ROMANIAN ACADEMY

School of Advanced Studies of the Romanian Academy

Institute of Cellular Biology and Pathology "Nicolae Simionescu"

PhD THESIS SUMMARY

IDENTIFICATION AND UTILIZATION OF FACTORS SECRETED BY MESENCHYMAL-DERIVED CELLS IN CUTANEOUS WOUND THERAPY: *IN VITRO* AND *IN VIVO* STUDIES

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Bucharest

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Keywords: chronic wounds, regenerative medicine, mesenchymal stromal cells, immortalization, fibroblasts, secretome, tridimensional culture, wound healing, re-epithelialization, angiogenesis

Total number of pages – 249

Number of figures in Part I – 15

Number of tables in Part I – 5

Number of figures in Part II – 99

Number of tables in Part II – 3

Number of bibliographic references – 338

Number of original papers published in ISI-indexed international journals – 4 (3 as main author, 1 as co-author)

Number of original papers in preparation – 2 (main author)

Book chapters – 1

Oral communications presented at international scientific events – 2

Posters presentations at national and international scientific events – 7 (3 as main author)

International stages completed during the doctoral programme – 1

Number of courses and seminars attended during the doctoral programme - 7

National awards received from UEFISCDI – 2

Registered national patents – 1 (submitted)

Scholarships obtained during the doctoral training programme and funding of the research activity – 2 (PhD scholarship from the Romanian Academy, scholarship from Romanian Ministry of Education for an international research stage)

Participation in national research grants – 4

Participation in international research grants – 2

CONTENT

INTRODUCTION AND GENERAL OBJECTIVES

Wound healing of cutaneous tissue is a physiological process, which involves a sequence of a several interconnected biological and molecular events in response to the occurrence of an injury, resulting in the reconstitution of the skin layers integrity and the restoration of homeostasis. During this dynamic and complex process, multiple biological pathways are activated and synchronized, with coordinated interactions between inflammatory cells, dermal fibroblasts, keratinocytes and endothelial cells (Paul & Sharma, 2004; Reinke & Sorg, 2012). At the same time, resident stem cells can undergo self-replication and differentiation into multiple cell types and can implicitly affect paracrine signaling at the wound level, all of which are crucial for tissue regeneration (Chen et al., 2009).

Depending on the duration of healing, skin wounds are classified into acute and chronic (healable and difficult to heal, respectively) (Robson et al., 2001). Acute wounds show well-defined signs of recovery within 4 to 12 weeks, the healing period depending on the extent of the injury. In some cases, it is impossible to effectively restore normal tissue structure and function, as the physiological wound healing process is interrupted, as a result of dysfunctions in the regulation of tissue repair responses, such as inflammation, angiogenesis, extracellular matrix deposition and cell recruitment. Thus, the inflammatory phase associated with excessive inflammation, the decreased of angiogenesis and the poor keratinocytes and fibroblasts (Fb) migration and proliferation are the key elements of chronic wounds (Falanga, 2005; Lazarus et al., 1994).

Currently, the incidence of chronic wounds continues to increase, representing a significant clinical challenge. Usually, adults with comorbidities such as venous insufficiency, diabetes, obesity, neuropathies, arterial insufficiency or presenting various limiting factors such as aging, infections, poor nutrition, immunosuppression, are affected. Chronic wounds are classified into three major categories: vascular ulcers, diabetic ulcers, and pressure ulcers (Nunan et al., 2014).

Despite the efforts made over time, chronic skin wounds and their healing represent major clinical challenges, which involve a considerable social and medical burden. However, there is significant interest in the use of stem cells in the management of this problem. In the last decades, regenerative medicine has gained considerable attention, based on the restoration, replacement or repair of traumatized cells, tissues and organs, thus offering possible treatment methods.

Mesenchymal stromal cells (MSCs) play an important role in this field. They are defined as a heterogeneous group of unspecialized spindle-shaped progenitor cells (Fb-like shape), of mesodermal origin, adherent to plastic under standard culture conditions and capable of self-renewal by division and differentiation into multiple cell lineages (Wei et al., 2013). These cells were first identified in bone marrow (BM). However, harvesting BM cells (BM aspirate) is an invasive and painful procedure, with a low yield of MSCs (Pittenger et al., 1999).

These limitations have driven the search for alternative sources of MSCs, including adipose tissue, Wharton's jelly, umbilical cord blood, placenta, amniotic fluid, and others (Keating, 2006; Ullah et al., 2015; Gottipamula et al., 2018; Eom et al., 2011). Among these, sources that enable minimally invasive harvesting and provide abundant cell yields, such as adipose tissue are preferable (Kuhbier et al., 2010).

