Scientific and Technical Report in extenso
Project 65PCCDI/2018
Stage IV/2021

The component projects within the 65PCCDI Complex Project 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 project 1**, murine experimental models were obtained and *in vivo* studies were performed in order to evaluate the osteogenic potential of the BM selected in the previous stages. As a complement and continuation of this study, the partners evaluated on these murine models, the immunomodulatory potential of the selected BM. Following the *in vitro* and *in vivo* tests performed within the project, the obtained data were analyzed, interpreted and an optimized protocol for *in vitro* evaluation of the proposed biomaterials was elaborated. These results illustrating the physico-chemical-biological characteristics of BM materials have been explained in detail in this report.

In **component project 2**, at this stage we continued the investigation of the pro-regenerative potential of SARN +/- nanodiamond (ND) and +/- NGF / HGF in *in vivo* implantation in Wistar rats after a sciatic nerve transection/excision injury. Implantation operations of all types of SARN revealed good biocompatibility, not being accompanied by inflammatory or rejection processes. Reconnection of the severed ends of the sciatic nerve with SARN appears to be a source of intense mechanical stress, which has been evidenced by degenerative processes in nerve fibers visible through electron microscopy. The information obtained at this stage of the project on SARN was the subject of a patent application filed with OSIM, and histochemistry images were included in the database of the Medical Center for Diagnosis and Treatment "Dr. Victor Babes" from the National Institute Victor Babes.

In **component project 3**, SECs were synthesized, optimized, and implanted in experimental murine models. The samples were taken and analyzed in order to observe the evolution of the soft tissue regeneration process using histological methods of analysis. In the following activities, these tissue samples were analyzed by molecular biology methods in order to evaluate some markers (perilipin, PPARγ, etc.) specific to the soft tissue regeneration process. Molecular biology studies have been deepened, and the post-transcriptional control mechanisms involved in *in vivo* regeneration have been studied, following which non-coding RNA species involved in the soft tissue regeneration process have been correlated. The results of these studies were analyzed and quantified by a patent application.

Within the **component project 4**, the studies started in stage III/2020 were continued and deepened. The therapeutic effects of STR on fibrous tissue using molecular biology techniques were studied in animal models. In the following activities, an experimental murine model was obtained in which the breast tumor was induced and subsequently excised. Biomaterials based on natural polymers enriched with an anti-tumor agent (paclitaxel) for post-mastectomy breast reconstruction have been synthesized and characterized. The SP materials were transplanted at breast level and the behavior of the murine models was observed for 28 days after the procedure. After 28 days, breast tissue samples were collected from all studied groups, and the efficiency of adipose tissue reconstruction at breast level assisted by the SP implant was analyzed by observing the gene and protein expression of molecular markers specific to the tissue regeneration process.

Activities covered in the IV/2021 stage of the project:

Activity: Act. 4.1 *In vivo* studies evaluating the osteogenic potential of selected BM (P2-IVB)
Activity: Act. 4.2 *In vivo* studies to highlight the immunomodulatory potential of BM (P2-IVB)
Activity: Act. 4.3 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 (IC-UB; P1-UPB; P2-IVB)
Activity: Act. 4.4 Identification and attribution of the intellectual property rights over the research results (IC-UB; P1-UPB; P2-IVB)
Activity: Act. 4.5. Dissemination of the results obtained in the project (IC-UB; P2-IVB)

Activity: Act. 4.6 In vivo evaluation of the capacity to facilitate the regeneration of peripheral nerves using SARN (P1-UB; P2-UPB; IC-IVB)
Activity: Act. 4.7 *In vivo* evaluation of the capacity to facilitate gastrocnemius muscle regeneration after SARN implantation (P1-UB; IC-IVB)
Activity: Act. 4.8 Documentation elaboration supporting the patent application at OSIM for SARN homologation (P1-UB; P2-UPB; IC-IVB)
Activity: Act. 4.9 Collaboration agreement for transfer of SARN and IHC databases to the economic operator (P1-UB; P2-UPB; IC-IVB)

