

Scientific and technical report in extenso
Project 65PCCDI/2018
Stage III/2020

The component projects within the Complex Project 65PCCDI are:

Component project 1: Osteoimmunomodulation as a predictive factor of bone tissue regeneration

efficiency (BONE)

Component project 2: Biocompatible system for assisting peripheral nerve regeneration (NERVE)

Component project 3: Cellular and molecular mechanisms involved in soft tissue regenerative processes (SOFT)

Component project 4: Modulation of the tumor microenvironment with intelligent systems for breast reconstruction (TUMOR)

In the III/2020 stage, the 65PCCDI councils, the IC-UB, P1-UPB, P2-IVB and P3-IOCN partnerships contributed to carrying out the structure design activity for each project. The results obtained at the scientific level are summarized below:

In this stage of the component 1 project, the procedure for obtaining AZ31 alloys coated with PCL fibers loaded with coumarin and ZnO, the technological flow and the material balance was performed. The aim was to evaluate the impact of the immune microenvironment generated by the AZ31 alloy on the process of osteoclastogenesis. Biological studies included the analysis of TRAP activity, the quantification of the number of multinucleated positive TRAP osteoclasts, as well as the analysis of actin ring formation. Thus, they were selected and characterized for Mg-based in vivo BM studies with those favorable osteoimmunomodular properties. Regarding the in vivo evaluation of the tissue integration potential of Mg-based alloys, in a first stage a subcutaneous implantation was performed in order to optimize the subsequent implantation procedures and highlight the inflammatory infiltrate. In the second stage, the implantation of biomaterials in bone defects performed surgically on the skull of adult CD1 mice was performed. Histopathological analysis was performed 3 and 6 months after implantation to assess osteogenic potential, but also immunomodulatory potential. Cumulatively, the results indicated that coating AZ31 alloy with PCL films and functionality with CM and / or ZnO endows the alloy with increased corrosion resistance, determines an appropriate inflammatory response, supports the osteogenic process and reduces osteoclastogenesis, thus demonstrating a potential promising bone regeneration.

In **component project 2**, the pro-regenerative potential of +/- nanodiamond (ND) and +/- NGF / HGF SARNs in in vivo implantation in Wistar rats after a sciatic nerve transection /

excision injury was investigated. Implantation operations of all types of SARN revealed a good biocompatibility, not being accompanied by inflammatory or rejection process. Reconnection of the severed ends of the sciatic nerve with SARN appears to be a source of intense mechanical stress, leading to severe autotomy processes incompatible with long-term survival. In particular, the presence of ND alone or ND & NGF / HGF seems to accentuate this mechanical stress: if at the implantation of simple SARN the animals can survive with manifestations of autotomy of medium intensity for 45 days, in the case of SARD-ND and SARN- ND / HGF-NGF autotomy phenomena were severe (onset 7-14 days), incompatible with long-term survival. The encouraging results are that simple SARN appears to improve the culture viability of large mechano-sensitive neurons and to reduce the increased amplitude of lesion-induced action potentials by reducing the activation of voltage-dependent Na⁺ currents and increasing the amplitude of K⁺-dependent currents. voltage.

Within **component project 3**, SEC materials were synthesized for preclinical studies in animal models. The design of the polymer-based controlled release (SEC) system for soft tissue regeneration was optimized. Thus, the double-layered hydroxide clays were modified to increase the degree of compatibility with the polymeric matrix. A new type of clay, montmorillonite (MMT), has been used for the development of controlled release systems with optimal characteristics. An *in vivo* evaluation of the adipogenic induction potential of HEMA / AMPSA / LDH and fibroin / sericin, respectively, was performed on CD1 mice, whose materials were implanted subcutaneously in the dorso-lumbar region. Samples collected 1 and 3 weeks after implantation were used to investigate the evolution of the regeneration process in soft tissues by histological techniques, confocal microscopy techniques, but also at the molecular level, by analyzing the gene expression of markers involved in regeneration. The *in vivo* regeneration process was also investigated by evaluating the post-transcriptional mechanisms involved.

Within the **component 4 project**, studies were performed on cell lines and animal models, in order to validate the molecular mechanisms involved in tissue regeneration processes and which observed the activation of immunological mechanisms and their negative impact on tissue regeneration processes. Therapeutic nanosystems such as liposomes with halofuginone and rhodamine were synthesized, characterized physico-chemically and evaluated at cellular and molecular effects. The data obtained on the cell lines are validated on the animal model using naked mice. At the same time, several generations of porous materials (scaffold), based on fish gelatin, were synthesized, intended for breast reconstruction and evaluated for the purpose of selecting the most effective systems and subsequently enriched with anti-tumor agent (paclitaxel). The effect of both the basic composition of the scaffold and the drug was evaluated *in vitro* conditions, by quantitative and qualitative methods. Subsequently, an *in vivo* experimental model was obtained to evaluate the efficiency of breast adipose tissue reconstruction with the proposed functionalized fish gelatin systems. In a last step, the murine models were sacrificed and the degree of tissue regeneration in the presence of the implant was

analyzed, at histological, protein and molecular level, by evaluating some markers specific to the regeneration process.