Initially, the differentiation capacity of MSCs was studied as a potential regenerative therapy for the healing of cutaneous tissue wounds. It has been demonstrated that MSC transplantation promotes tissue repair primarily through paracrine effects rather than direct cell replacement. This paracrine effect is attributed to the secretome, which comprises soluble factors (such as cytokines and chemokines) and extracellular vesicles released by the cells into the extracellular space. Consequently, current research is focused on developing novel acellular therapeutic strategies that harness the MSC secretome to promote wound healing by stimulating

angiogenesis, modulating inflammation, reducing scar formation, and inducing re-epithelialization (Zarei & Soleimaninejad, 2018; Guillamat-Prats, 2021; Ahangar et al., 2020). Compared to cell-based therapy, the acellular therapy is less immunogenic and carries a minimal risk of tumor formation (Vizoso et al., 2017; Lu et al., 2017; Ha et al., 2020), at the same time offering advantages in terms of handling and safety.

Cells exhibit a limited number of population doublings *in vitro*, eventually leading to the onset of senescence and, consequently, cell death. To overcome this limitation, telomerase reactivation techniques are employed to preserve telomere length, enabling cells to undergo an unlimited number of divisions. Alternatively, methods based on inactivation of proteins involved in cell cycle regulation can be used. Cells obtained through these methods are genetically and phenotypically similar or identical to their original counterparts. This process, known as ‘‘cell immortalization’’ has been made possible by understanding the molecular mechanisms that drive this process *in vivo*, particularly in cancer (Ivanković et al., 2007; Chalak et al., 2024; de Bardet et al., 2023).

Fibroblasts (Fb) and MSCs share several common characteristics: spindle-shaped morphology, localization in connective tissue, proliferative potential, expression of specific surface markers, multipotency, lack of telomerase activity, and similar gene expression patterns. Furthermore, both cell types play analogous roles in immune regulation. Due to these similarities, Fb could serve as a promising alternative to MSCs for clinical applications, such as tissue regeneration and skin wound healing. They can be easily isolated in large quantities from various tissues, including skin, adipose tissue, and gingiva. In contrast, the most commonly used source of MSCs, bone marrow (BM), provides relatively limited material for expansion and requires invasive collection methods. Moreover, fibroblasts exhibit a significantly shorter population doubling time compared to MSCs, with senescence typically occurring after more than 50 population doublings (Huang et al., 2010). At the same time, the culture media requirements for expanding and maintaining Fb *in vitro* culture are not complex.

On the other hand, the regenerative potential of mesenchymal cells is influenced by various factors, such as the tissue of origin, the age of the donor, the culture conditions or the presence of inflammatory factors in the body (Ferreira et al., 2018). For this reason, several *in vitro* MSC preconditioning strategies, such as culturing under hypoxic conditions, in a 3D

system or treatment with inflammatory factors, have been used to stimulate their secretory properties (Haider and Ashraf, 2010; Lu et al., 2010). Moreover, fibroblasts cultured in the form of aggregates were assigned the concept of "nemosis" by Vaheri et al. (Vaheri et al., 2009). More specifically, this notion is defined as a way of activating cells, which is characterized by increased production of COX-2 (Cyclooxygenase-2), secretion of prostaglandins, proteinases, chemotactic cytokines, HGF (Hepatocyte Growth Factor), VEGF (Vascular endothelial growth factor) and expression of activated nuclear factor-kappa B (Vaheri et al., 2009; Enzerink et al., 2010). Thus, through this process, the paracrine properties of Fb can be improved, which can be the basis for therapeutic strategies that use the secretome ("cell-free" therapies).

Also, the generation of MSCs from stem cells seems to offer the unique opportunity to overcome most of the obstacles that currently exist regarding the large-scale use of MSCs as an innovative therapy. Over time, it has been reported that induced pluripotent stem cells (iPSCs) can be differentiated into MSCs, as well as Fb. They originate from a single clone of iPSCs, presenting high homogeneity for clinical applications. Moreover, their biological performance is more stable and predictable, as the molecular pattern of native MSC changes subtly between different batches.

Thus, **the aim** of this doctoral thesis was to evaluate the effects of the secretome derived from human mesenchymal cells on the wound healing process, both *in vitro* and *in vivo* on a murine wound model. To achieve this goal, several **objectives** have been proposed, utilizing numerous cell sources, all of human origin.

Objective 1. Obtaining and characterizing human mesenchymal stromal cells (MSCs) derived from bone marrow and adipose tissue, as well as primary human dermal fibroblasts or obtained from human induced pluripotent stem cells.

