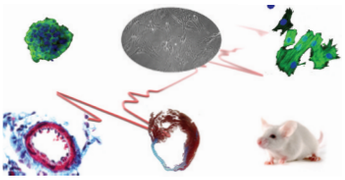


STEM CELL BIOLOGY LABORATORY

***Alexandrina Burlacu**, PhD*
HEAD OF LABORATORY

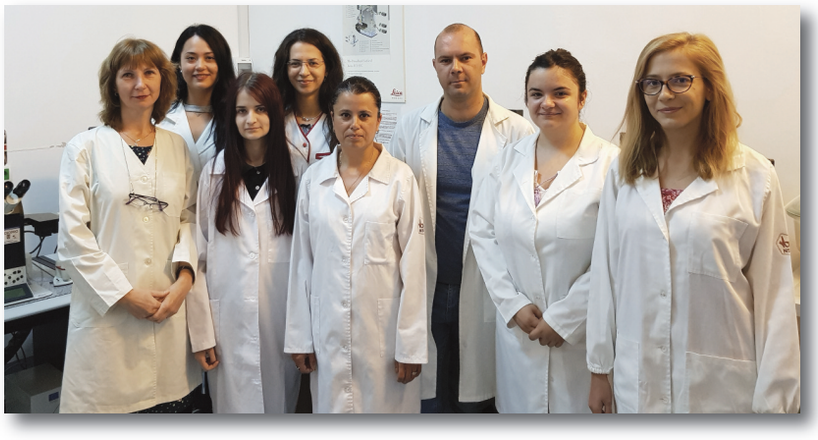
***Mihai Bogdan Preda,** PhD GROUP LEADER*

STAFF

***Ana-Maria Catrina,** PhD*
***Sînziana Popescu,** PhD Student*
***Ana Mihaela Lupan,** PhD Student*
***Evelyn Gabriela Rusu,** PhD Student*
***Cătălina Marinescu,** Technical Assistant*
***Roxana Vlădulescu,** Technical Assistant*
***Ioana Leti,** Master Student*
***Carmen Neculachi,** Master Student*

CORE LABORATORY UNITS:

Flow Cytometry - responsible: *Alexandrina Burlacu*
Confocal Microscopy -responsible: *Alexandrina Burlacu* and *Mihai Bogdan Preda*
Whitley H35 Hypoxystation/ Seahorse Analyzer -responsible: *Mihai Bogdan Preda*



STEM CELL BIOLOGY LABORATORY

Alexandrina Burlacu, *PhD*

Head of Laboratory
E-mail: *sanda.burlacu@icbp.ro*

Major position/appointments and professional training

- Principal Investigator, Scientific Researcher grade I
- Member of the Scientific Council of ICBP-NS
- PhD Coordinator
- Supervision of Graduate Students and Postdoctoral Fellows
- Expert Evaluator for National and International Grants
- Invited Peer Reviewer for International Scientific Journals

MAJOR RESEARCH INTERESTS

- **Regenerative therapies for injured heart**
- **Mesenchymal stem cell-based approaches for type 1 diabetes**

SPECIAL TECHNICAL EXPERTISE

- Flow-cytometry analysis and cell sorting
- Confocal microscopy
- Animal models of myocardial infarction and hind limb ischemia
- Ex vivo platforms mimicking cell-cell functional interactions in cardiac tissue

PUBLICATIONS

32 scientific articles, with more than 1000 citations (Google Scholar).

SELECTED NEW FINDINGS OF THE LABORATORY

- Concurrent transplantation of Endothelial Colony Forming Cells and factors released by cultured Circulating Angiogenic Cells enhanced the outcome of angiogenic therapy, compared to any single element.
- Mesenchymal Stem Cells and Endothelial Progenitor Cells have several distinct, yet complementary, paracrine effects, so that the combination of these two cell populations produced many benefits after transplantation.
- Subcutaneous transplantation of Mesenchymal Stem Cells could be considered as a safe and non-invasive procedure to induce protection and repair of the ischemic heart.
- Short treatment of Mesenchymal Stem Cells with 5-azacytidine resulted in a restricted differentiation potential with concomitant increased chondrogenic commitment.
- An insult may cause apoptosis or necrosis in endothelial cells, as a function of the intensity rather than its nature.
- Cardiomyocyte apoptosis was not induced by ischemia per se, but rather by the oxidants from the surrounding environment at the time of reperfusion.