Activity: Act. 4.10 Studies for *in vivo* evaluation of the ability to facilitate peripheral nerves' regeneration using SARN (P1-UB; P2-UPB; IC-IVB)

Activity: Act. 4.11 SEC Synthesis for Preclinical Animal Studies (P1-UPB)
Activity: Act. 4.12 Investigation of the dynamics of soft tissue regeneration process by histological techniques (P2-IVB)
Activity: Act. 4.13 Gene expression profile investigation in regenerated tissue fragments compared to the normal profile of specific genes (IC-UB)
Activity: Act. 4.14 Evaluation of protein expression of markers specific to soft tissue regeneration by microscopic (confocal) and/or quantitative (IC-UB) techniques
Activity: Act. 4.15 Evaluation of post-transcriptional mechanisms involved in the regenerative process *in vivo* (P3-IOCN)
Activity: Act. 4.16 Data analysis. Results dissemination. Elaboration of necessary documentation for supporting the patent application at OSIM (IC-UB, P1-UPB, P2-IVB, P3-IOCN)

Activity: Act 4.17 Study of the therapeutic effects of STR *in vivo* (IC-IOCN)
Activity: Act 4.18 Generation of *in vivo* experimental model to evaluate the efficiency of adipose tissue reconstruction at breast level with the SP system (P2-UPB, P3-IVB)
Activity: Act 4.19 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 4.20 Analysis of the degree of tissue regeneration in animal models studied in the presence of the SP implant-evaluation of gene expression profile (P1-UB)
Activity: Act 4.21 Analysis, integration, correlation of experimental data (IC-IOCN, P1-UB, P2-UPB, P3-IVB)
Activity: Act. 4.22 Dissemination of results (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**	**Reference**
P1	**ISI Articles**	- Negrescu A.M., Necula M.G., Gebaur A., Golgovici F., Nica C., Curtis F., Iovu H., Costache M., Cimpean A. 2021, In vitro macrophage immunomodulation by poly(ε-caprolactone) based-coated AZ31 Mg alloy, *International Journal of Molecular Sciences*, 22 (2), 909. Doi: 10.3390/ijms22020909. - Negrescu A.M., Cimpean A. 2021, The state of the art and prospects for osteoimmunomodulatory biomaterials, *Materials*, 2021, 14(6), 1357. Doi: 10.3390/ma14061357.

	Conference communication	- Negrescu A.M., Cimpean A., Evaluarea *in vitro* a potentialului imunomodulator al aliajului AZ31 acoperit cu biofilme pe baza de poli(ε-caprolactona), Sesiunea de comunicari stiintifice a studentilor Facultatii de Biologie, editia 2021, Bucuresti, 28 mai, Volumul de rezumate-pag. 55 (comunicare orala).
P2	**ISI Articles**	- Olaret E., Dragusin D.M., Serafim A., Lungu A., Selaru A., Dobranici A., Dinescu S., Costache M., Boerasu I., Vasile B.S., Steinmüller-Nethl D., Iovu H., Stancu I.C., 2021, Electrospinning fabrication and cytocompatibility investigation of nanodiamond particles-gelatin fibrous tubular scaffolds for nerve regeneration, *Polymers*, 13, 407. Doi: 10.3390/polym13030407. - Isvoranu G, Manole E, Neagu M., 2021. Gait Analysis Using Animal Models of Peripheral Nerve and Spinal Cord Injuries. *Biomedicines*. 9(8), 1050. Doi: 10.3390/biomedicines9081050
	Conference communication	- Armășescu FV., Ghenghea MS., Gheorghe RO., Stancu I., Olaret E., Isvoranu G., Neagu M., Costache M., Ristoiu V., I_{Nav} currents during sciatic nerve reconstruction guided by a Nerve Regeneration Assistance System (NerveRAS), 14th Göttingen Virtual Meeting, March 22-30, 2021 - Ghenghea MS., Armășescu FV., Gheorghe RO., Stancu I., Olaret E., Isvoranu G., Neagu M., Costache M., Ristoiu V., The functioning of voltage gated (IKdr) K+ channels restores faster after a peripheral nerve lesion in the presence of a biocompatible nanomaterial support system, 14th Göttingen Virtual Meeting, March 22-30, 2021
P3	**Manuscript in evaluation**	-Serban M., Ignat S., Dinescu S., Radu I., Zaharia C., Costache M., Hermenean A. *In vitro* and *in vivo* evaluation of HEMA/AMPSA/MMT hydrogels enriched with sericin and fibroin in the context of soft tissue engineering applications, *Nanomaterials*, in evaluare -Radu I., Zaharia C., Ignat S., Serban M., Dinescu S., Iovu H., Costache M., Characterization and in vitro biocompatibility of HEMA/AMPSA/LDH designed for adipose tissue engineering, *Polymers*, in evaluare
	Conference comunication	- Ignat S.R., Dinescu D., Radu I., Zaharia C., Serban M., Costache M., Biocompatibility analysis of HEMA/AMPSA/LDH 3D bioconstructs designed for soft tissue reconstruction, Annual Scientific Meeting of Victor Babes Institute, The 12th National Pathology Symposium, 21-24.11.2019, Bucuresti, Romania, Abstract Book, p57 (comunicare orala). - Serban M., Ignat S., Dinescu S., Radu I., Zaharia C., Hermenean A., Costache M., HEMA/AMPSA hydrogel enriched with sericin and fibroin proved efficiency for soft tissue engineering applications, 2nd World Congress on Smart Materials and Structures, 29-30.09.2021, Webinar (poster).