Objective 2. *In vitro* testing of the secretome from an immortalized line of adipose tissue-derived MSCs (ADSCs) following its effect on the processes involved in skin wound healing.

Objective 3. Comparative study of the properties of human dermal fibroblasts and human bone marrow-derived MSCs cultured in two- (2D) and three-dimensional (3D) systems.

Objective 4. *In vitro* testing of the effect of the secretome from primary dermal fibroblasts and fibroblasts derived from human induced pluripotent stem cells cultured in 2D and 3D systems on the processes involved in skin wound healing.

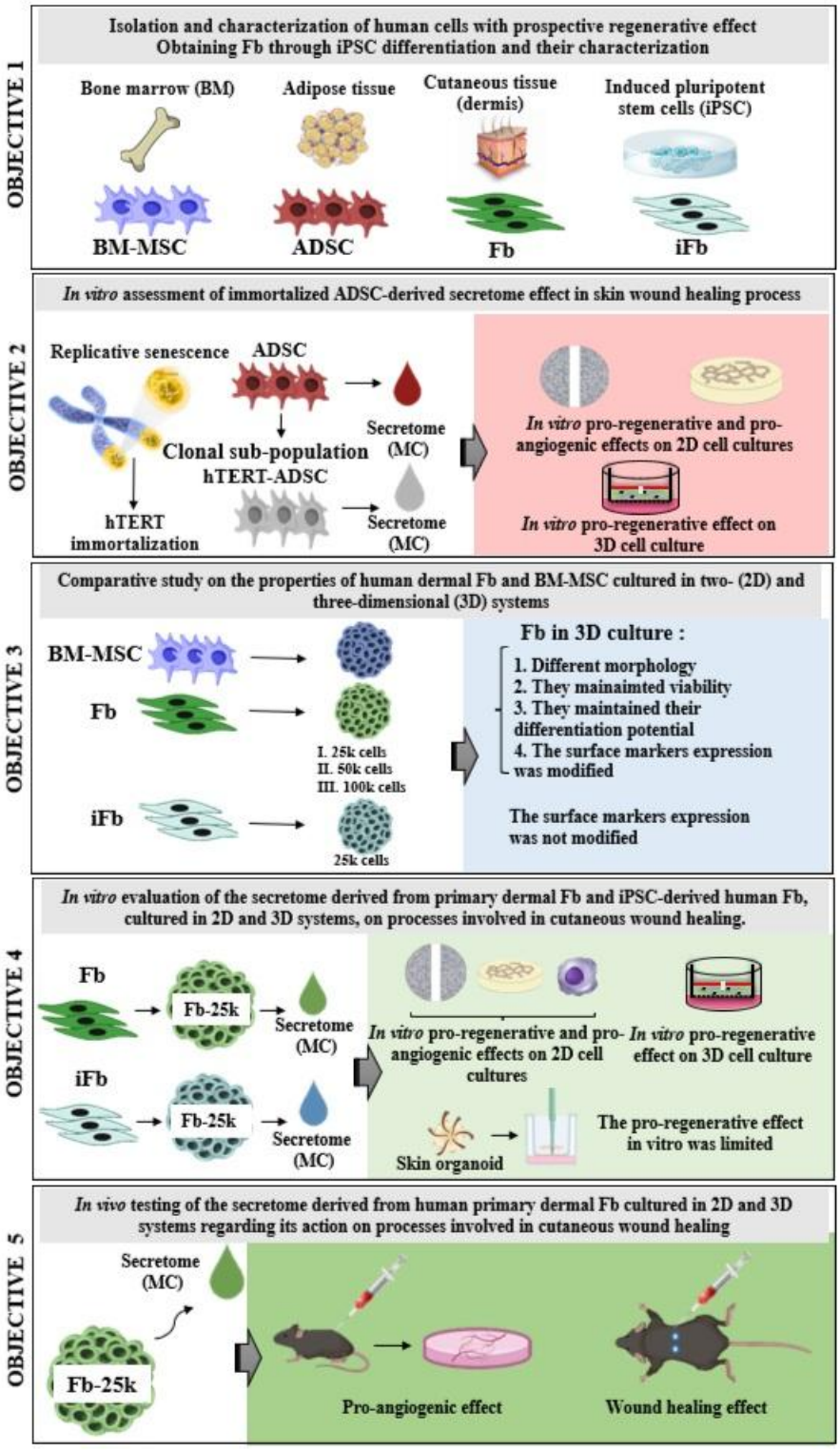
Objective 5. *In vivo* testing of the secretomes from primary human dermal fibroblasts cultured in 2D and 3D systems regarding their action on the processes involved in skin wound healing.

