ROMANIAN ACADEMY
School of Advanced Studies of the Romanian Academy
"Nicolae Simionescu" Institute of Cellular Biology and Pathology

PhD THESIS SUMMARY

THE CELLULAR THERAPY OF ISCHEMIC TISSUES: MESENCHYMAL STROMAL CELLS AND ENDOTHELIAL PROGENITOR CELLS DUAL TRANSPLANT

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Oral presentations at international scientific events: 1

National awards: 4

Specializations and courses carried out during the doctoral program: 5

Scholarships obtained during the doctoral program: 2 (doctoral scholarship from the Romanian Academy and Fulbright scholarship for research)

Participation in national research projects: 3

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Introduction and general objectives of the doctoral thesis

Cardiovascular disease (CVD) is the leading cause of death worldwide, with more than 18.6 million victims annually. Of these, more than 80% are due to ischemic heart disease and cerebrovascular disease. Although the programs to inform the population about the risk factors and prevention are successful, the incidence and spread of these pathologies are constantly increasing (Roth et al., 2018). The burden of cardiovascular disease can be felt not only in the quality of life of citizens but also in economic terms, given that in 2015 CVD alone cost the European Union over 210 billion euros (Elizabeth Wilkins, 2017).

According to Eurostat data, approximately 58% of the deaths that occured in Romania in 2016 were due to CVD, with a higher incidence among women (64.9%) than in the case of men (51.6%). The most common medical risk factor is currently considered to be elevated systolic blood pressure, while poor diet is the main behavioral factor associated with CVD mortality. According to the conventional medical perspective, the pathophysiology of ischemic heart disease is multifactorial, based on coronary microvascular dysfunction, severe vasoconstriction, inflammatory and / or atherogenic processes. Indeed, although the gradual onset of ischemia is favored by the typical processes of aging, the medical attention is most often directed at atherosclerotic disease and coronary artery occlusion, culminating in the manifestation of acute myocardial infarction (Elizabeth Wilkins, 2017; Severino et al., 2020).

Ischemic myocardial damage results in loss of cardiomyocyte contractility and mechanical dysfunction, leading to heart failure over time. Considering that the heart has a negligible regenerative capacity, with an annual cardiomyocytes turnover of only 1-2%, strategies that induce regeneration and functional recovery of the heart are necessary (Hashimoto et al., 2018; Turner et al., 2020). To date, the reduced regenerative capacity of cardiomyocytes has been approached from multiple angles, one of the most promising ways being cellular therapy. This involves the direct administration of live cells to a patient, most often by injection, in order to achieve a therapeutic effect. Beneficial mechanisms that can be triggered by cell therapy include tissue regeneration, restoring compromised biological functions, and increasing the body's ability to fight infections (Wang et al., 2021). Numerous preclinical studies have strengthened the potential of cellular therapy in the myocardial recovery process post-infarction, which has accelerated the transition to clinical trials. However, to date, the results of clinical trials have

suggested that the inefficient grafting of transplanted cells to the damaged site is a significant shortcoming of the cell therapies, limiting the beneficial effects induced by the cells administered over time (Fernández-Avilés et al., 2017; Pittenger et al., 2019). For this reason, the need to develop an effective therapy remains relevant and is the target of many innovative biomedical approaches. These are part of the field of tissue engineering and many use cells, biomaterials and complex architectures obtained through 3D printing technology. Most likely, through the progress of the individual fields, it will be possible to outline new solutions for the treatment of cardiovascular diseases.

Considering the impact of myocardial infarction on the global population, the present thesis aimed to develop a cellular strategy to aid cardiac recovery post-infarction. The main hypothesis underlying the research was that the simultaneous administration of two cell types, individually noted for their reparative properties, could produce beneficial effects superior to mono-therapies. Also, in an attempt to develop a 3D structure compatible with cell encapsulation in the future, we optimized the formulation of a hydrogel that can be later applied in tissue engineering.

The structure of the doctoral thesis

The thesis is structured in two parts: the first, entitled "Current state of knowledge", describes recent advances related to cardiovascular disease, myocardial infarction and cell therapy, focusing on mesenchymal stromal cells (MSCs) and endothelial colony-forming cells (ECFCs), these representing the two cell types used in the experimental part. Essential concepts in the field of tissue engineering and hydrogels are also explained.

The second part of the thesis contains original contributions and is divided into two major studies.