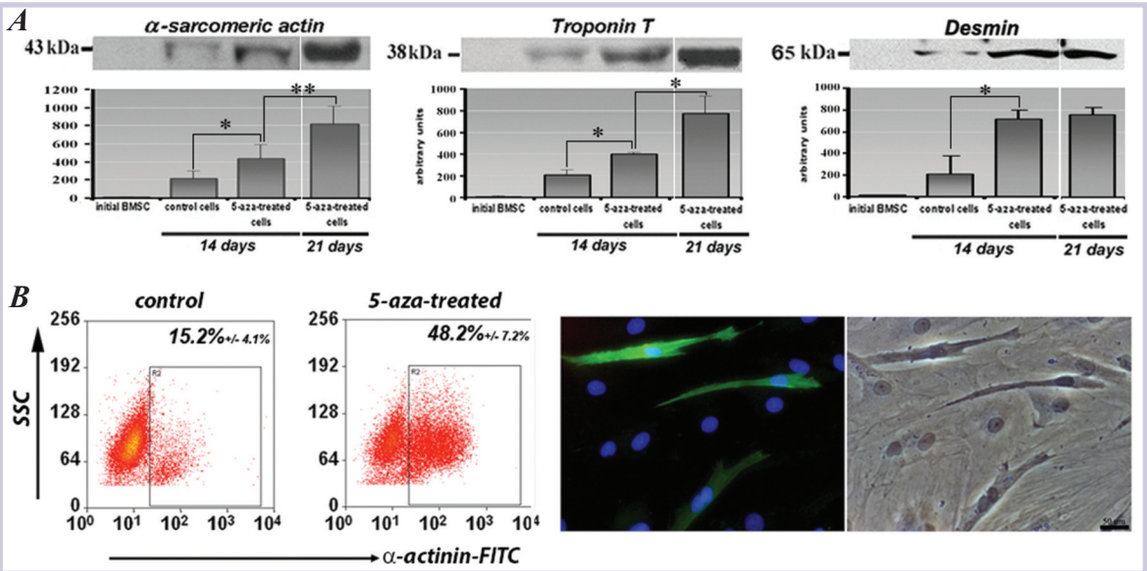
PREVIOUS PROJECTS / PUBLICATIONS /

1. THE SIGNALING PATHWAYS INVOLVED IN THE OXIDATIVE STRESS-INDUCED APOPTOSIS OF ENDOTHELIAL CELLS

The role of reactive oxygen species (ROS) in the pathogenesis of vascular diseases was well established, but few data existed on the mechanisms by which ROS induced endothelial cells (EC) death. Our results showed that (i) oxidative stress induced EC death either by apoptosis or necrosis and (ii) the mechanisms of EC death differed as a function of the oxidative stress intensity. Thus, the same insult can cause apoptosis and/or necrosis, as a function of the intensity rather than the nature of the offense (Burlacu A et al., Severity of oxidative stress generates different mechanisms of endothelial cell death. Cell and Tissue Research. 2001; 306: 409-416).

2. IN VITRO DIFFERENTIATION OF ADULT BONE MARROW STROMAL CELLS INTO CARDIO-MYOCYTES

In the attempt to increase the myogenic commitment of bone marrow stromal cells (BMSC), we investigated the extent of conversion induced by the demethylation agent 5-azacytidine. Our results demonstrated a promoting effect of 5-azacytidine on the expression of muscle-specific proteins and genes in BMSC in culture.

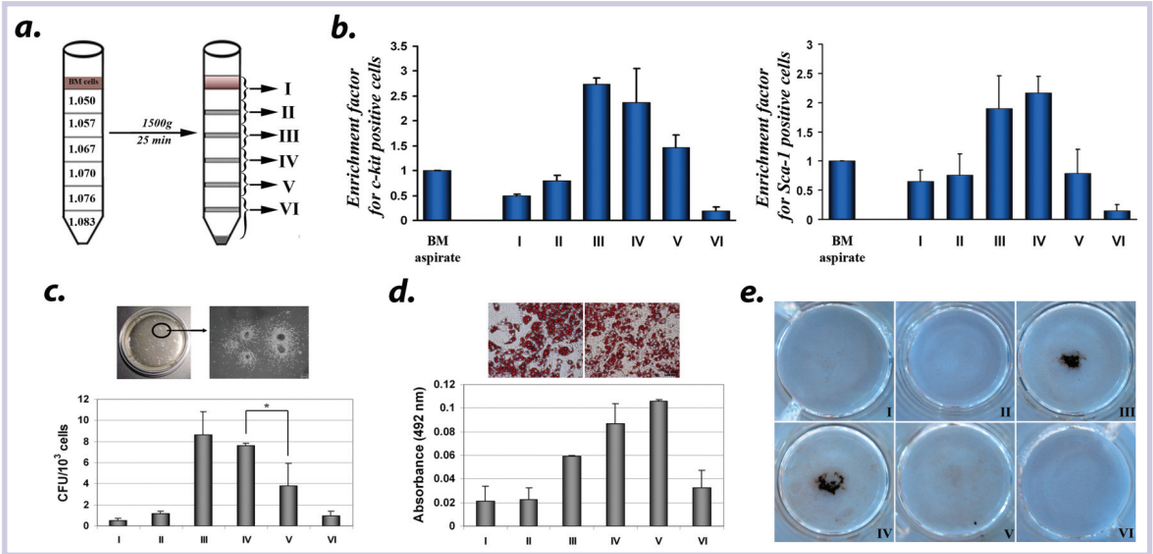


Priming of BMSC to cardiomyogenic differentiation with 5-azacytidine. (A) Western blot analysis of cardiac-specific proteins in the initial BM aspirate, untreated and 5-azacytidine-treated BMSC. (B) Flow-cytometry analysis (left) and ICC staining (right) of α-actinin staining in 5-azacytidine-treated cells after 14 days.