THESIS STRUCTURE

The thesis is structured in **9 chapters**, comprising theoretical notions regarding the current state of knowledge, as well as the original results obtained (the original contributions part).

The first section (“Current state of knowledge”) is structured in ***4 chapters*** and comprises up-to-date data from the literature regarding the subject of this thesis. More precisely, **chapter I.1** presents the issue of cutaneous wounds, focusing on the anatomical and physiological foundations of the cutaneous tissue, the stages of wound healing along with a brief presentation of the disruption of the physiological process encountered in the case of chronic cutaneous wounds, as well as current therapeutic approaches. **Chapter I.2** focuses on the defining characteristics of the contribution of the secretome originating from MSCs in the healing process of cutaneous wounds, focusing on the definition of MSCs, the description of the secretome composition secreted by these cells but also the presentation of current methods for boosting its activity. Also, studies in the literature suggesting the use of secretome from MSCs as a potential therapy for skin wounds are presented. **Chapter I.3** focuses on the concept of using fibroblasts as an alternative to MSCs, presenting the similarities between them, as well as existing studies on the applicability of the secretome from fibroblasts as a possible therapy for skin wounds. In **chapter I.4** are presented the potential directions for obtaining an inexhaustible source of mesenchymal cells: cell immortalization, as well as their generation from iPSCs. Some of the data presented in this section are part of a review article published this year in an ISI-indexed journal: **Gheţu, D-.M.**, Raymond, K., Titorencu, I., Simionescu, M. **2025.** Innovative Strategies: Use of Stromal Cell-Derived Secretome for Chronic Wound Therapy. *International Journal of Molecular Sciences*, *26*(12), 5609. DOI: 10.3390/ijms26125609 (IF/2024 = 4.9, Q1, AIS = 1.121).

The second section ("<u>The original contributions</u>") is structured into ***5 chapters*** based on the 5 objectives stated above. Thus, to achieve the proposed objectives we used various techniques such as cell cultures, flow cytometry, molecular biology, histology and animal experiments which are described in the ''Materials and Methods'' sections of each study. The content of the chapters is outlined in the figure below and briefly presented as follows.



The schematic representation of the content of the five chapters (objectives) in the second part of the thesis ("Original Contributions") is presented below. CM: conditioned medium. hTERT: telomerase reverse transcriptase (catalytic subunit of telomerase).

Chapter II.1 of the thesis is associated with the first objective (Objective 1), being entitled **"Obtaining and characterizing human mesenchymal stromal cells (MSCs) derived from bone marrow and adipose tissue, as well as primary human dermal fibroblasts or obtained from human induced pluripotent stem cells".** It presents the isolation of primary cells with regenerative potential for wound healing (MSCs derived from BM and adipose tissue and Fb isolated from dermis) and their characterization in terms of specific markers and the capacity to differentiate towards mesodermal cell lines: adipogenic, osteogenic and chondrogenic lines. Moreover, it is also focused on obtaining Fb by differentiating from iPSCs and characterizing them in terms of specific surface and intracellular markers (such as vimentin and collagen). The results show the success of isolating primary cells that express specific markers and have the ability to differentiate towards mesodermal cell lines. Fb were also obtained by differentiating iPSCs (iFb), expressing specific surface and intracellular markers. These cells were used in *in vitro* and *in vivo* experiments to achieve the other proposed objectives, which aim to use the secretome (conditioned medium) as an "acellular" therapy for skin wounds.

Chapter II.2 (Objective 2) is entitled **"*In vitro* testing of the secretome from an immortalized line of adipose tissue-derived MSCs (ADSCs) following its effect on the processes involved in skin wound healing**". It presents the generation of an immortalized line of ADSCs (clonal subpopulation) by lentiviral transduction of the gene encoding the reverse transcriptase (catalytic component) of human telomerase (hTERT) to overcome the phenomenon of replicative senescence and the *in vitro* evaluation of the effects of the secretome (conditioned medium) from these cells on key processes associated with skin wound healing. Briefly, the impact of the conditioned medium on the proliferation and migration of keratinocytes, Fb and endothelial cells, as well as on angiogenesis (processes that occur during the proliferative phase of wound healing), were reported. At the same time, the supporting effect on the re-epithelialization process of a wound made on an organotypic human skin culture model was also demonstrated. The results presented in this chapter were included in a paper published in an ISI indexed journal: **Iacomi, D. M.**, Roşca A. M., Ţuţuianu, R., Neagu, T. P., Prună, V., Simionescu, M., Titorencu, I. **2022**. Generation of an immortalized human adipose-derived mesenchymal stromal cell line suitable for wound healing therapy. *International Journal of Molecular Sciences*, 23(16):8925. DOI: 10.3390/ijms23168925 (IF/2022 = 5.6, Q1, AIS = 1.028).

