

Scientific and technical report in extenso Contract 65PCCDI/2018

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 II/2019 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**, coatings were made and characterized of Mg alloy (AZ31) based on polycaprolactone fibers (Mg-PCL indicated and CPO), to overcome electrospinning, care and support and healing activation - coumarin (Mg-PCL_cumarina indicated and CPO_1) or with ZnO (Mg-PCL_ZnO indicated and CPO_2) and both compounds simultaneously (Mg-PCL_cumarina_ZnO indicated and CPO_3). Also, synthetic / natural polymer (PCL / chitosan) polymer coating hybrid consisting of three layers of synthetic polymer with glutaraldehyde used for a lattice was made, the next layer containing then natural polymer (PCL-GA-CS). In turn, hybrid cover and charged with ZnO (PCL-GA-CS-ZnO). BM analysis can favor the biological characterization of the analysis by their antimicrobial activities and the osteoimmune environment generated by interaction with MFG line, RAW 264.7. Analysis of morphology (fluorescence marking of actin), proliferation vats (LIVE / DEAD tests, CCK-8), dosage is NOT released in the environment (Griess reagent) and pro-anti-inflammatory cytokines by ELISA assays, assessment of cellular ROS level (with 2', 7'-dichlorofluorescein diacetate), analyzes the processing of autophagy activation, and evaluates by RT-qPCR a level of gene expression of some osteogenic and osteoclastogenic factors. In order to analyze the impact of the immune micromedia created by the BMs developed on the process of osteogenesis, a comparative study was performed on pre-osteoblasts grown in the standard culture medium and under average conditions for a macrophage-exposed macrophage-septum, collagen synthesis by staining with Sirius Red, and expresses a specific gene expressed during osteoblast differentiation (OB) (osteopontin, osteocalcin and osteonectin).

Within the **component project 2**, the synthesis, characterization and in vitro and in vivo evaluation of the efficiency of the Assistance System of the Peripheral Nerve Regeneration (SARN) - Generation 2 in the process of regeneration of the peripheral nerves took place. All the objectives of this stage were achieved, respectively, the synthesis / manufacture of 3D artificial nerve conduction and its ability to support in vivo regeneration (rats) of the injured sciatic nerve. The results obtained from the in vitro biocompatibility tests showed that SARN - Generation 2 was compatible with both the cells of the primary neuron cuticle and the human mesenchymal stem cells. Also, it has been demonstrated that SARN - Generation 2 has antimicrobial activity.

Preliminary studies have been obtained regarding the ability of SARN - Generation 2 to support peripheral nerve regeneration. The obtained results were disseminated by publishing in specialized magazines and by participating in national and international conferences.

Within the **component project 3**, new systems were developed based on four types of materials: synthetic polymers (poly (2-hydroxyethyl methacrylate), poly (2-acrylamide-2-methyl-1-propansulfonic acid) (PHEMA / PAMPSA)), natural polymers (fibroin and sericin), hydrophilic clays, double layered hydroxides (LDHs) and active biomolecules (growth factors, adhesion factors and chemotactic factors). The resulting hydrogels were characterized from a physico-chemical and morphological point of view. The biocompatibility evaluation tests showed good results for the synthesized materials and two were selected for the following studies. The two hydrogels show antimicrobial activity tested on the microbial strains *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Also, the inflammatory profile induced by these materials was evaluated by protein microarray. In the last step, the two hydrogels were evaluated in terms of the potential to support adipogenic differentiation, both at the gene level and at the protein level.

Within the **component project 4**, the properties of the materials from generation 2 were improved, consisting of six types of samples, based on nanocellulose (CN), alginate (A) or pectin (P), enriched with two different drug concentrations (1% respectively 5%). The evaluation of the effect of the anti-tumor agent was evaluated by observing the inflammatory profile, which indicated a higher rate of pyroptosis, in the 3D systems composed of the proposed materials and the tumor cells, as compared to the controls performed on normal cells. The ability of the material to support adipogenic differentiation of hASC, both in the absence and in the presence of tumor conditions, was followed by the ability to accumulate neutral lipids in the cytoplasm, by evaluating the expression profile of perilipine and PPAR γ 2. The results indicated that adipogenic differentiation is supported by a higher yield of CNP material. The gene expression profile of p53 and NF κ B, presented by the cells of the MDA-MB 231 line exposed to the treatment incorporated in the proposed materials, indicated a significant increase of p53 and NF κ B in the samples with the highest drug concentration. The evaluation of the tumor microenvironment after the interaction with the hASC-SP system confirmed the cytotoxic effect, with the decrease of the viability of hASC cells and breast carcinoma cells.