Priming of BMSC to cardiomyogenic differentiation may have significant applications in cellular approaches to ameliorate muscle loss after myocardial ischemia (Rosca AM, Burlacu A. Effect of 5-azacytidine: evidence for alteration of the multipotent ability of mesenchymal stem cells. Stem Cells Dev. 2011; 20:1213-1221, Burlacu A et al., Promoting effect of 5-azacytidine on the myogenic differentiation of bone marrow stromal cells. Eur J Cell Biol. 2008; 87: 173-184).

3. ISOLATION OF A BONE MARROW POPULATION ENRICHED IN STEM AND PROGENITOR CELLS

In an attempt to enrich the progenitor cell pool from bone marrow, we separated bone marrow cells based on their physical properties. We showed that a small size subpopulation could be isolated by Percoll gradient centrifugation, which was enriched in c-kit+ and Sca-1+ progenitor cells, and had an increased clonogenic capacity under specific conditions. Furthermore, this subpopulation could be a source of MSC, because it generated in vitro hematopoietic cell-free MSC cultures (Rosca A-M, Burlacu A. Isolation of a mouse bone marrow population enriched in stem and progenitor cells by centrifugation on Percoll gradient. Biotechnol Appl Biochem. 2010;55: 199-208).



Separation of bone marrow cells in sub-populations. a) Schematic representation of the separation of bone marrow cells into six fractions by centrifugation on a discontinuous Percoll gradient. b) Enrichment of fractions III and IV in progenitor cells (c-kit⁺ and Sca-1⁺ cells). c) Evaluation of differentiation potential of each fraction using the CFU assay. A representative field of fraction IV was displayed above the diagram. d) Spectrophotometric quantification of lipid accumulation in the six fractions after culturing the cells in adipogenic medium. The upper panel depicts a representative picture of Oil Red staining in fractions III and IV. e) Light microscopy of von Kossa staining in the six fractions after culture in osteogenic medium.

4. DEFINED-SIZE EMBRYOID BODIES FORMED IN THE PRESENCE OF SERUM REPLACEMENT INCREASES THE CARDIAC DIFFERENTIATION EFFICIENCY OF MOUSE EMBRYONIC STEM CELLS

The pluripotent nature of embryonic stem (ES) cells makes them powerful tools in cell replacement therapy for severe degenerative diseases, such as heart failure. We had generated two ES cell lines from RAP and C57Bl/6 mice. These cells expressed pluripotency markers and induced teratomas when injected into syngeneic mice, which made them suitable for differentiation into CMCs.

We found that EBs formed as a result of in vitro ES cell aggregation generated contractile tissue in direct correlation with the initial number of ES cells. Furthermore, the presence of Knock-out Serum Replacement (KO-SR)

during ES cell aggregation resulted in less compacted EBs and increased cell differentiation into CMCs compared to the presence of Fetal Bovine Serum (FBS). In conclusion, cardiac differentiation of ES cells is dependent on the size and the degree of compaction of EBs, and the presence of KO-SR during initiation of EBs may lead to improved cardiogenic differentiation of ES cells (Preda MB et al., Defined-size embryoid bodies formed in the presence of Serum Replacement increases the efficiency of the cardiac differentiation of mouse embryonic stem cells. Tissue Cell. 2013; 45: 54-6).

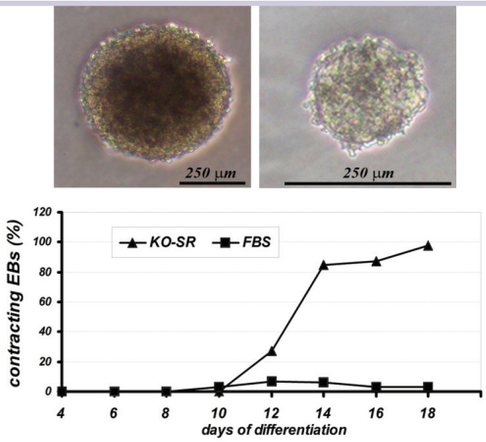


Diagram illustrating the time-course development of contractility in EBs initiated in the presence of FBS and KO-SR. Upper images show the phase-contrast microscopy for comparative illustration of the compaction and the size of EBs derived from 500 ES cells in the presence of FBS (left) and KO-SR (right).

