

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 stage 1/2018, at the level of the 65PCCDI consortium, the partners IC-UB, P1-UPB, P2-IVB and P3-IOCN contributed to the achievement of the scientific activities proposed within each component component. All assumed result indicators (scientific stage report, 1 website, 1 scientific communication, 3 working protocols, improvement of research services through equipment upgrades, 1 consortium conference), as well as additional contributions (3 conference communications national / international, as well as 4 publications in preparation, accepted or already published in ISI quoted journals). Regarding the human resource to be employed, the contests for the vacant positions were organized (12), following that in December and January they will be validated and the employment formalities will be finalized. The research services offered by the partner institutions on the ERRIS platform have been improved by purchasing equipment for quantitative measurement of the motor performance of laboratory animals (CatWalk XT) (P2-IVB) and the upgrade of confocal microscopy equipment (A1 Nikon Eclipse) with appropriate software (NIS Elements C software) and other components required for operation at maximum performance parameters (IC-UB). The results obtained at the scientific level are summarized below:

Within the component project 1, the current strategies for obtaining biomaterials with favorable osteoimmunomodulatory properties were synthesized. The UPB partner designed coatings of the selected magnesium alloy (AZ31) using electrochemical techniques and electrospinning technique. It was chosen to cover the Mg alloy with conductive polymers (polypyrrole) and degradable polymers (PLGA). The results of the tests revealed that the coating of magnesium alloy with polymeric film leads to the decrease of the corrosion speed, a fact proven both by the electrochemical impedance spectroscopy and the potentiodynamic polarization tests. The evaluation of the biocompatibility of the materials of interest by carrying out the cytotoxicity tests by direct and indirect contact according to ISO 10993-5 norms were evaluated using MC3T3-E1 cells, by examining the viability and by analyzing the cell proliferation. Indirect cytotoxicity tests demonstrated the biocompatibility of single or polymer coated Mg alloy. Direct contact studies have shown, however, that polypyrrole coating exerts cytotoxic effects, recommended for future studies being the use of PLGA to improve the corrosion behavior of Mg and, implicitly, biocompatibility. Testing of antibiofilm activity, performed at this stage only on PLGA-based coatings, showed a more pronounced proliferation for *Staphylococcus aureus* in the case of

unmodified or PLGA-coated alloy compared to PLGA-ZnO modified surface. By the presence of ZnO nanoparticles, the antibacterial effect was substantially enhanced.

Within the component project 2, the synthesis, characterization and in vitro evaluation of the efficiency of an Assistance System of the Peripheral Nerve Regeneration (SARN) in the regeneration process of the peripheral nerves took place. All the objectives proposed during this stage were achieved, respectively obtaining the first generation of material with medical applicability. In vitro cell biocompatibility assays confirmed the biocompatibility of SRNA with respect to cells from primary neuronal culture but also from mesenchymal stem cells; the same tests confirmed a proportional relationship of biocompatibility with the concentration of nanoparticles of nanodiamonds in the composition of materials. The methodological flow of experimental model testing in vivo (mice) was optimized. The purchase of equipment to increase the institutional capacity was made and the equipment was put into operation in stage 1.

Within the component project 3, a controlled release system (SEC) was obtained based on microbial polymers with high biocompatibility for regeneration of soft tissues. The material obtained contains Poly (3-hydroxybutyrate-co-3-hydroxivalerianate) (PHBHV), a natural polymer-sericin and a synthetic hydrophilic polymer that offers dimensional stability, polyacrylamide. The chemical modification by oxidation of PHBHV was revealed by FTIR-ATR analysis. The combination of the three components resulted in 5 test compositions in this stage. The biocompatibility tests showed a low cell viability in PHBHV compositions I.1, II.1 and II.4 compared to the experimental control, as well as an increased cell mortality rate, except for PHBHV composition II.2. Consequently, only the PHBHV II.2 composition can be considered suitable for further optimization in order to continue in vitro studies. Also, tests were performed, tests were made for encapsulation and drug release - silver sulfadiazine, a compound with antibiotic properties.

Within the component project 4, two generations of materials were synthesized; The first generation of materials was based on nanocellulose (CN), pectin (P) or alginate (A), in various proportions. Based on the biocompatibility tests, the optimal ratio between components at 1: 3 was determined (CN: P / A). The second generation of materials aimed to incorporate an antitumor agent - 5fluorouracil - in different concentrations in materials and to investigate the effect of the released product on the cultures of breast carcinoma cells. The results indicated the 2B and 4B compositions as being the most effective in decreasing the viability of the tumor cells. The collection of samples and the enrichment of the fund of the biobank with tissue samples taken from patients with breast carcinoma were optimized and the therapeutic transport systems (STR) were developed and characterized.