The first study, entitled "Dual cell therapy improves myocardial recovery after infarction by reciprocal modulation of MSC and ECFC functions," investigated the therapeutic potential of mesenchymal stromal cells in the presence of endothelial colony-forming cells. This first study had two main objectives:

1. The evaluation of the efficacy of dual MSC+ECFC transplant compared to MSC mono-transplantation in restoring cardiac function in mice with induced myocardial infarction;

2. To investigate the molecular mechanisms by which ECFC modulates MSC and vice versa.

To meet the objectives of this study, we used both in vitro experimental models and an animal model of myocardial infarction, performed on mice. The experiments involved biochemistry techniques such as ELISA and Western blotting, molecular biology techniques such as qRT-PCR, cell culture studies and characterization by flow cytometry. The detailed description of the protocols can be found in the "Materials and methods" section related to this chapter of the thesis.

The original results of this study showed that:

- Dual transplantation of MSC and ECFC improves cardiac function after myocardial infarction, compared to the therapy based only on MSC;
- Dual cell therapy (MSC+ECFC) promotes the expression of Conexin 43 and Integrin alpha5 in infarcted hearts, suggesting a superior integration of cells in the extracellular matrix;
- MSC and ECFC have distinct secretory profiles, with angiogenic effect;
- The interaction with ECFC induces in MSC the expression of the fibronectin receptor, indicating an improved ability of the co-transplanted cells to graft into the host tissue;
- The direct contact between MSC and ECFC modulates their secretory activity, especially growth factors and cytokines involved in angiogenesis, in the organization of the extracellular matrix and in the inflammatory response.

These results prove the effectiveness of a dual approach as a therapy for the post-infarction myocardium, by mutually modulating the beneficial properties specific to each cell type. This conclusion confirms the initial hypothesis that the simultaneous use of MSCs and ECFCs, individually recognized for their repair potential, has beneficial effects superior to MSC-based mono-therapy.

The second study included in the "Original Contributions" section of the doctoral thesis is entitled "Obtaining and testing a cytocompatible matrix for cell encapsulation" and aimed to include mesenchymal stromal cells in the 3D structure of a photopolymerizable hydrogel with biomedical applicability. This study was conducted during a Fulbright research internship in the

Department of Chemical and Biomolecular Engineering at the University of California, Los Angeles.

To achieve this goal we employed chemical methods for the synthesis and polymerization of the hydrogel, mechanical tests to characterize its properties as well as experiments on cell cultures to validate the cytocompatibility of the matrix. The specific details of these experiments can be found in the section "Materials and methods" related to this chapter of the doctoral thesis.

The results of this study showed that:

- The functionalization of gelatin with methacrylate groups allows the photopolymerization of the hydrogel in an externally controlled process that is dependent on light exposure;
- The duration of the photopolymerization determines the mechanical properties of the hydrogel, in a relation of direct proportionality;
- The hydrogel is stable in terms of degradation in aqueous conditions;
- The photopolymerization of the hydrogel on the skin determines the adhesion to it, an important parameter for the in vivo applicability of biomaterials;
- The hydrogel obtained is highly cytocompatible and the cells encapsulated in it have a high viability during the first 5 days of encapsulation;
- The internal structure of the hydrogel allows the spreading of cells, an essential property of the matrices used in cell encapsulation.

In conclusion, the original results obtained in this doctoral thesis indicate a higher in vivo efficacy of dual MSC + ECFC cell therapy compared to MSC-based mono-therapy. Moreover, the data indicate a reciprocal regulation of the two cell types, with the ultimate effect of stimulating the grafting ability of MSCs as well as individual repair processes. The results obtained in the second study demonstrate the versatility of the photopolymerized hydrogel and its compatibility with MSC encapsulation, indicating its biomedical applicability.

These studies confirm and expand the huge potential of cell therapies in regenerative medicine and the impact it could have on the fight against cardiovascular disease.

Perspectives

There is currently a significant need for therapeutic solutions to reverse the dramatic effects of myocardial infarction on the architecture and function of the heart, in the attempt to slow down the transition to heart failure. With the primary goal of aiding cardiac regeneration, significant progress has been made in recent years in both biomedicine and seemingly distant fields.