CURRENT PROJECTS

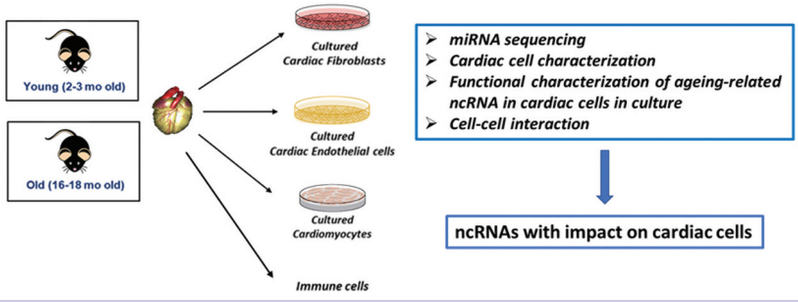
1. FUNCTIONAL ANALYSIS OF CELL-CELL INTERACTIONS IN THE AGED MYOCARDIUM

Ageing is a highly complex and poorly-understood process characterized by a gradual decrease in physiological function of all cells and organs, leading to the increased incidence of the prevalent diseases such as cardiovascular diseases.

One of the main hallmarks of ageing is progressive cardiac fibrosis. While many studies focused on cardiomyocyte loss as a leading cause of cardiac fibrosis, poor information exists about the changes affecting cardiac fibroblasts in aged individuals. Furthermore, less is known about the ability of cardiac fibroblasts and immune system cell populations to rejuvenate aged myocardium.

Our project aims at discovering a strategy for healthy-ageing through a better understanding of the significance and contribution of interaction between cardiac fibroblasts and other cellular and non-cellular components of the heart to age-associated cardiac fibrosis.

The objectives are: (i) Generate a cardiac fibroblast-specific miRNA blueprint of the ageing heart; (ii) Define the predictive value of miRNAs in cardiac ageing by functional characterization and cell-cell interactions; (iii) Validate the importance of miRNA in age-related cardiovascular pathways by studying various animal models of heart failure.



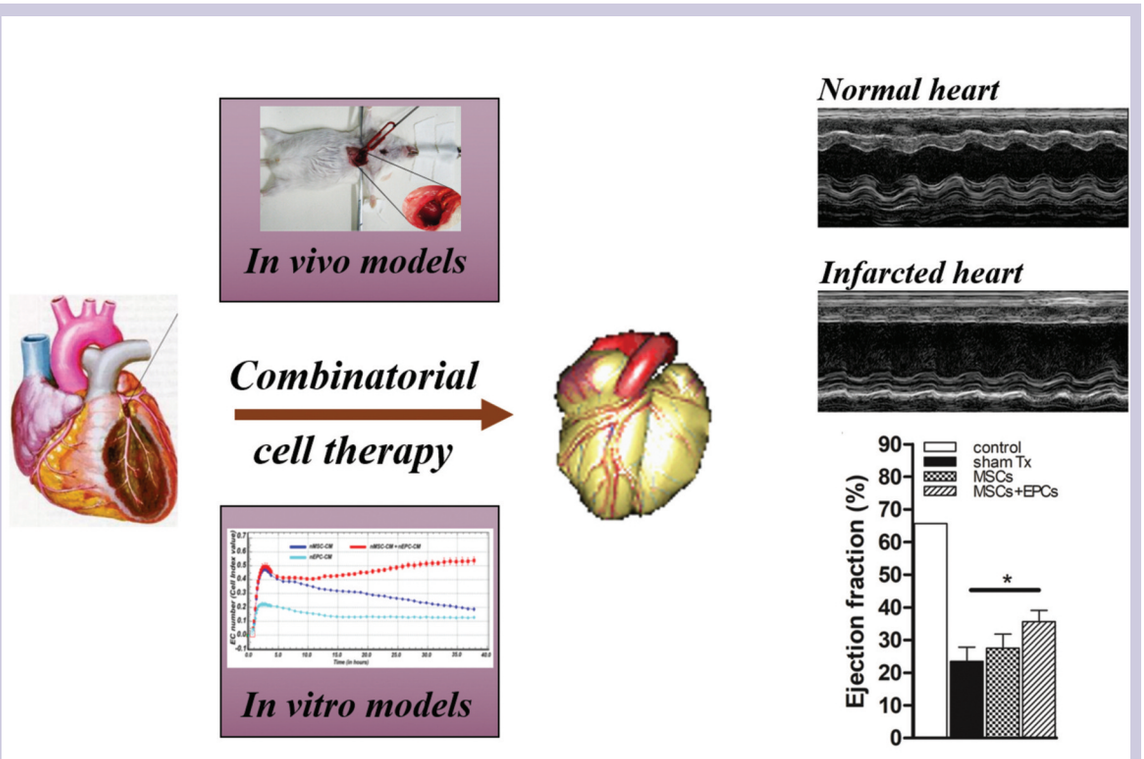
Schematic representation of the work flow used to identify and study ageing-related fibroblast-specific miRNAs.

