multipotent and can be maintained in culture without losing their potential. This cell line represents a valuable source of stem cells in the regenerative medicine. **Funding:** This work was supported by the Romanian Ministry of Education and Research, IDEA Program (grant number 250/2007).

# *Calendula officinalis* extracts stimulate adhesion molecules expression and *in vitro* motility of human endothelial progenitor cells

**Florin Iordache, Ph.D. Student**[1], Marilena Lupu[1], Cosmin Buzila[1], Eugen Andrei[1], Andrei Constantinescu[1], Aneta Pop[2], Horia Maniu[1]

[1] Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

[2] University of Agronomical Sciences and Veterinary Medicine, School of Veterinary Medicine, Bucharest, Romania

florin.iordache@icbp.ro

**Introduction:** The promise of cell therapy has advanced the use of adult stem cells for the development of novel approaches to induce impaired tissues repair, such as the stimulation of their proliferation and adhesion properties to promote regeneration of injured endothelium. **Objective:** The aim of this study was to stimulate endothelial progenitor cells (EPCs) with *Calendula officinalis* (Marigold) extracts in order to increase their proliferation and the expression of adhesion molecules responsible for their attachment to the damaged vascular tissue. **Materials and Methods**: For EPCs isolation, umbilical cord blood derived mononuclear cells were separated by Histopaque gradient centrifugation and cultured in an endothelial differentiation medium. EPCs characterization was done by light microscopy and flow cytometry. Cell proliferation was determined by MTS assay after stimulation for 24-48 hours with Marigold extracts. Expression of adhesion molecules (ICAM-1, VE-cadherin, P-selectin, PCAM-1, VCAM-1) and those involved in EPCs chemotaxis and angiogenesis (CXCR4, Tie-2) was determined by RT-PCR. To determine cell motility *in vitro* a wound-healing assay was employed. **Results:** Marigold extracts stimulated cell proliferation in a concentration dependent manner. We also observed an increased expression of molecules involved in cell adhesion (ICAM-1, PCAM-1, VCAM-1, VE-cadherin, P-selectin), as well as in chemotaxis and angiogenesis (CXCR4, Tie-2). Furthermore, Marigold extracts increased cell motility, as compared to control. **Conclusion and Relevance:** This study demonstrates the usefulness of Marigold extracts to increase EPCs proliferation and adhesion molecules expression, suggesting that they may facilitate EPCs attachment to injured endothelium.

# Paracrine properties of human endothelial progenitor cells under hypoxic conditions

**Gabriela Grigorescu, Ph.D. Student**[1], Alexandrina Burlacu [1,2]
[1] Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
[2] "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania
gabriela.grigorescu@icbp.ro