Activities covered in the II/2019 stage of the project:

Activity: Act. 3.1 Evaluation of the process of osteoclastogenesis induced by the conditioned environment obtained by exposing macrophages to developed biomaterials (IC-UB)

Activity: Act. 3.2 Selection of BM that shows the best biological performance in in vitro studies (IC-UB)

Activity: Act. 3.3 Demonstration of technology for obtaining a coating with favorable IOM properties on Mg-based biomaterials (P1-UPB)

Activity: Act. 3.4 In vivo studies evaluating the osteogenic potential of selected BM (partial activity) (P2-IVB)

Activity: Act. 3.5 In vivo studies to highlight the immunomodulatory potential of BM (partial activity) (P2-IVB)

Activity: Act. 3.6 Evaluation of the correlation of in vitro tests with in vivo tests, establishment of the in vitro evaluation protocol that leads to the highest degree of prediction of the in vivo efficiency of developed BM (partial activity) (IC-UB; P1-UPB; P2-IVB)

Activity: Act. 3.7. Dissemination of the results obtained in the project (partial activity) (IC-UB; P2-IVB)

Activity: Act. 3.8: In vivo evaluation of the capacity to facilitate the regeneration of peripheral nerves using SARN (partial activity): (a) Manufacture of SARN for in vivo testing and supply to partners; (b) Assessing the ability to facilitate the regeneration of peripheral nerves by investigating the functional properties of neurons in the spinal ganglia; (c) Assessing the ability to facilitate the regeneration of peripheral nerves with behavioral tests that quantify pain sensitivity. (IC-IVB; P1-UB; P2-UPB)

Activity: Act. 3.9: In vivo evaluation of the capacity to facilitate the regeneration of the gastrocnemius muscle after implantation of SARN (partial activity): Evaluation of the regeneration capacity of the gastrocnemius muscle after regeneration of the sciatic nerve. (IC-IVB; P1-UB; P2-UPB)

Activity: Act. 3.10: Realization of the documentation supporting the patent application at OSIM for SARN approval (partial activity). (IC-IVB; P1-UB; P2-UPB)

Activity: Act. 3.11: Collaboration agreement for transfer of SARN and IHC databases to the economic operator (partial activity). (IC-IVB; P1-UB; P2-UPB)

Activity: Act. 3.28: Studies for the in vivo evaluation of the capacity to facilitate the regeneration of peripheral nerves using SARN: (a) Carrying out the in vivo implantation operation of SARN with controlled release with inducing factors of nerve regeneration; (b) Evaluation of the ability to facilitate the regeneration of peripheral nerves by investigating the histological features of the regenerated area (partial activity). (IC-IVB; P1-UB; P2-UPB)

Activity: Act. 3.12 Synthesis of the SEC for preclinical studies on animal models -partial activity- (P1-UPB)

Activity: Act. 3.13 In vitro experimental model generation for the study of soft tissue regeneration and surgical application of SEC to the general experimental model (IC-UB)

Activity: Act. 3.14 Monitoring the soft tissue regeneration process related to the implant over time (since the application of SEC on the damaged tissue) (P2-IVB)

Activity: Act. 3.15 Investigation of the dynamics of the regeneration process at the level of soft tissues by histological techniques -partial activity- (P2-IVB)

Activity: Act. 3.16 Investigating the gene expression profile in regenerated tissue fragments compared to the normal profile of specific genes -partial activity- (IC-UB)

Activity: Act. 3.17 Evaluation of protein expression of markers specific to soft tissue regeneration by microscopic (confocal) and / or quantitative (IC-UB) techniques

Activity: Act. 3.18 Evaluation of the post-transcriptional mechanisms involved in the in vivo regenerative process -partial activity- (P3-IOCN)

Activity: Act. 3.19 Data analysis. Dissemination of results -partial activity- (IC-UB, P1-UPB, P2-IVB, P3-IOCN)

Activity: Act 3.20 - Development of experimental models for exposure to STR-loaded therapeutic agents (IC-IOCN)

Activity: Act 3.21 - Characterization of functional changes of cells exposed to STR treatment (IC-IOCN).

Activity: Act 3.22 - Molecular profile characterization for cells exposed to STR treatment (IC-IOCN).

Activity: Act 3.23 - Study of the therapeutic effects of STR in vivo (IC-IOCN).

Activity: Act 3.24 - Generation of in vivo experimental model to evaluate the efficiency of adipose tissue reconstruction at the breast level with the SP system. Murine model with breast tumor, in which the tumor is surgically excised and the reconstruction of adipose tissue with SP is desired (P2-UPB, P3-IVB).

Activity: Act 3.25 - Analysis of the degree of tissue regeneration in animal models studied in the presence of the SP-implant at histological and protein level (P3-IVB).

Activity: Act 3.26 - Analysis of the degree of tissue regeneration in animal models studied in the presence of the SP implant-study of the gene expression profile (P1-UB).