Chapter II.3 (Objective 3) entitled **"Comparative study on the properties of human dermal fibroblasts and human bone marrow-derived MSCs cultured in two-dimensional (2D) and three-dimensional (3D) systems**" involved the comparative analysis of primary human BM-MSCs and dermal Fb (as a much more accessible alternative cell source)/differentiated from iPSCs cultured in a 3D system (as a strategy to improve secretory capacity), more precisely in cell aggregates (spheroids) obtained by culturing cell suspension in „hanging drops". The spheroids/primary cells in their composition were analyzed in terms of morphology, viability, secretion of extracellular matrix components, presence of adhesion molecules, but also maintenance of specific surface markers and differentiation potential towards the adipogenic, osteogenic and chondrogenic lineages. The results showed that both primary cell types have the ability to aggregate into spheroids after 72 hours, detecting the secretion of extracellular matrix elements, the maintenance of differentiation potential, but also some morphological changes and the presence of specific surface markers. From the point of view of viability, no major changes were observed following the aggregation process. Fb differentiated from iPSCs also formed spheroids, maintaining their specific surface markers following aggregation. The original data contained in this study are part of a paper in preparation.

In **chapter II.4** (Objective 4) - **"*In vitro* testing of the effect of the secretome from primary dermal fibroblasts and from fibroblasts derived from human induced pluripotent stem cells cultured in 2D and 3D systems on the processes involved in skin wound healing**" - the results obtained regarding the *in vitro* evaluation of the effects of the conditioned medium from primary dermal Fb cultured in 3D systems (spheroids) on the key processes associated with skin wound healing: inflammation, angiogenesis and re-epithelialization, compared to the effects of the conditioned medium from Fb cultured in monolayer (2D) are presented. It was shown that the secretion of pro-angiogenic factors was increased by three-dimensional (3D) cultivation of primary human dermal Fb in the form of spheroids (25.000 cells/spheroid- 25k), improving the migration and proliferation processes, as well as re-epithelialization at the wound level on a 3D model of organotypic skin culture in the presence of the conditioned medium from these cells. Thus, the results obtained confirm that the secretome from these cells cultured in 3D system represents a possible treatment strategy for wound healing. At the same time, the data regarding the evaluation of the secretome obtained from iPSC-derived Fb (iFb) cultured in both

2D and 3D system (25000 cells/spheroid) are also presented, but no effect on stimulating the re-epithelialization on a 3D model of a skin equivalent obtained from organoids was obtained. The original data included in this study are part of a paper in preparation.

The last chapter of the original section, **chapter II.5** (objective 5) – "***In vivo* testing of the secretomes from primary human dermal fibroblasts cultured in 2D and 3D systems regarding their action on the processes involved in skin wound healing**" is associated with the *in vivo* evaluation of the effects of the conditioned medium from primary dermal Fb cultured in 3D systems (spheroids consisting of 25000 cells) in terms of angiogenesis and the re-epithelialization process, compared to the effects of the conditioned medium from Fb cultured in monolayer. In short, the results showed the beneficial effect of the secretome from primary human dermal Fb on angiogenesis and re-epithelialization of skin wounds, using the Matrigel plug assay technique and, respectively, a skin wound model on mice. The original data contained in this study are part of a paper in preparation.

Finally, the thesis concludes with the corresponding bibliography.

PERSONAL CONTRIBUTIONS

LIST OF PUBLISHED AND COMMUNICATED PAPERS DURING THE DOCTORAL PROGRAMME

Papers published in ISI journals (first author)

1. **Gheţu, D.-.M**, Raymond, K., Titorencu, I., Simionescu, M. **2025.** Innovative Strategies: Use of Stromal Cell-Derived Secretome for Chronic Wound Therapy. *International Journal of Molecular Sciences, 26*(12), 5609. DOI: 10.3390/ijms26125609 (IF/2024 = 4.9, Q1, AIS = 1.121).

2. Sorca†, B. V., Kaya, D. A., Kaya, M. G. A., Enăchescu, M., **Gheţu†, D.-M.**, Enache, L.-B., Boeraşu, I., Coman, A. E., Rusu, L. C., Constantinescu, R., Titorencu, I. **2025**. Bone Fillers with Balance Between Biocompatibility and Antimicrobial Properties. *Biomimetics*, 10(2), 100. DOI: 10.3390/biomimetics10020100 (IF/2023 = 3.4, Q2, AIS = 0.567).