**Introduction:** Blood flow recovery is an important goal in cellular therapy for complete restoring of the ischemic tissue function. The behavior of the endothelial progenitor cells (EPC), which generally contribute to vessel formation, either directly or by paracrine effects, can be modified by hypoxic conditions such as those found in infarcted myocardium. Thereby, **the aim** of this study was to evaluate the pro-angiogenic properties of EPC after exposure to hypoxia. **Materials and Methods:** EPC were isolated from human peripheral blood by culturing the mononuclear cell fraction in MV2 medium supplemented with specific growth factors (VEGF, IGF, FGF and EGF) for 7 days. At the end of this period, cells were characterized both morphologically (phase-contrast microscopy and FACS analysis to establish the profile of surface markers) and functionally (the colony-forming ability in the presence of bovine pituitary extract, BPE). Hypoxia was induced by incubating the cells in MV2 medium and 1% $O_2$ atmosphere. After 24 hours, the conditioned medium (CM) was saved and cells were checked for angiogenic activity or processed for Western-blot assay. Proangiogenic abilities were assessed by in vitro angiogenesis assay on Matrigel or alternatively, by the capacity to stimulate the proliferation of EA.hy926 endothelial cells. **Results:** Isolated cells expressed both markers of the monocyte-macrofage lineage (CD14, CD45) and endothelial cells (KDR, VEGFR1, VE-Cadherin, CD31) and have the specific pattern of "early outgrowth EPC" as described elsewhere. In the presence of BPE cells were able to form specific colonies. Pro-apoptotic protein Bax was increased in EPC under hypoxic conditions; however, its level was still below that noticed in mature endothelial cells. On contrary, the secretion of SDF and expression of its receptor CXCR4 were increased in EPC compared to those in mature EC, and were maintained at high levels even after hypoxia exposure. CM obtained from hypoxic cells induced the formation of capillary tube-like structures on Matrigel in vitro, even if at a lower extent than normal cells (15 vs.33 polygonal structures per microscope field). Nevertheless, CM from hypoxic cells lessened the ability to increase the mobility of endothelial cells in a wound-healing assay, suggesting a possible decline in their functional properties. **Conclusions:** The functional properties of EPC are partially preserved in hypoxic conditioned, but still superior to mature endothelial cells. The axis SDF/CXCR4 involved in cellular mobility and engraftment was maintained intact under hypoxia, demonstrating the ability of EPC to mobilise and engraft to the damaged sites and survive the local hypoxic conditions.

**Acknowledgement:** One of the authors (A.B.) acknowledges the financial support of European Social Fund – „Cristofor I. Simionescu" Postdoctoral Fellowship Programme (ID POSDRU/89/1.5/S/55216).

# Characterization of cryopreserved stem cells isolated from human umbilical cord blood

**Cosmin Buzilă, M.D., Ph.D. Student**, Florin Iordache, Marilena Lupu, Eugen Andrei, Horia Maniu
Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
cosmin.buzila@icbp.ro

**Introduction:** Cord blood (UCB) units are intensely used in the field of hematopoietic stem cell transplantation. In order to evaluate the engraftment efficiency or to predict the transplant outcome, standardized and accredited procedures for UCB units processing must be established, for research and cellular therapy applications. **Objectives:** To establish the (**1**) collection, (**2**) characterization and (**3**) cryopreservation procedures for human UCB units; (**4**) quality manual design and standard operating procedures (SOPs). **Materials and Methods: (A)** Isolation and Cryopreservation **–** UCB units (n=56) were harvested with mother's informed consent, using a dedicated kit, and tested for: HBV; HCV; HIV1,2; CMV; HTLV, *T. pallidum* and *T. gondii*. Mononuclear cells (MNC) fraction was obtained by ***separation on density gradient Histopaque*** and cryopreserved in vials (1.6 ml cell suspension/vial), in Iscove's Modified Dulbeco's Medium (IMDM) supplemented with 20% fetal calf serum (FCS), 10% DMSO and 1% antibiotic. Seven out of 56 freshly isolated UCB units have been processed for ***volume reduction with the Sepax automated system*** (Biosafe, CH) and cryopreserved in 10% DMSO (final volume = 20 ml/cryobag). **(B)** Cell cultures – Cell samples obtained from freshly isolated (Control) and revitalized units, either separated on density gradient or processed with Sepax, were cultured in IMDM with 20% FCS for 20 days, in normoxic conditions. (**C)** Characterization of freshly isolated and revitalized cells has been performed by: (**i**) ***Flow cytometry*** (MoFlo FACS, Dako, DK), using antibodies against CD34, CD38, CD45, CD90, CD105 and HLA-DR; (**ii**) ***Real-time PCR for telomerase activity quantification***; (**iii**) ***Colony-forming cell assay*** by plating 20.000 cells/ml in methylcellulose. **Results:** The average harvested UCB volume was 95 ml/sample. ***Immunophenoltyping*** of the cryopreserved cells revealed a similar profile after revitalization, as compared to the freshly harvested cells: $CD34^{+}/CD45^{+}/CD38^{+}/HLA\text{-}DR^{low}$. ***Telomerase activity*** of cryopreserved UCB-derived MNCs did not change after revitalization, as compared to control; moreover, ***colony-forming capacity*** of cryopreserved cells was kept after revitalization. Therefore, the quality manual and SOPs concerning UCB units collection and bacteriology/virology testing have been generated. We also obtained preliminary data necessary to design the cryopreservation and characterization algorithms for the UCB cells. **Conclusions:** The UCB-derived cells maintained their viability, immunophenotype, and capacity to proliferate after cryopreservation. Therefore, these characterized, cryopreserved stem cells could now serve for experimental cell therapy studies. Our final goal is to apply our collection, characterization, cryopreservation, and quality control algorisms to UCB units that will be deposited in a Stem Cell Bank accredited according to the Good Laboratory Practice, Good Clinical Practice and Good Manufacturing Practice standards.