P4	**ISI Articles**	- Balahura L.R., Dinescu S., Balas M., Cernencu A., Lungu A., Vlasceanu G.M., Iovu H., Costache M., 2021. Cellulose nanofiber-based hydrogels embedding 5-FU promote pyroptosis activation in breast cancer cells and support human adipose-derived stem cell proliferation, opening new perspectives for breast tissue engineering, *Pharmaceutics*, 13 (8), 1189. Doi: 10.3390/pharmaceutics13081189. - Cernencu A.I., Lungu A., Dragusin D., Stancu I.C., Dinescu S., Balahura R., Mereuta P., Costache M., Iovu H., 2021, 3D Bioprinting of biosynthetic nanocellulose-filled GelMA inks highly reliable for soft tissue-oriented constructs, *Materials*, 14 (17), 4891. Doi: 10.3390/ma14174891. - 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
	Manuscript in evaluation	- Jurj A., Berindan-Neagoe I., Braicu C., 2021, Application of bioprinted models to study of tumor microenvironment interaction, in evaluare. - Jurj A., Soritau O., Munteanu M., Groza M., Cojocneanu R., Zanaoaga O., Cismaru A., Braicu C., Berindan-Neagoe I., 2021, Cellular and molecular alteration of mammary epithelial cells by cellular reprogramming cell culture, in evaluare.
	Conference communication	- Balahura L.R., Dinescu S., Lazar A., Cernencu A., Lungu A., Costache M., 5-fluorouracil-enriched nanocellulose and pectin biomaterials determined inflammasome complex activation and ROS production in breast cancer cells, 45th FEBS Congress, 3-8.07.2021. Online (poster). - Balahura L.R., Dinecu S., Costache M., Smart biocomposites encapsulating methotrexate designed for cancer therapy, TRANSCOLONCAN COST Action – Workshop „Challenges of tumor profiling in translational research", 20-21.09.2021, Bucuresti, Romania (comunicare orala). - Balahura L.R., Dinescu S., Cernencu A.I., Lungu A., Costache M., Efectul 5-fluorouracil asupra celulelor tumorale mamare cultivate in materiale tridimensionale pe baza de nanoceluloza, Sesiunea de comunicari stiintifice a studentilor Facultatii de Biologie, 28.05.2021, Bucuresti, Romania (comunicare orala). - Jurj A., Raduly L., Braicu C., Berindan-Neagoe I.. Adipocyte-derived exosomes promote the progression of triple negative breast cancer cells *in vitro*, 5th ACTC- Advances in Circulating Tumor Cells "Liquid biopsy is its the best", 22-25.09.2021, Kalamata, Grecia (poster).