3. **Iacomi, D. M.**, Roşca A. M., Ţuţuianu, R., Neagu, T. P., Prună, V., Simionescu, M., Titorencu, I. **2022**. Generation of an immortalized human adipose-derived mesenchymal stromal cell line suitable for wound healing therapy. *International Journal of Molecular Sciences*, 23(16):8925. DOI: 10.3390/ijms23168925 (IF/2022 = 5.6, Q1, AIS = 1.028).

Papers published in ISI journals (co-author)

1. Ţuţuianu, R., Roşca, A. M., **Iacomi, D. M.**, Simionescu, M., Titorencu, I. **2021**. Human mesenchymal stromal cell-derived exosomes promote *in vitro* wound healing by modulating the biological properties of skin keratinocytes and fibroblasts and stimulating angiogenesis. *International Journal of Molecular Sciences*, 22(12), 6239. DOI: 10.3390/ijms22126239 (IF/2021 = 5.924, Q1, AIS = 1.064).

Collaborator in chapter published in an international monography

1. Roșca, A.M., Ţuţuianu, R., **Ghetu, D.M.**, Titorencu, I., **2023.** Mesenchymal stromal cells for wound healing therapy: From expectations to reality. In: K.H. Haider, ed. *Handbook of Stem Cell Applications*. Singapore: Springer. DOI: 10.1007/978-981-99-0846-2_53-1.

Papers in preparations

1. **Gheţu, D.-M.**, Ţuţuianu, R., Titorencu, I., Roşca, A.M. The secretome of human dermal fibroblasts spheroids supports skin regeneration by promoting the remodeling of the extracellular matrix on a full thickness wound mouse model.
2. **Gheţu, D.-M.,** Ramovs, V., Flesseman, M., Titorencu, I., Raymond, K., A Novel Full Thickness In Vitro Human Wound Model Based on Organoid-derived Skin Equivalent.

Presentations at international scientific manifestations

Oral presentations (2):

1. **Gheţu D.M.**, Roşca A.M., Ţuţuianu R., Neagu T.P., Prună V., Simionescu M., Titorencu I., Aggregates of dermal fibroblasts and bone marrow mesenchymal stromal cells exhibit different characteristics: implications for regenerative therapy, "*International Conference and XXXIX Scientific Session of the Romanian Society of Cell Biology*", October 21-23, 2022, Cluj, Romania.

2. **Iacomi D. M.**, Roşca A.-M., Ţuţuianu R., Prună V., Titorencu I., Simionescu M., Generation and characterization of an immortalized human adipose mesenchymal stromal cell line, International Conference under the aegis of the Romanian Academy - 42nd Anniversary Symposium of the Institute of Cellular Biology and Pathology "Nicolae Simionescu" held jointly with 38th annual scientific session of The Romanian Society for Cell Biology, November 4-6, 2021, online event.

Poster presentations (2):

1. **Gheţu D. M.,** Ţuţuianu R., Albu Kaya M. G., Roşca A. M., Titorencu I., Development of three-dimensional intestinal organotypic models using various collagen-based dermal equivalents", poster presented at the international conference "*Applications of Chemistry in Nanosciences and Biomaterials Engineering (NanoBioMat)*", June 25-27, 2025 (Summer Edition), online event.
2. **Gheţu D.M.,** Ţuţuianu R., Roşca A.M., Simionescu M., Titorencu I., Three-dimensional aggregation stimulates the pro-angiogenic properties of adult human dermal fibroblasts, poster presented at the anniversary symposium with international participation "*Aspiration, Inspiration, and Innovation in Exploring New Frontiers in Biomedical Research*" – organized on the occasion of the 45th anniversary of the Institute of Cellular Biology and Pathology "Nicolae Simionescu", December 17-18, 2024, Bucharest, Romania.
3. **Gheţu D.M.**, Roşca A.M., Ţuţuianu R., Prună V., Simionescu M., Titorencu I., Human dermal fibroblasts and bone marrow mesenchymal stromal cells display different characteristics when cultured in 3D settings, poster presented at the annual scientific symposium of the Institute of Cellular Biology and Pathology "Nicolae Simionescu" with international participation "*43 years on the never-ending road of cardiovascular discoveries*", December 8-9, 2022, Bucharest, Romania.