**Financial support:** PNCDI-II grant No. 41-032/2007-2010 from the Romanian Ministry of Education and Research.

# Fractalkine-CX3CR1 interaction on chemotaxis of monocytes towards smooth muscle cells activated with resistin ± high glucose; potential application for cell therapy with CX3CR1+ progenitor cells

**Viorel Simion, Master Student,** Ana-Maria Gan, Daniela Stan, Monica Parvulescu, Manuela Calin, Elena Dragomir, Ileana Manduteanu
Laboratory of Biopathology and Pharmacology of Inflammation, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania
viorel.simion@icbp.ro

**Introduction:** Fractalkine (FK) and its specific receptor CX3CR1 are involved in the directional migration of various cells to atherosclerotic sites. Recently, resistin emerged as a significant local and systemic regulatory cytokine implicated in inflammation and vascular endothelial cell dysfunction. **Objective:** Since in humans, serum resistin level is increased in diabetes and monocytes are an important source of resistin production, we examined the effect of high glucose concentration (25 mM) on resistin expression in monocytes, and their chemotaxis towards smooth muscle cells (SMCs). **Methods:** The U937 cell line was employed to to generate monocytes by incubation with either 8-bromo-cAMP (1mmol/l), TNF-α (10ng/ml), resistin (100ng/ml), or high glucose(HG, 25mM) for 24 or 48h. SMC were incubated with resistin (100ng/ml) and HG concentration (25mM); after 24h the conditioned medium was collected and used for the chemotaxis assay using Boyden chambers with 5uM-diameter filter pores. To assess whether FK is specifically involved in monocytes chemotaxis, in some experiments, the FK receptor, CX3CR1, expressed on monocytes, was blocked with an anti-CX3CR1 antibody prior to the chemotaxis assay. The monocytes migrated to the lower compartment of the Boyden chamber were counted under an inverted microscope. **Results: (i)** monocytes chemotaxis to the conditioned medium from SMC incubated with resistin ± HG was significantly higher compared to untreated SMCs; blocking the CX3CR1 receptor on monocytes **(ii)** significantly decreased the monocyte chemotaxis to conditioned medium from SMCs treated with resistin, and **(iii)** non-significantly decreased the monocyte chemotaxis to conditioned medium from SMCs treated with either HG or resistin+HG. **Conclusion:** The results suggest that resistin±HG increases the expression of monocytes chemoattractant molecules in human SMCs, and that FK expression in SMCs, induced by resistin, can induce chemotaxis of human monocytes. **Further goals:** Since there is indication that FK interacts with CX3CR1 present on bone marrow progenitor cells that are a source of SMC progenitor cells, we propose to study the impact of FK-$CX_3$CR1 interaction on human progenitor cells recruitment into the injured vessel wall, and their differentiation into SMC and endothelial cells, with potential applications in atherosclerosis therapy.