2. ISCHEMIC TISSUE ENGINEERING BY COMBINATORIAL TRANSPLANT AIMING TO GAIN MUTUAL BENEFITS FOR GRAFT SURVIVAL AND HOST TISSUE REPAIR

Although stem cell-based therapy for ischemic muscle regeneration has reported several functional improvements, they were recently demonstrating to be transient and attributed mostly to the paracrine effects stimulating angiogenesis and cell survival.

We are focusing on the development of an effective approach for cell therapy, based on simultaneous use of several stem/progenitor cell populations to provide mutual benefits for both grafted cells and ischemic tissue, by improving the retention of transplanted cells into ischemic tissues and stabilization of the newly-derived blood vessels. This novel approach is transferable to the clinic and expected to produce significant improvements in the life quality of patients with heart failure and to decrease the social economic burden of ischemic heart disease with direct implications for human health.

Our objectives are*:* (i) To characterize the cross-talk between Mesenchymal Stem Cells (MSCs) and Endothelial Progenitor Cells (EPCs) in vitro; (ii) to assess the spatial and temporal contribution of MSC and EPC to graft survival, when used as individually or simultaneously transplanted cells in ischemic tissue models; (iii) to quantify the capacity of combinatorial transplant of MSC and EPC to enforce the regenerative process of the ischemic tissues.



Combinatorial cell therapy using EPCs and MSCs produced enhanced benefits for ischemic myocardial function, in comparison to the single therapy.

3. AN IMMUNOLOGICAL APPROACH TO TYPE 1 DIABETES USING MESENCHYMAL STROMAL CELLS

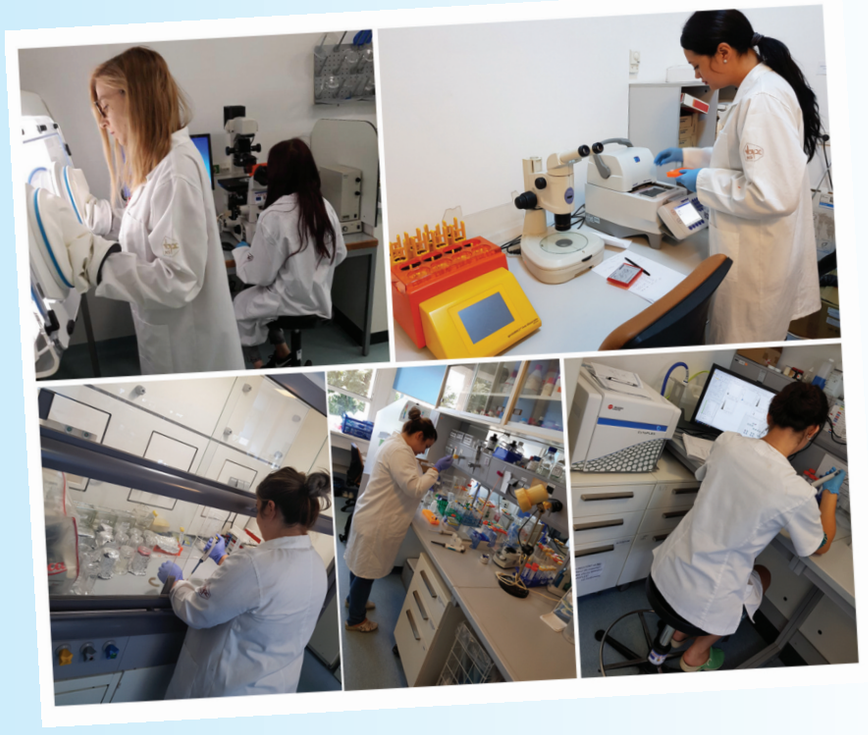
A multi-laboratory project- DIABETER,
Coordinator: *Dr. Nadir Askenasy*, Schneider Children's Medical Center of Israel;
ICBP Coordinators: *Acad. Dr. Maya Simionescu*, *Dr. Alexandrina Burlacu*

Although MSC therapy has been yet unsuccessful in generating robust recovery of endogenous insulin production for the treatment of diabetes, their versatile mode of function may in essence mediate both immunomodulation, islet recovery, and even the conversion to insulin-producing cells.