In **project 1**, the partners were involved in carrying out the following activities:

Act. 1.1 Establishing the strategy for obtaining biomaterials with osteoimmunomodulatory properties (IC-UB; P1-UPB; P2-IVB)

Act. 1.2 Design of new bo-interfaces by covering and functionalizing the surface of a biodegradable Mg alloy (P1-UPB)

Act. 1.3 Obtaining by surface treatment techniques (electrospinning, electrochemical) the surfaces capable of inducing bone tissue regeneration and their advanced characterization (P1-UPB)

Act. 1.4 Electrospinning coating of the surface of Mg alloys with hybrid materials loaded with biomolecules that accelerate the process of bone tissue regeneration and their advanced characterization (P1-UPB)

Act. 1.5 Standardized in vitro evaluation of the cytocompatibility and anti-microbial and anti-biofilm activity of materials (IC-UB; P2-IVB)

In project 2, the partners involved carried out the following activities:

Activity: A1.6 (P1-UB; P2-UPB) referred to the Optimization of working methods for testing the regenerative processes in the peripheral nerves through (a) Induction of a continuity defect in the peripheral nerves through the transection / resection of the nerve in vivo sciatic; (b) Monitoring of experimental animals to confirm the validity of the experimental model; (c) Defining the parameters of synthesis and functioning of the Assistive System of Peripheral Nerve Regeneration (SARN).

Activity: A1.7 (P1- UB; P2-UPB; IC-IVB) which included (a) Synthesis / manufacture of SARN components - Generation 1: (a1- Synthesis of Nano-SARN component - Generation 1 and a2- Manufacture of nerve conduit artificial 3D-Nerv - Generation 1); (b) Advanced microstructural, physico-chemical and stability characterization under relevant physiological conditions of Nano-SARN and 3D-Nerv components - Generation 1; (c) In vitro testing of the ability to release growth factors into the sequential system by SARN - Generation 1; (d) In vitro validation of SARN biocompatibility - Generation 1.

Within the Project 3- "Cellular and molecular mechanisms involved in the regeneration processes of soft tissues" (acronym SOFT), the project partners were involved in carrying out the following activities corresponding to stage 1/2018:

Activity: A 1.8 - Optimization of culture conditions for cell types to be used for in vitro testing (IC-UB)

Activity: A 1.9 - Establish the experimental design suitable for soft tissue reconstruction in correlation with the existing needs in the clinic (IC-UB)

Activity: A 1.10 - Design of a controlled release system based on microbial polymers with high biocompatibility for soft tissue regeneration (SEC) (P1- UPB) Activity: A 1.11 - Obtaining and characterizing the basic support with controlled morphology of the controlled release system (P1-UPB)

Activity: A 1.12 - In vitro evaluation of the cytocompatibility of SEC system components (IC-UB, P2- IVB)

Activity: A 1.13 - Dose screening for active substances that will be sequentially released from the SEC system (IC-UB)

Activity: A 1.14 - Improvement of UB infrastructure by upgrading confocal equipment and other equipment (IC-UB)

Activity: A 1.15 - Optimization of the working protocol and analysis methods regarding posttranscriptional mechanisms involved in tissue regeneration (P3-IOCN)

During **project 4**, the partners IC-IOCN, P1-UB, P2-UPB and P3-IVB carried out the following activities:

Activity: Act 1.16 - Harmonization and optimization of experimental techniques (IC-IOCN, P1-UB, P2-UPB and P3-IVB)

Activity: Act 1.17 - Experimental and material design for the development of a three-dimensional porous (scaffold) controlled release system that encompasses the cellular component, intended for tissue reconstruction of mammary tumor post-resection, called permanent substitute (SP) (P1-UB, P2-UPB)

Activity: Act 1.18 - Assessment of the biocompatibility of different compositions of SP materials in order to select the most efficient working systems (P1-UB)

Activity: Act 1.19 - Evaluation of the inflammatory status of SP materials components (P3-IVB)

Activity: Act 1.20 - Biological sample collection and tissue and cell biobanking (IC-IOCN)

Activity: Act 1.21 - Development and characterization of therapeutic transport systems (STR) (IC-IOCN)

Dissemination of results

In this stage, the scientific results were disseminated to the scientific environment through 2 publications in ISI journals and 4 communications at national/internationals congresses and conferences.