Poster presentations at national and international scientific manifestations (co-author)

1. Ţuţuianu R., **Gheţu D.M.**, Titorencu I., Roşca A.M., Conditioned Medium Derived from Fibroblasts Cultured in Spheroids Promotes Skin Regeneration in a Murine Model of Cutaneous Wound", poster presented at the scientific event ARSAL Symposium 2024 "*Severity Assessment*

and the 3R Concept in Projects Using Animals in Experimentation'', March 29, 2024, organized by the Romanian Association for Laboratory Animal Science and hosted by the National Institute of Research and Development in Pathology and Biomedical Sciences "Victor Babeș'', Bucharest, Romania.

2. Prună V., **Gheţu D.M.**, Vrânceanu D., Cotruţ C., Vlădescu (Dragomir) A., Titorencu I., Enhanced osteogenic differentiation of human adult mesenchymal stem cells cultured on plate-like hydroxyapatite coatings, poster presented at the international conference, "*Applications of Chemistry in Nanosciences and Biomaterials Engineering (NanoBioMat)*", June 28-30, 2023 (Summer Edition) – online event.

3. Ţuţuianu R., Roşca A.M., **Gheţu D.M.**, Prună V., Albu Kaya M., Evaluation of mesenchymal cell-derived products as therapeutics for skin regeneration, poster presented at the scientific event "*1st NETSKINMODELS Network event*" February 14-18, 2023, Bratislava, Slovakia.

4. Rădulescu A. L., **Iacomi D.M.**, Florea G., Roşca A. M., Tutuianu R., Titorencu I., Obtaining and characterization of mesenchymal stem cells derived from adipose tissue, poster presented at the conference dedicated to International Microorganism Day (ZIM), September 17, 2021, Bucharest, Romania.

REGISTERED NATIONAL PATENTS

1. OSIM application no. A/00370 (25.06.2021): "Composite polymer hydrogels with antibacterial and wound-healing properties and method for their preparation''. Authors: Simionescu M., Roşca A.M., Titorencu I.D., **Iacomi M.D.**, Ţuţuianu R., Pruna V., Lasca I., Chercherita I.A., Neagu P.T., Mogoantă L., Mogosanu G.D., Pirici N.D., Streba C.T., Birca A.C., Burdusel A.C., Stoica A.E., Grumezescu A.L., Chircov C..

COURSES AND SEMINARS ATTENDED DURING THE DOCTORAL PROGRAMME

1. Training School ''3D Skin Cultures'' of the COST Action CA21108 – European Network for Skin Engineering and Modeling (NETSKINMODELS) – June 29 to July 2, 2025, Nijmegen, The Netherlands.

2. Prof. Dimitris Kardassis: Lectures to the students (Lipoprotein pathways and their roles in the pathophysiology of atherosclerosis; Transcriptional Regulation of Cardiovascular Genes: Basic principles and methodologies *in vitro* and *in vivo*; Hormone Nuclear receptors: from basic biology to clinical exploitation in CVD), November 14-18, 2024, Institute of Cellular Biology and Pathology "Nicolae Simionescu'', Bucharest, Romania.

3. Training course completed at the "Carol Davila'' University of Medicine and Pharmacy in Bucharest, entitled "Laboratory Animal Science'', held between January 30 and February 10, 2023.

4. INTERA-1 Summer School "Encapsulation of cells and drugs: materials, procedures and applications'', May 13-14, 2021, Institute of Cellular Biology and Pathology "Nicolae Simionescu'', Bucharest, Romania.

5. INTERA Online Workshop "Recent advances in the field of obtaining nanovectors for gene transfection'', April 22, 2021, Institute of Cellular Biology and Pathology "Nicolae Simionescu'', Bucharest, Romania.

6. THERAVALDIS Online Workshop "Diabetes in cardiovascular diseases; pathogenic mechanisms and targeted therapies'', November 27, 2020, Institute of Cellular Biology and Pathology "Nicolae Simionescu'', Bucharest, Romania.

7. DIABETER Online Workshop "A new therapeutic tool in autoimmune diabetes: Mesenchymal Stromal Cell'', November 20, 2020, Institute of Cellular Biology and Pathology "Nicolae Simionescu'', Bucharest, Bucharest, Romania.

INTERNATIONAL STAGES

1. International research stage abroad, lasting 9 months (September 2023 – May 2024), at Leiden University Medical Center, the Netherlands, and supported by a scholarship awarded by the Romanian Ministry of Education in accordance with Government Decision no. 118/2023.