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

Activity: Act. 2.1 Monitoring of stabilization and degradation of modern and unmodified alloys and physiological media (SBF, PBS) (P1-UPB)

Activity: Act. 2.2 Highlighting the release profiles of biomolecules used for coating functionalization (P1-UPB)

Activity: Act. 2.3 Selection of optimum coatings in terms of biointerfaces (antimicrobial protection, cytocompatibility) and stability in physiological environments, release of biologic active molecules (IC-UB; P1-UPB)

Activity: Act. 2.4 Inflammatory response of MFG to selected BM (IC-UB)

Activity: Act. 2.5 ROS and NO production as a result of the MFG-BM interaction (IC-UB)

Activity: Act. 2.6 Active autophagy and macrophage (IC-UB)

Activity: Act. 2.7 Expression of osteogenic and osteoclastic factors in MFG exposed to BM (IC-UB)

Activity: Act. 2.8 The biochemical, ocular, and molecular involvement of immune cells in the modulation of osteogenesis by BM of interest (IC-UB)

Activity: Act. 2.9 Optimizing the procedures for implantation of biomaterials with potential for osteointegration (P2-IVB)

Activity: Act. 2.10 Dissemination of the results obtained in the project (IC-UB; P1-UPB; P2-IVB)

Activity: Act. 2.11 - (IC-UB; P1-UPB; P2-IVB) Synthesis / manufacture of SARN components - Generation 2: (a) Synthesis of Nano-SARN component - Generation 2; (b) Manufacture of 3D-Nerv artificial nerve conduit - Generation 2; (c) Advanced microstructural, physico-chemical and physiological conditions characterization relevant for a Nano-SARN and 3D-Nerv component - Generation 2; (d) In vitro testing of the release capacity of growth factors in the secreted system for SARN - Generation 2; (e) In vitro validation of SARN biocompatibilities - Generation 2; (f) In vitro validation of antiseptic capacity of SARN - Generation 2.

Activity: Act. 2.12 - (IC-UB; P1-UPB; P2-IVB) Preliminary studies for the in vivo evaluation of the ability to facilitate peripheral nerve regeneration with the help of SARN: (a) Optimizing the steps of implanting a model animal SRNA; (b) Evaluation of peripheral nerve regeneration assisted by SARN at histological level; (c) Evaluation of peripheral nerve regeneration assisted by SARN by investigating the degree of expression of genes associated with the type of regeneration.

Activity: Act. 2.13 - Assembly of SEC components by loading with growth factors, adhesion factors and chemoattractants (P1-UPB)

Activity: Act. 2.14 - Physical-chemical and morphological characterization of the SEC platform (P1-UPB)

Activity: Act. 2.15 - Demonstration of the functionality of the SEC platform through release tests in specific environments (P1-UPB)

Activity: Act. 2.16 - In vitro investigation of the biocompatibility of the SEC assembled system (IC-UB)

Activity: Act. 2.17 - Evaluation of antimicrobial properties of SEC (IC-UB)

Activity: Act. 2.18 - In vitro evaluation of anti-inflammatory efficacy of SEC (IC-UB)

Activity: Act. 2.19 - In vitro investigation of the SEC's ability to support the process of hASC differentiation and soft tissue regeneration. (IC-UB)

Activity: Act. 2.20 - Evaluation of posttranscriptional mechanisms involved in soft tissue regeneration processes (P3-IOCN)

Activity: Act. 2.21 Dissemination of results (IC-UB, P1-UPB, P2-IVB, P3-IOCN)

Activity: Act 2.22 - Synthesis and characterization of polymeric materials intended for permanent reconstruction (SP) of breast tissue using natural / synthetic macromolecular compounds. Optimizing stability for the permanent character of the implant. Immobilization of bioactive compounds. Efficacy tests for bioactivation and controlled release of bioactive principles under in vitro acellular conditions (P1-UPB)

Activity: Act 2.23 - Investigation of the biocompatibility of SP materials with adipose tissue stem cells for breast reconstruction

Activity: Act 2.24 - Evaluation of inflammatory profile of SP material (P2-IVB)

Activity: Act 2.25 - Investigating the ability of SP material to support adipogenic differentiation of hASC (sist hASC-SP) (IC-UB)

Activity: Act 2.26 - Characterization of the inflammatory status of the tissue taken after the mastectomy (P3-IOCN)

Activity: Act 2.27 - Exposure of the hASC-SP system to the tumor microenvironment derived from breast carcinoma and the analysis of the changes in the gene expression profile and inflammation following the interaction (IC-UB, P2-IVB)

Activity: Act 2.28 - Evaluation of the tumor microenvironment after the interaction with the hASC-SP system (P1-UB, IC-IOCN)

Activity: Act 2.29 - Integration and analysis of experimental data (P3-IOCN, IC-UB, P1-UPB, P2-IVB)

Activity: Act 2.30 - Dissemination of results

Dissemination of results

In this stage, the scientific results were disseminated to the scientific environment through 8 publications in ISI journals, 1 international book chapter and 17 communications at national/international congresses and conferences.