The objective of the project is to develop a clinically-relevant cell therapeutic approach to cure diabetic autoimmunity by targeted delivery of apoptotic signals using MSCs as vehicles (killer MSCs). The approach is based on adenovirus-mediated transient expression of death ligands, such as Fas-ligand (FasL), tumor necrosis factor-α (TNF) and TNF-related apoptosis-inducing ligand (TRAIL), in MSCs.

In addition to evaluation of the therapeutic impact of killer MSCs on progression of autoimmunity, we aim at determining pathways of MSC navigation to the pancreatic islets, reciprocal interactions between MSCs and apoptotic ligands, the impact of immunomodulation on pancreatic structure and regenerative capacity and the immunological consequences of therapy.

We seek to provide a proof of concept that targeted delivery of death ligands to sites of inflammation modulates and/or abrogates autoimmunity by depletion of pathogenic cells and restoration of self-tolerance.



STEM CELL BIOLOGY LABORATORY

Mihai Bogdan Preda, *Ph.D.*

Group leader of Laboratory
E-mail: *bogdan.preda@icbp.ro*

Special technical expertise

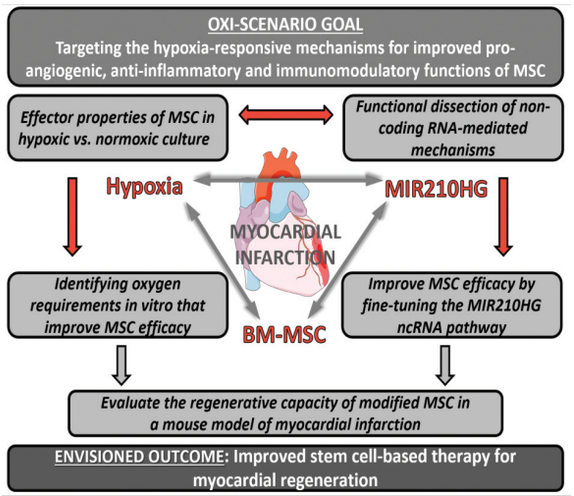
- In vivo fluorescence and bioluminescence imaging
- Immunofluorescence and Confocal microscopy
- Animal models of hind limb ischemia and myocardial infarction
- Isolated heart perfusion using Langendorff system
- Ultrasound imaging – echocardiography
- Stem cell culture

CURRENT PROJECTS

1. ACTIVATION OF HYPOXIA-SIGNALING PATHWAYS IN MSCS TO ENFORCE THEIR REGENERATIVE CAPACITY

In this project, we hypothesized that modulating the culture conditions so as to better mimic the in vivo environment would produce MSCs with higher therapeutic potential. To assess our hypothesis, three specific objectives are envisioned: (i) Assessing the effector functions (pro-angiogenic, anti-inflammatory and immunomodulatory) of MSCs cultured in hypoxic environments; (ii) Uncover the way by which hypoxia-signaling pathways interfere with the effector properties in MSCs; (iii) Evaluation of the migration efficacy and regenerative capacity of hypoxia-cultured human MSCs in a clinically-relevant animal model of myocardial infarction.

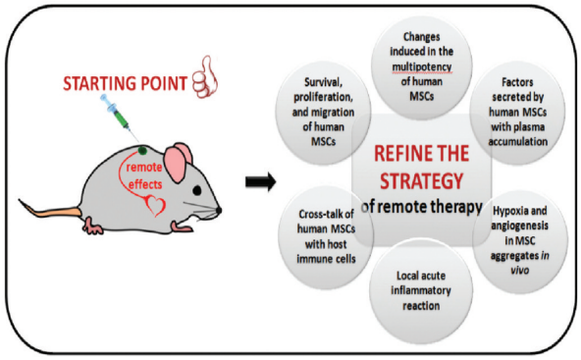
The importance of this project resides in its potential to produce new advances on the molecular mechanisms of hypoxia-responsive pathways in MSCs, with direct implications for human health. Uncovering the "real" mechanisms in MSC biology would be critical in order to improve their engraftment efficacy and therapeutic value.



Although MSCs have a native potential to promote regeneration in the injured tissue, increasing evidence points towards a rather abnormal behavior of cultured MSCs. A possible explanation is the transition of the cells from a low oxygen environment, where they normally reside in vivo, into an atmospheric oxygen environment for in vitro expansion before therapeutic use.

2. REMOTE TRANSPLANTATION OF MESENCHYMAL STROMAL CELLS AS AN ADJUVANT THERAPY FOR MYOCARDIAL REPAIR

Despite the enormous effort to find therapies able to modify the unfavorable course of heart disease after myocardial infarction, specific treatments able to reduce the acute myocardial damage and to promote efficient repair are missing. Stem cell therapy stands as a possible therapeutic approach for ischemic heart disease, however functional improvements reported to date in almost all clinical trials are only moderate positive. Cell delivery methods used in the clinical trials are generally associated with poor survival and engraftment, possibly caused by a local inflammatory environment. Besides, the delivery of stem cells directly into the myocardium is a rather invasive method and is associated with certain risks for the patient. Thus, the inefficiency of the existing approaches for cell transplant demands the development of novel strategies for the treatment of acute and chronic myocardial ischemia.