## Specific Session Moderators

**Session 1: Iacob Checiu**, Department of Biology, School of Chemistry-Biology-Geography, University of Western Timisoara, Timisoara, Romania, icheciu@cbg.uvt.ro

**Session 2: Octavian Voiculescu**, Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, United Kingdom, ogv20@cam.ac.uk

**Session 3: Kurt Pfannkuche**, Institute for Neurophysiology, University of Cologne, Cologne, Germany**,** kurt.pfannkuche@uni-koeln.de

**Session 4 and Special Session Part I: Irinel Popescu**, Center of Gastroenterology and Hepatology, "Fundeni" Clinical Institute, Bucharest, Romania, irinel.popescu@icfundeni.ro

**Session 5: Nicanor I. Moldovan**, Davis Heart and Lung Research Institute, Ohio State University, Columbus, USA**,** Nicanor.Moldovan@osumc.edu

**Session 6: Ioan Ovidiu Sirbu**, Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germania, ovidiu.sirbu@uni-ulm.de

**Special Sessions Part I and II: Octavian Popescu**,"Babeş-Bolyai" University, Cluj-Napoca, Romania, opopescu@biolog.ubbcluj.ro

## Speakers from Diaspora

**Ioan Ovidiu Sirbu**, Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germania, ovidiu.sirbu@uni-ulm.de

**Tudor Fulga**, Cell Biology Department, Harvard Medical School, Boston, USA
Tudor_Fulga@hms.harvard.edu

**Victor Luria,** Department of Genetics and Development, Columbia University Medical Center, New York, USA**,** avl@columbia.edu

**Nicanor I. Moldovan**, Davis Heart and Lung Research Institute, Ohio State University, Columbus, USA**,** Nicanor.Moldovan@osumc.edu

**Mihaela Crisan**, Erasmus MC Stem Cell Institute, Medical Faculty Rotterdam, Netherlands**,** m.crisan@erasmusmc.nl

**Mara E. Pitulescu**, Department of Tissue Morphogenesis, Max–Planck–Institute for Molecular Biomedicine, and Faculty of Medicine, University of Münster, Münster, Germany, mpitule@gwdg.de

**Catalin Toma**, University of Pittsburgh Medical Center, Pittsburgh, USA
tomacx@UPMC.EDU

**Elisa Liehn**, Institute for Molecular Cardiovascular Research, Aachen, Germany
eliehn@ukaachen.de

**Octavian Voiculescu**, Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, United Kingdom, ogv20@cam.ac.uk

**Adrian Salic**, Department of Cell Biology, Harvard Medical School, Boston, USA**,**
adrian_salic@hms.harvard.edu

## Speakers from Romania

**Maya Simionescu**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, maya.simionescu@icbp.ro

**Mihnea Ioan Nicolescu**, "Victor Babes" National Institute of Pathology, Bucharest, Romania, and Department of Cellular and Molecular Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, mihnea.nicolescu@gmail.com

**Catalin Gabriel Manole**, Cellular and Molecular Medicine Department, "Carol Davila" University of Medicine and Pharmacy , Bucharest, Romania, and "Victor Babes" National Institute of Pathology, Bucharest, Romania, catalin.manole@jcmm.org

**Horia Maniu**, Cell Therapy Laboratory, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania**,** horia.maniu@icbp.ro

**Felix Mircea Brehar**, "Bagdasar Arseni" Clinical Hospital, Bucharest, Romania, felixbrehar@yahoo.com

**Marilena Lupu**, Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania**,** marilena.lupu@icbp.ro

**Razvan Iacob**, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania, razvan.iacob@icfundeni.ro

**Mihaela Chivu**, "Stefan S. Nicolau" Institute of Virology, Bucharest, Romania, mihaela.chivu@virology.ro

**Irina Titorencu**, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania and Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology, "Nicolae Simionescu", Bucharest, Romania, irina.titorencu@icbp.ro

**Oana Gavriliuc**, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania, oana_gav@yahoo.com

**Alexandrina Burlacu**, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania and Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu" Bucharest, Romania, sanda.burlacu@icbp.ro