Activity: Act 3.27 - Analysis, integration, correlation of experimental data (IC-IOCN, P1-UB, P2-UPB, P3-IVB).

Dissemination of results

In this stage, the scientific results were disseminated to the scientific environment:

Project component	Type of dissemination	Referinta
P1	BDI Articles	- A.M. Negrescu, M.G. Necula, M. Costache, A. Cimpean, 2020. „In vitro and in vivo biological performance of Mg-based bone implants”, Rev. Biol.Biomed. Sci. 3(1), 11-41. - M-E. Voicu, F. Golgovici, 2020, „ A biointerface growth at immersion of a biodegradable magnesium alloy in simulated body fluid”, UPB Sci.

		Bull. B 82(2), 57-68, ISSN 1454-233.
	ISI Article in evaluation	- A.M. Negrescu, M.G. Necula, A. Gebaur, F.A. Golgovici, C. Nica, C. Filis, H. Iovu, M. Costache, A. Cimpean, „In vitro macrophage immunomodulation by the PCL based-coated AZ31 Mg alloy”, Int. J. Mol. Sci. (FI:4,556)
P2	International Book Chapter	- The Cellular and Molecular Patterns Involved in the Neural Differentiation of Adipose-Derived Stem Cells in “Advances in Experimental Medicine and Biology” book, Springer Nature, 2020; DOI: 10.1007/5584_2020_547. - Schwann cell plasticity in peripheral nerve regeneration after injury, In book: Schwann Cells, InTech Open, 2020, DOI: 10.5772/intechopen.91805
P3	ISI Articles	- Lazar A.D., Dinescu S., Albu-Kaya M.G., Gharbia S., Hermenean A., Costache M., 2020, Release of the Non-Steroidal Anti-Inflammatory Drug Flufenamic Acid by Multiparticulate Delivery Systems Promotes Adipogenic Differentiation of Adipose-Derived Stem Cells, Materials, 13(7), 1550, doi 10.3390/ma13071550 - Vasile E., Radu I.C., Galateanu B., Rapa M., Hudita A., Jianu D., Stanescu P.O, Cioflan H., Zaharia C., 2020, Novel Nanocomposites Based on Bacterial Polyester/LDH-SDS Clay for Stem Cells Delivery in Modern Wound Healing Management, Materials, 13(20), 4488, doi: 10.3390/ma13204488
P4	ISI Articles	- Neagu M., Constantin C., Popescu I.D., Zipeto D., Tzanakakis G., Nikitovic D., Fenga C., Stratakis C.A., Spandidos D.A., Tsatsakis A.M., 2019, Inflammation and metabolism in cancer cell – mitochondria key player, Frontiers in Oncology, 9, 348. DOI: 10.3389/fonc.2019.00348. IF=4.416. - Lungu A., Cernencu A. I., Dinescu S., Balahura R., Mereuta P., Costache M., Syverud K., Stancu I. C., Iovu H., 2021, Nanocellulose-enriched hydrocolloid-based hydrogels designed using a Ca ²⁺ free strategy based on citric acid, Materials & Design, 197, 109200. DOI: 10.1016/j.matdes.2020.109200. IF=5.770. - Balahura L.R., Selaru A., Dinescu S., Costache M., 2020, Inflammation and inflammasomes - pros and cons in tumorigenesis, Journal of Immunology Research, 2020, 2549763. DOI: 10.1155/2020/2549763. IF=3.327. - Dobre E.G., Dinescu S., Costache M., 2020, Connecting the Missing dots: ncRNAs as critical regulators of therapeutic susceptibility in breast cancer, Cancers, 12, 9, 2698. DOI:10.3390/cancers12092698, IF= 6.370.
	Manuscript in evaluation	- Cernencu A.I., Lungu A., Dragusin D., Stancu I.C., Dinescu S., Balahura R., Mereuta P., Costache M., Iovu H., 3D Bioprinting of biosynthetic nanocellulose-filled inks highly reliable for soft tissue-oriented constructs
	BDI Articles	- Neagu M., Bostan M., Constantin C., 2019, Protein microarray technology: Assisting personalized medicine in oncology, World Academy of Sciences Journal, 1, 113-124. - Balahura L.R., Dinescu S., Costache M., 2020, The interconnection between the inflammasome and breast cancer, Reviews in Biological and Biomedical Sciences, 3(1)43-54.
	Conference communication	- Balahura L.R., Dinescu S., Costache M., Activation and involvement of inflammasome in tumor progression, A 49-a Conferința a Societății de Imunologie din România, 30.09-3.10.2020, România. - Isvoranu G., Surcel M., Munteanu A.N., Pirvu I.R., Neagu M.T., Caracteristici fenotipice ale celulelor NK induse de prezența celulelor tumorale, A 49-a Conferința a Societății de Imunologie din România,

		<p>30.09-3.10.2020, România. - Balahura L.R., Dinescu S., Costache M., Molecular changes in breast cancer development during inflammasome activation, EMBO-FEBS Lecture Course "Systems biology of cancer: promises of artificial intelligence". 28.09-2.10.2020.</p>
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