Through an interdisciplinary approach, I envision to continue the study on MSC+ECFC dual cell therapy and intersect it with the optimized hydrogel in the second part of the thesis. Thus, it will be possible to test the cells in vivo by administration in encapsulated form, as a patch, compared to the classic cell suspension injection and to determine the optimal delivery method. I also anticipate in vitro encapsulation experiments using MSCs and ECFCs that highlight not only the secretory abilities of the two cell types, but also their ability to arrange in native tissue-like structures.

Such results will be able to determine whether the administration of cells in a 3D protective matrix is sufficiently advantageous to prolong the effectiveness of cell therapies over time.

Personal contributions

List of papers published and communicated during the doctoral program

Paper published in an ISI indexed journal - lead author

S. Popescu, M.B. Preda, C.I. Marinescu, M. Simionescu, A. Burlacu. Dual Stem Cell Therapy Improves the Myocardial Recovery Post-Infarction through Reciprocal Modulation of Cell Functions. Int J Mol Sci. 2021 May 26; 22 (11): 5631. doi: 10.3390 / ijms22115631. (Impact factor: 5.92, Q1)

Papers published in ISI indexed journals - co-author

M.B. Preda, A.M. Lupan, C.A. Neculachi, L.I. Leti, I.M. Fenyo, S. Popescu, E.G. Rusu, C.I. Marinescu, M. Simionescu, A. Burlacu. Evidence of mesenchymal stromal cell adaptation to local microenvironment following subcutaneous transplantation. J Cell Mol Med. 2020 Aug 12; 24 (18): 10889–97. doi: 10.1111 / jcmm.15717. (Impact factor: 5.31, Q1)

C.I. Marinescu, M.B. Preda, C.A. Neculachi, E.G. Rusu, S. Popescu, A. Burlacu. Identification of a Hematopoietic Cell Population Emerging From Mouse Bone Marrow With Proliferative Potential In Vitro and Immunomodulatory Capacity. Front. Immunol. 2021 12: 698070. doi: 10.3389 / fimmu.2021.698070 (Impact factor: 7.56, Q1)

Abstract published in an ISI indexed journal

S. Popescu, A.M. Lupan, M.B. Preda, M. Simionescu, A. Burlacu, "Dual stem cell therapy improves cardiac function after experimental myocardial infarction". Circulation Research. 2020; 127: A492, DOI: 10.1161 / res.127.suppl_1.492 (Impact factor: 17.36, Q1)

Contributions at international and national scientific events - first author

S. Popescu, M.A. Publik, B. M. Preda, "A novel neutrophil-like in vitro model for the study of human neutrophil polarization". The 38th International Conference and Annual Scientific Session of the Romanian Society for Cell Biology, November 4-6, 2021. Virtual event - poster presentation

S. Popescu, AM Lupan, M.B. Preda, M. Simionescu, A. Burlacu, "Dual stem cell therapy improves cardiac function after experimental myocardial infarction". American Heart

Association Basic Cardiovascular Science Scientific Sessions 2020. July 27–30, 2020. Virtual Event. - Poster presentation

S. Popescu, AM Lupan, M.B. Preda, A. Burlacu, M. Simionescu "Cross-talk between Mesenchymal Stromal Cells and Endothelial Progenitors modulates the expression of angiogenesis related molecules" - The 36th Annual Scientific Session of the Romanian Society for Cell Biology and the 10th National Congress with International participation, 2018, Craiova, Romania - Oral presentation.

S. Popescu, M.B. Preda, A. Burlacu. "Assessment of the proper ratio between endothelial progenitor cells and mesenchymal stromal cells that confers superior angiogenic effects in vitro." 9th National Congress with International Participation and 35th Annual Scientific Session of the Romanian Society for Cell Biology, June 6-12, 2017, Iasi, Romania - Poster presentation

S. Popescu, M.B. Preda, A. Burlacu "Assessment of the proper ratio between endothelial progenitor cells and mesenchymal stromal cells that confers superior angiogenic effects in vitro" European Society of Cardiology "Basic Science Summer School", 17-25 June 2017, Nice, France - Poster presentation

Contributions at international and national scientific events - co-author

M.B. Preda, S. Popescu, R. Tutuianu, A.M. Rosca, M. Simionescu, A. Burlacu. "Hypoxia regulates the pro-angiogenic effect of subcutaneously transplanted mesenchymal stromal cells." EMBO Conference: The Molecular and Cellular Basis of Regeneration and Tissue Repair. 15-19 September 2018. Valletta, Malta;

M.B. Preda, S. Popescu, R. Tutuianu, A.M. Rosca, M. Simionescu, A. Burlacu. "Hypoxia regulates the pro-angiogenic effect of subcutaneously transplanted mesenchymal stromal cells." Keystone Symposia meeting on Therapeutic Targeting of Hypoxia-Sensitive Pathways. 10-14.04.2018. University of Oxford Mathematical Institute, Oxford, UK.