**Simona Dima**, Center of Gastroenterology and Hepatology, "Fundeni" Clinical Institute, Bucharest, Romania, simona.dima@icfundeni.ro

## International Speakers

**Dimitry Spitkovsky**, Institute of Neurophysiology, University of Cologne, Cologne, Germany, dimitry.spitkovsky@uni-koeln.de

**Roland Adelmann**, Pediatric Cardiology Department, University of Cologne, Cologne, Germany, and Institute for Neurophysiology, University of Cologne, Cologne, Germany**,** roland.adelmann@uk-koeln.de

**Kurt Pfannkuche**, Institute for Neurophysiology, University of Cologne, Cologne, Germany**,** kurt.pfannkuche@uni-koeln.de

## PARTICIPANTS

**Adriana Georgescu**, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania and Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, adriana.georgescu@icbp.ro

**Rodica Cristescu**, Lasers Department, National Institute for Lasers, Plasma & Radiation Physics, Bucharest-Magurele, Romania, rodica.cristescu@inflpr.ro

**Mihai Bogdan Preda**, Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, bogdan.preda@icbp.ro

**Ana-Maria Rosca**, Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, ana-maria.rosca@icbp.ro

**Florin Iordache**, Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, florin.iordache@icbp.ro

**Gabriela Grigorescu**, Laboratory of Adult and Embryonic Stem Cell Biology, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, gabriela.grigorescu@icbp.ro

**Cosmin Buzila**, Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, cosmin.buzila@icbp.ro

**Viorel Simion**, Laboratory of Biopathology and Pharmacology of Inflammation, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, viorel.simion@icbp.ro

**Luminita Simion**, "Grigore T. Popa"University of Medicine and Pharmacy, Iasi, Romania, luminitasimion@yahoo.com

**Mircea Leabu**, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, and "Victor Babes" National Institute of Pathology, Bucharest, Romania, mleabu@jcmm.org

**Vlad Badilita**, IMTEK, University of Freiburg, Freiburg, Germany, vlad.badilita@gmail.com

**Andrei Eugen**, Laboratory of Fetal and Adult Stem Cell Therapy, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, andrei.eugen@icbp.ro

**Otilia Adina Postea**, Department of Pharmacy, Center for Drug Research, Ludwig Maximilians-University, Munich, Germany, otilia.postea@cup.uni-muenchen.de

**Imre Benedek**, University of Medicine and Pharmacy, Targu Mures, Romania, imrebenedek@yahoo.com

**Nicoleta Alexandru**, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania and Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, nicoleta.alexandru@icbp.ro

**Alina Constantin**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, alina.constantin@icbp.ro

**Monica Raicu**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, monica.raicu@icbp.ro

**Mirela Balan**, National Institute for Research-Development in Biological Sciences, Bucharest, Romania, mire.balan@gmail.com

**Ana-Maria Stanciuc**, National Institute for Research-Development in Biological Sciences, Bucharest, Romania, annastanciuc@yahoo.fr

**Stergios Karamitros**, ANTISEL Selidos Ro SRL – Scientific Equipment Solutions, Romanian Office, Bucharest, Romania, stergios.karamitros@antisel.ro

**Mihaela Androne**, ANTISEL Selidos Ro SRL – Scientific Equipment Solutions, Romanian Office, Bucharest, Romania, mihaela.androne@antisel.ro

**Mihai Pandele**, ANTISEL Selidos Ro SRL – Scientific Equipment Solutions, Romanian Office, Bucharest, Romania, mihai.pandele@antisel.ro

**Catalina Klumpner**, MEDIST Life Science, Bucharest, Romania, catalina.klumpner@medist-ls.ro

**Sorin Grigorescu**, National Institute for Lasers, Plasma and Radiation Physics, Bucharest-Magurele, Romania, sorin.grigorescu@inflpr.ro