AWARDS

1. Award offered by the Ministry of Education and Research and UEFISCDI ,,Scientific Achievements – Original Article'', PN IV, Program 5.2 - Human Resources – Subprogram 5.2.3 – Support – List 1 – First applications submitted for articles in 2021 and 2022_13.03.2023 for the article ''Generation of an immortalized human adipose-derived mesenchymal stromal cell line suitable for wound healing therapy'' published in the journal ,,International Journal of

Molecular Sciences'', Volume 23 (16), 8925, IF/2022 = 5.6 (**Iacomi, D. M.**, Roşca, A. M., Ţuţuianu, R., Neagu, T. P., Prună, V., Simionescu, M., Titorencu, I.).

2. Award offered by the Ministry of Education and Research and UEFISCDI ,,Scientific Achievements – Original Article'', Subprogram 1.1 - Human Resources - Awarding of Research Results - Articles, Competition 2021, Evaluation Results_List 2 - Award Applications Submitted for Articles Published in 2021_18.11.2021 – for the article ''Human mesenchymal stromal cell-derived exosomes promote *in vitro* wound healing by modulating the biological properties of skin keratinocytes and fibroblasts and stimulating angiogenesis" published in the journal ,,International Journal of Molecular Sciences'', Volume 22(12), 6239, 2021, IF/ 2021 = 5.924 (Ţuţuianu, R., Roşca, A. M., **Iacomi, D. M.**, Simionescu, M., Titorencu, I.).

SCHOLARSHIPS OBTAINED DURING THE DOCTORAL TRAINING PROGRAMME AND FUNDING OF THE RESEARCH ACTIVITY

SCHOLARSHIPS

1. Scholarship awarded by the Ministry of Education for a 9-month research stage abroad (September 2023 – June 2024) at Leiden University Medical Center, the Netherlands, in accordance with Government Decision no. 118/2023.
2. PhD scholarship – School of Advanced Studies of the Romanian Academy (2020–2023).

COLLABORATIONS IN RESEARCH GRANTS

Collaborator in the following national grants:

1. PN-IV-P7-7.1-PED-2024-1446

Proiect experimental demonstrativ

Title: *Innovative antimicrobial collagen based three dimensional scaffolds for craniofacial reconstruction*

Period: 2025-2027

Coordinator: Institute of Cellular Biology and Pathology "Nicolae Simionescu'', Bucharest

Project director: Dr. Irina Titorencu

Partner: LMS PLASTIC SURGERY CLINIQUE SRL – Dr. Ana Căruntu

2. **PN-III-P1-1.1-TE-2021-1344**

Research project for encouraging the establishment of young independent research teams

Title: *Enhancing the paracrine properties of fibroblasts by three-dimensional aggregation for chronic wounds therapy* - FIBROTHER

Period: 2022-2024

Project director: Dr. Roşca Ana-Maria

3. **PN-III-P2-2.1-PED-2021-4275**

Experimental Demonstration Project

Title: *Optimization of human mesenchymal stem cells interaction with innovative biomimetic structures for tissue engineering applications* - BioMimCells

Period: 2022-2024

Coordinator: University Politehnica of Bucharest

Project director: Dr. ing. Diana Maria Vrânceanu

Partners:

Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest - Partner Coordinator: Dr. Irina Titorencu

National Institute for Research and Development in Optoelectronics (INOE 2000), Bucharest - Partner Coordinator: Dr. Alina Vlădescu

4. **PN III PCCDI 45/2018**

Complex projects carried out in Research, Development and Innovation consortia

Title: *Bioactive Nanostructures for Innovative Therapeutic Strategies* - NANOLIFE

Period: 2018-2020

Coordinator: University of Medicine and Pharmacy of Craiova

Proiect director: Dr. Laurenţiu Mogoantă

Partners:

University of Medicine and Pharmacy "Carol Davila" Bucharest – Partner Coordinator: Dr. Ioan Lascăr

Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest – Partner Coordinator: Dr. Irina Titorencu

University Politehnica of Bucharest - Partner Coordinator: Dr. Alexandru Grumezescu

Collaborator in international grants:

1. PNRR–III-C9-2022-I8 CF 197/15.11.2022

***Romania's National Recovery and Resilience Plan*) - Project funded by the European Union**

Title: *Enchancing the endogenous anti-oxidant and cholesterol removal potential by gene editing in fatty liver disease; pre-clinical studies* - THERAGENLIV

Period: 2023-2026

Project director: Prof. Shlomo Sasson

2. European Cooperation in Science and Technology

***CA21108 - European Network for Skin Engineering and Modeling* (NETSKINMODELS)**

Period: 2022-2026

Project director: Prof. Sandrine Dubrac

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