M.B. Preda, S. Popescu, R. Tutuianu, A.M. Rosca, M. Simionescu, A. Burlacu. "Hypoxia regulates the pro-angiogenic effect of subcutaneously transplanted mesenchymal stromal cells."

INTERNATIONAL COURSE & ISRMS 2nd CONGRESS, June 14-17, 2017, Bucharest, Romania.

Specializations and courses conducted during the doctoral program

2021 - Virtual Workshop: "Cell-Matrix Interaction and Mechanobiology in Regenerative Medicine", Tissue Engineering and Regenerative Medicine International Society, June 10-11, 2021

2019 - "Micro- and Nanotechnologies for Medicine Workshop: Emerging Frontiers and Applications", University of California, Los Angeles, Los Angeles, USA July 8-12, 2019

2019 - Molecular Basis of Vascular Biology Course (MCD BIO 224-1), University of California, Los Angeles, Los Angeles, USA, March - June 2019

2018 - TOEFL Certification - English Language ibt Test: score 106/110

2017 - "Techniques to validate the isolation of adipose derived stem cells and their differentiation evaluation" Course - the Second Congress of the International Society of Regenerative Medicine and Surgery (ISRMS), June 14-17, 2017, Bucharest, Romania

Awards received during the doctoral program

2021 - UEFISDCI PN-III-P1-1.1-PRECISI-2021 - Awarding research results - articles - S. Popescu, M.B. Preda, C.I. Marinescu, M. Simionescu, A. Burlacu. Dual Stem Cell Therapy Improves the Myocardial Recovery Post-Infarction through Reciprocal Modulation of Cell Functions. Int J Mol Sci. 2021 May 26; 22 (11): 5631. doi: 10.3390 / ijms22115631. (Impact factor: 5.92)

2021 - UEFISDCI PN-III-P1-1.1-PRECISI-2021 - Awarding research results - articles - C.I. Marinescu, M.B. Preda, C.A. Neculachi, E.G. Rusu, S. Popescu, A. Burlacu. Identification of a Hematopoietic Cell Population Emerging From Mouse Bone Marrow With Proliferative Potential In Vitro and Immunomodulatory Capacity. Front. Immunol. 2021 12: 698070. doi: 10.3389 / fimmu.2021.698070 (Impact factor: 7.56)

2020 - UEFISDCI PNCDI III - Awarding research results - articles - M.B. Preda, A.M. Lupan, C.A. Neculachi, L.I. Leti, I.M. Fenyo, S. Popescu, E.G. Rusu, C.I. Marinescu, M. Simionescu, A. Burlacu. Evidence of mesenchymal stromal cell adaptation to local microenvironment following subcutaneous transplantation. J Cell Mol Med. 2020 Aug 12; 24 (18): 10889–97. doi: 10.1111 / jcmm.15717. (Impact factor: 5.31)

2020 - American Heart Association Basic Cardiovascular Science Scientific Sessions 2020: Paul Dudley White International Scholar Award for the Highest Ranked Abstract from Romania

2018 - Award for the best oral presentation (3rd place) - "The 36th Annual Scientific Session of the Romanian Society for Cell Biology and the 10th National Congress with International participation", 2018, Craiova, Romania

Scholarships and research funding received during the doctoral program

2018 - 2019 - Research Fellowship - Fulbright Student Award to the United States

2016 - 2019 - Doctoral Scholarship - School of Advanced Studies of the Romanian Academy

Collaborator in national and international grants:

INNATE-MI PN-III-P4-ID-PCCF-2016-0172: Targeting innate immune mechanisms to improve risk stratification and to identify future therapeutic options in myocardial infarction.

Co-SuSTaIn PN-III-P2-2.1-PED-2016-1881: Consolidating the Subcutaneous Transplantation of Mesenchymal Stem Cells as a Warranted Therapy for Myocardial Infarction.

ComTIsM PN-II-RU-TE-2014-Contract 83/2014 Ischemic tissue engineering by combinatorial transplantation: assembly of parts that induce both graft survival and host tissue repair.

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