Schematic representation of the behavior and effects of MSCs in remote transplant therapy.

Taking advantage of the trophic effects of mesenchymal stromal cells (MSCs) and the possibility of evoking myocardial protection through extra-cardiac approaches, we hypothesized that remote transplantation of MSC might confer protection of the heart against ischemia injury. In this project, we first demonstrated that MSC, when subcutaneously transplanted at a site remote from the heart, improved the outcome of ischemia-reperfusion injury, both ex vivo and in vivo and we have introduced the principle of cardioprotection by remote stem cell transplantation (Preda et al., Stem Cells 2014). This study has brought evidence that remote transplantation of MSCs is a safe and a minimally invasive procedure able to provide protection against acute ischemic injury. Transplanted MSCs secrete molecules that are possibly involved in an endocrine-like manner. These results unravel new facets regarding the behavior of MSCs after in vivo transplantation and may contribute significantly to the development of cell remote transplantation as a viable option for ischemia-reperfusion injury and other clinical applications.

Currently, we are expanding our studies beyond the proof-of-concept to understand the behavior of MSCs after subcutaneous transplantation and find the optimal conditions and cellular mechanisms by which remote transplantation of MSCs confer cardioprotection after myocardial infarction.

The therapeutic approach proposed here (remote transplantation of MSCs) offers several advantages compared to other strategies of cell transplantation. Firstly, our proposed approach is minimal invasive, almost painless, does not require general anaesthesia and does not imply any blood loss. This is a major advantage because it reduces the overall procedural risk in patients affected by heart failure. Secondly, this approach can be repeated and many doses can be administrated periodically without any major risk for the patients and without being diluted by other tissue. Thirdly, the clinical use of the remote therapy may represent an adjuvant therapeutic option in combination with conventional therapies in order to add further benefits, or it may be applied as a stand-alone therapy (alternative to conventional therapies) for patients with increased surgical risk.

COLLABORATIONS OF THE LABORATORY

INTERNATIONAL

- Schneider Children's Medical Center of Israel (Prof. Dr. Nadir Askenasy)
- University of Aachen, Germany (Dr. Elisa Liehn)
- Indiana University School of Medicine (Prof. Mircea Ivan, MD, PhD)
- Hannover Medical School (Dr. Thomas Thum)
- Humanitas Research Hospital, Milano, Italy (Dr. Gianluigi Condorelli)
- Maastricht University, The Netherlands (Dr. Leon de Windt)
- Institute of Basic Medical Sciences, University of Oslo, Norway (Dr. Jan Øivind Moskaug)
- University of Groningen, The Netherlands (Dr. Marco Harmsen)

NATIONAL

- National Institute for Non-ferrous and Rare Metals, Bucharest (Dr. Roxana Piticescu)
- INCD Victor Babes, Bucharest (Dr. Valeriu Cismasiu)
- University Emergency Hospital, Bucharest (Dr. Radu Eugen)
- Institute of Biochemistry, Bucharest (Dr. Stefan Szedlacsek)
- Emergency Hospital Floreasca, Bucharest (Prof Maria Dorobantu/Dr. Miruna Micheu)
- "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (Dr Veronica Mocanu)

GRANTS AWARDED BY COMPETITION

2017-2020: Exploring new pathways in age-related heart diseases, acronym **EXPERT** (ERA-CVD - Joint Transnational Call 2016- Transnational Research Projects on Cardiovascular Diseases; Project Manager: Dr. Thomas Thum; ICBP Coordinator: Dr. Alexandrina Burlacu)

2016-2020: Improve institutional competitiveness in the field of type 1 diabetes by developing an innovative concept of immunotherapy based on mesenchymal stromal cells, acronym **DIABETER** (POC-A1-A1.1.4-E-2015, Project Manager: Dr, Nadir Askenasy; Scientific Coordinators: Dr. Maya Simionescu, Dr. Alexandrina Burlacu)

2018-2020: Interference with hypoxia-signaling pathways in mesenchymal stem cells prior to transplantation as a strategy to enhance myocardial recovery post infarction, acronym **OXI-SCENARIO** (PN-III-P1-1.1-PD-2016-1903; Contract no 133/2018; Project Coordinator: Dr. Mihai Bogdan Preda)

2017-2018: Consolidating the Subcutaneous Transplantation of Mesenchymal Stem Cells as a Warranted Therapy for Myocardial Infarction, acronym **Co-SuSTaIn** (PN-III-P2-2.1-PED-2016-1881; Contract no 251PED/2017; Project Coordinator: Dr. Mihai Bogdan Preda)

