Short Scientific report on the implementation of the project
PN-III-P1-1.1-PD-2019-0937 (contract no. PD 225/2021) entitled
"Biodegradable and Bioactive 3D Composite Scaffolds for Bone Regeneration in Osteoporosis"
Phase 2 – 2022

The main objectives of phase 2 – 2022 were the incorporation of bioactive principles into the structure of three-dimensional composite scaffolds, the evaluation of their citocompatibility *in vitro* and the obtaining of an osteoporosis model, *in vivo.*

The concept of bone tissue engineering, already introduced since Phase 1 of the project, is based on three components: a scaffold, cells and bioactive principles. According to the National Cancer Institute definition, "a bioactive compound is a type of chemical found in small amounts in plants and certain foods (such as fruits, vegetables, nuts, oils, and whole grains). Bioactive compounds have actions in the body that can promote good health and are continuously studied in the prevention of cancer, heart disease, and other diseases" [1]. They can be used on their own, or incorporated into certain controlled release systems, in the case of tissue engineering in supports. On one hand, the bioactive principles are released in a controlled manner as the scaffold biodegrades, and on the other hand, the scaffold itself becomes bioactive.

Most often, some growth factors, which have an essential role in the process of bone remodeling in the human body, were used as bioactive principles, for example: bone morphogenetic proteins - 2 (BMP-2) or transforming growth factor (TGF-β), or even some hormones involved in the same process, for example: parathormone (PTH). Studies on the use of PTH have shown tumoral effect in rats after long-term use, while BMP-2 can lead to abnormal repair of fractured bone and certain inflammatory processes. Moreover, the cost of both is very high [2]. Drugs of the statin class can compensate for all of the previously mentioned shortcomings of growth factors, making them promising candidates for incorporation into scaffolds for bone tissue engineering [3]. Given these theoretical aspects, for the present phase there were selected two statins: simvastatin (SIM) and rosuvastatin (ROS). These were incorporated into the structure of the three-dimensional scaffolds – beads, by two different methods: directly in the material synthesis stage or by diffusion, respecting the same amount of drug/sphere, in order to perform an adequate comparative analysis.

From the physico-chemical evaluation of the beads (behavior in simulated biological fluids, morphology analysis, chemical structure analysis) it was observed that both drugs and both methods of incorporation are effective and no differences were observed in terms of their morphology or chemical structure.

In vitro evaluation of the cytocompatibility of the spheres was carried out using the MTT assay and the Calcein-AM assay on two different cell lines (human tumor keratinocytes – A431 line and human osteosarcoma – MG-63 line), and it was found that the beads were free of cytotoxicity and does not in any way influence the morphology and density of cells, both those functionalized with drug (ROS) and those without drug.

The evaluation of the osteogenic potential of the beads (with or without incorporated bioactive principles) was studied from the perspective of their ability to support mineralization *in vitro*. The formation of calcium phosphate deposits in osteoblasts (MG-63) was followed, in the presence of the beads compared to their absence (control), using the Alizarin Red test. It was observed that at all three contact times (14, 21 and 28 days), calcium phosphate deposits were formed, colored red/brown by the dye used, but only in the case of the wells where beads were included, in the control wells, they were

absent. At 28 days, a greater number of such deposits was observed, which indicates a gradual process, benefic for the bone regeneration process.

The last important activity at this phase was to obtain an animal model of osteoporosis in the rat, an excellent preclinical model for postmenopausal osteoporosis.

The deliverables associated to the objective of phase 2:

- ✓ Composite three-dimensional scaffolds (beads) functionalized with bioactive principles, citocompatible *in vitro* and posing the capacity to sustain mineralization *in vitro*;
- ✓ An *in vivo* osteoporosis model (rat);
- ✓ One article published: Gardikiotis I, Cojocaru FD *, Mihai CT, Balan V, Dodi G. *Borrowing the Features of Biopolymers for Emerging Wound Healing Dressings: A Review*. International Journal of Molecular Sciences **2022**, 23 (15), 87782022, https://doi.org/10.3390/ijms23158778, (*corresponding author) **FI=6.208**.
- ✓ One article under review (submitted also to International Journal of Molecular Sciences, **FI=6.208**)
- ✓ Poster at ***Annual Meeting 2022, Cost Action CA18103, INNOGLY - Innovation with Glycans,*** University of Applied Sciences and Arts of Southern Switzerland (SUPSI), Lugano, Switzerland, May 4-6th 2022, *Fabrication and properties of polysaccharide based bone substitute beads: a comparative study*, authors: Florina Daniela Cojocaru, Ioannis Gardikiotis, Aurelian Rotaru, Elena Rezus, Gianina Dodi;
- ✓ Poster at ***9th International Conference "Biomaterials, Tissue Engineering & Medical Devices" BIOMMEDD'2022***, Bucharest (Romania), July 20-22nd, 2022; *Composite beads as scaffolds for bone regeneration: physico-chemical characterization and in vitro behaviour*; authors: F.D. Cojocaru, Gianina Dodi, Ioannis Gardikiotis, Aurelian Rotaru, Liliana Vereștiuc, Elena Rezus;
- ✓ Oral presentation at ***4th International Conference on Materials: Advanced and Emerging Materials,*** Barcelona, Spain, 19-21st, 2022, *Polysaccharides-calcium phosphates beads for the treatment of osteoporotic fractures,* authors: Florina Daniela Cojocaru, Elena Rezus, Ioannis Gardikiotis, Aurelian Rotaru, Gianina Dodi
- ✓ 1 activity report.

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[1] https://www.cancer.gov/publications/dictionaries/cancer-terms/def/bioactive-compound

[2] Maia FR, Bastos AR, Oliveira JM, Correlo VM, Reis RL. Recent approaches towards bone tissue engineering. Bone. 2022 Jan;154:116256. doi: 10.1016/j.bone.2021.116256. Epub 2021 Nov 12. PMID: 34781047.

[3] Brittain SB, Gohil SV, Nair LS. Statins as bioactive molecules to support bone regeneration. Curr Med Chem. 2014;21(25):2980-8. doi: 10.2174/0929867321666140601202933.