**Alexandru Corlan**, University Emergency Hospital, Bucharest, Romania, alexandru@corlan.net

**Bogdan Alexandru Popescu**, "Prof. Dr. C. C. Iliescu" Institute of Cardiovascular Diseases, Bucharest, Romania, bogdan.a.popescu@gmail.com

**Loredan Stefan Niculescu**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, loredan.niculescu@icbp.ro

**Adrian Manea**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, adrian.manea@icbp.ro

**Madalina Fenyo**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, madalina.fenyo@icbp.ro

**Madalina Dumitrescu**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, madalina.dumitrescu@icbp.ro

**Gabriela Costache**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, gabriela.costache@icbp.ro

**Daniela Stan**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, daniela.stan@icbp.ro

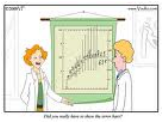
**Ana-Maria Gan**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, anca.gan@icbp.ro

**Miruna Nemecz**, Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania, miruna.nemecz@icbp.ro

**Dana Niculae**, Radiopharmaceuticals Department, "Horia Hulubei" National Institute for Physics and Nuclear Engineering, Bucharest, Romania, niculaedana@yahoo.com

# ORGANIZERS

**Organizing Committee:**
Acad. Maya Simonescu
Dr. Horia Maniu
Dr. Marilena Lupu
Dr. Ioan Ovidiu Sirbu



The Posters will be displayed throughout the workshop.
Poster discussions will be held during the dedicated Poster Session.

Coffee and lunch breaks, during both workshop days,
will be offered by the organizers.

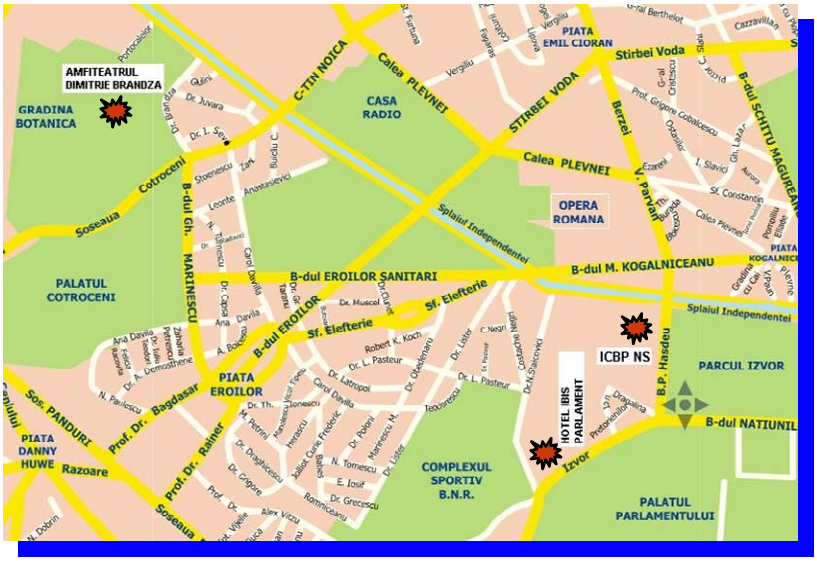
**Graphical Assistance:**
Marilena Daju
Mihaela Scheian

**Technical Assistance:**
Drd. Eugen Andrei

**Organizing Assistance:**
Drd. Eugen Andrei
Drd. Cosmin Buzila
Viorel Simion

**Workshop location:**
School of Biology, University of Bucharest, Botanical Garden,
1-3 Aleea Portocalilor, Bucharest
**Hotel location:**
Ibis Parlament, 82-84 Izvor Street, Phone: (+4)021-401-1000

**Contact:**
Dr. Marilena Lupu
marilena.lupu@icbp.ro
Phone: (+4)0741-781-153




## *SPONSORI*

**MEDIST Life Science**

**ANTISEL Selidos Ro SRL**
**Scientific Equipment Solutions**