PED-149-217 (DAPHYTBONE)

Enhancement of bone defect repair by mussel-inspired TiO_2-based nanostructures functionalized with bioactive phytomolecules

Phase 2 summary-

Phase objectives:

1. *In vitro* evaluation of the inflammatory response generated by RAW 274.7 macrophages in the presence of PDA / NDA coated Ti / TiO2 nanostructures and loaded with ICA.
2. Obtaining of the demonstrator product (ICA functionalized PDA-coated Ti/TiO_2 pins);
3. Development of an *in vivo* experimental model for osseointegration of the demonstrator product.

In spite of extensive application in the orthopedic and dental areas, implants made of titanium (Ti) and its alloys often fail because of their bioinert character, poor osteoinduction, and passive integration at the bone-implant interface. Therefore, various methods have been developed to improve the initial stability of bone implants through topography changes and by increasing surface bioactivity. ICA functionalized polydopamine/nitrodopamine (PDA/NDA)-coated TiO_2 nanotubes (NT) and nanofibers (NF) were developed and tested in the project as economic alternatives with the purpose to improve osteointegration of Ti implants. Furthermore, recent studies have highlighted that nanostructured surfaces reduce the inflammatory response of macrophages resulting in a limitation of chronic inflammation and preventing implant failure. In this context, one of the aims of this project was to determine the inflammatory potential of NT or NF coated metal surfaces, as such or functionalized with ICA. The cellular response confirmed that topography dictates changes in macrophage behavior, independent of surface chemistry. In addition, the results obtained have highlighted that ICA can have an anti-inflammatory effect when it is immobilized on TiO_2 nanostructures. The evaluation of the inflammatory response and the examination of osteoblast response showed in both cases that the surfaces covered with NT had superior biological behavior compared to the NF. Thus, maintaining the macrophages in contact with NT-coated surfaces resulted in the release of a lower level of pro-inflammatory mediators in the culture medium compared to the NF-coated surfaces.
Collectively, *in vitro* data suggested that surface modification by electrochemical anodization may be a more effective approach to improve osteointegration of metallic implants than electrospinning. Therefore, for the demonstration and validation activities, UPB (University Politehnica of Bucharest) prepared ICA functionalized PDA-coated Ti/NT (pins) implants which were further implanted in Wistar rats. For implant anodization, conditions previously optimized experimental in Stage 1 of the project, were used and ICA was loaded by direct dropping. Four types of implants were prepared for *in vivo* studies: unmodified Ti implants, NT coated implants, NT coated implants functionalized with PDA, and NT implants coated with PDA and loaded with ICA. The implantation of these materials in rat's femur by a third party, represented by the University of Agronomic Sciences and Veterinary Medicine of Bucharest, has been made without complications following the optimal protocol of anesthesia and surgical approach. Daily

clinical monitoring and radiographs performed at 30 and 90 days post-implantation revealed a good tissue tolerance for all the tested materials. The results of histological analysis showed a more pronounced fibrotic reaction around the unmodified Ti implant compared with the coated implants at 30 days post-implantation, whereas after 90 days no inflammatory or infectious (bacterial) reaction was observed, suggesting good tolerability for all the implanted materials. However, despite the modification of the surface characteristics, the bone regeneration process, analyzed at 3 months after implantation, is not improved around the nanotubular surfaces releasing icariin. This result is surprising since tissue compatibility was more favorable for the nanostructured and functionalized ICA implant at 30 days post-implantation. For elucidation of this contrasting behavior is necessary to extend the time interval for implantation to 6 and 12 months.