2015-2017: Ischemic tissue engineering by combinatorial transplant; piecing together the puzzle to gain mutual benefits for graft survival and host tissue repair, acronym **COMTISM** (PN-II-RU-TE-83/2015, Project Coordinator Dr. Alexandrina Burlacu)

2014-2017: Novel orbital implant with high biocompatibility and proliferation rate, acronym **ORBIMPLANT** (PN-II-PT-PCCA-2013-4-0584, Project Manager: Prof. Liliana Voinea; ICBP Coordinator: Dr. Alexandrina Burlacu)

2012-2016: Preclinical model of cell therapy employing protein tyrosine phosphatase-

microRNA, acronym **THERION** (PN-II-PT-PCCA- 79/2012, Project Manager Dr. Alexandrina Burlacu)

2010-2013 - Development of new strategies to improve cell survival and differentiation of stem cells after transplantation into the ischemic myocardium, (PN-II-RU-TE-1, Proiect Coordinator: Dr. Alexandrina Burlacu)

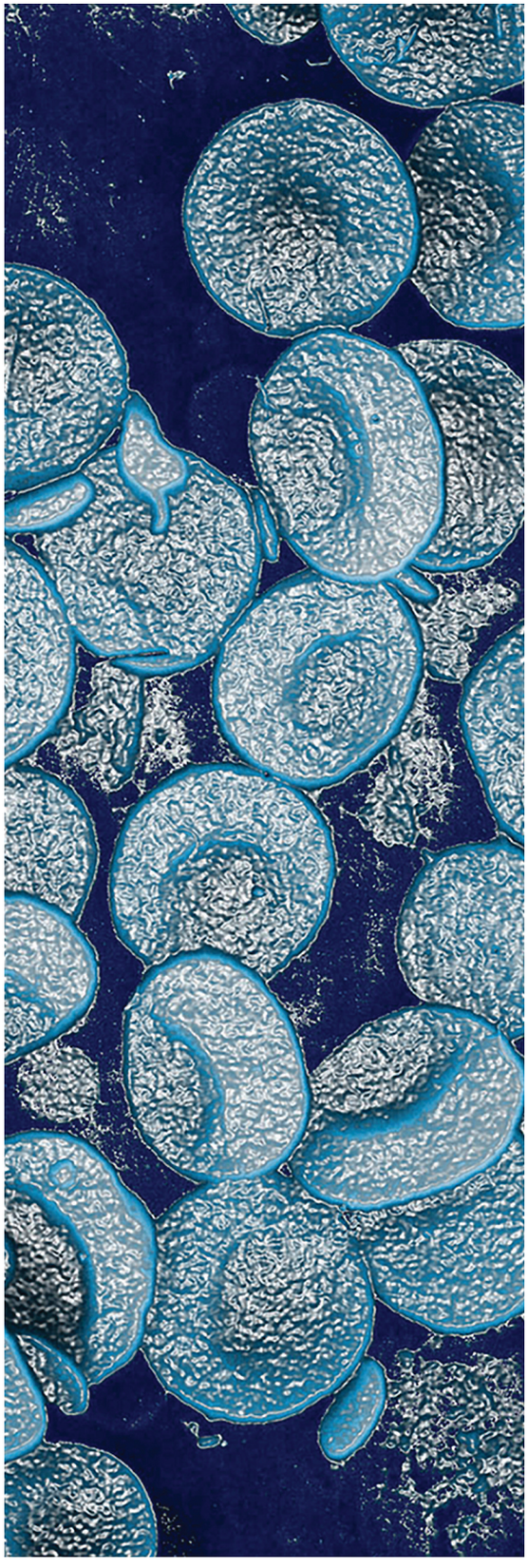
2007-2010- Comparative analysis of molecular signals involved in differentiation of mice embryonic stem cells and adult multipotent progenitor cells, acronym **CARDIOSTEM** (PN-II-ID-PCE-2007-1, Project Manager: Acad. Maya Simionescu; Scientific Coordinator: Dr. Alexandrina Burlacu)

PATENT OF THE LABORATORY

Burlacu A, Mitroi DN, Preda MB, Pleșu M, Roșca A-M, Grigorescu G, Popa M, Corotchi C, Droc I, Gussi IL. *Ex vivo* procedure for engraftment of stem cells into viable slides of human cardiac tissue, patent application, *State Office for Inventions and Trademarks, 2013*, A/00845.

AWARDS

- **Romanian Academy award in Biological Sciences,** 2014 (Alexandrina Burlacu)
- **Herbert Berler-Barbu award - Nicolae Cajal Foundation & Romanian Academy of Medical Science,** 2013 (Alexandrina Burlacu)
- **Prize for scientific debut –Romanian Academy and National Science & Art Foundation**, 2002 (Alexandrina Burlacu)



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