

Habilitation Thesis
***Developing strategies for in vitro biocompatibility assessment
of novel biomaterials***

-Abstract-

CÎMPEAN ANIȘOARA

Acknowledging that every material aiming to be used in biomedical applications requires to be screened for its biocompatibility, the main purpose of the present habilitation thesis entitled “**Developing strategies for in vitro biocompatibility assessment of novel biomaterials**” is to show the strategies developed in my last ten years of research for assessing the cell response to novel biomaterials. The thesis is based on several original studies published in ISI journals, studies that were financed by national and international research projects led by me these years.

The main body of the habilitation thesis is represented by **Part I “Scientific and Professional Achievements”** that describes the main scientific contributions. This part is organized in four chapters.

Chapter 1 is dedicated to the *assessment of in vitro biocompatibility of the novel low modulus β -type titanium alloys*: i) Ti-25Ta-25Nb; ii) Ti-23Nb-0.7Ta-2Zr-0.5N, and iii) the “Gum Metal” Ti-23Nb-0.7Ta-2Zr-1.2O. Among metallic materials used as bone substitutes, β titanium alloys have gained an increasing importance but their biological performance has not yet extensively investigated. Considering that the osteoblasts and macrophages are key cells involved in the implant osseointegration process, the developed biomaterials were tested by using established osteoblast and macrophage cell lines. The obtained results were compared to the *in vitro* performance of Ti-based conventional metallic biomaterials widely used for biomedical applications, such as commercial pure titanium (cpTi) or Ti-6Al-4V alloy, and revealed that the newly developed alloys exhibited increased bone tissue compatibility.

The first aim of the Chapter 2 *focussing on the surface modification of Ti alloys* to improve their biological performance was to *investigate the biocompatibility behavior of the nitrogen-implanted Ti alloys* (cpTi, Ti-6Al-4V, Ti-25Ta-25Nb). In this work, an original ion implantation technique developed and patented by Quertech Ingénierie, France was used to modify surface properties of Ti alloys. This novel technology provided the Ti alloys with a hardness increase, reduction of the friction coefficient, and high corrosion resistance. Furthermore, cell culture experiments with human fetal osteoblasts (hFOB 1.19 cell line) have shown that, in general, the cytocompatibility of N-implanted samples was enhanced in comparison to that of the corresponding non-treated specimens. Thus, all the characteristics observed for nitrogen-implanted Ti-alloys make these biomaterials very interesting for biomedical applications

Another study aimed to *evaluate the biocompatibility of various TiO₂ thin films deposited on Ti substrates using metal-organic chemical vapor deposition (MOCVD) technique*. Biocompatibility was evaluated in culture of human gingival fibroblasts (HGF-1 cell line) by addressing cell viability, cytomorphology, adhesive cell-biomaterial interactions mediated through fibronectin matrix, proliferation capacity and potential for degradation of the extracellular matrix, as indicators of the biological performance of studied materials. This study confirmed that the LP-MOCVD technique is well adapted to modifying the surface of materials used in dental implantology.

The last section of the Chapter 2 provides an *overview on the host response to biomaterial implantation with emphasis on macrophage implication*. This overview continues with a study approaching *the macrophage (RAW 264.7 cell line) behavior on TiO₂ nanotubes* with respect to cell morphology, cytokine and chemokine gene expression/ protein secretion, induction of foreign body giant cells (FBGCs) and NO release. Our findings show that lipopolysaccharide (LPS)-induced macrophage activation can be mitigated by nanotube surface as compared to cpTi and suggest that a nanostructured surface is tolerated best in a surgical environment at risk of bacterial contamination. Also, a study on the *macrophage inflammatory response to polypyrrole (PPy) films synthesized on Ti-6Al-7Nb alloy* by potentiostatic polymerization in the presence of poly(sodium 4-styrenesulfonate) (NaPSS), t-octylphenoxy polyethoxyethanol (Triton X-100) and N-dodecyl-β-D-maltoside (DM) surfactants was undertaken. It was demonstrated that the amplitude of macrophage activation increased in the following order: PPy/PSS<PPy<PPy/Triton X-100<PPy/DM.

Chapter 3 presents a *literature review on adipose tissue engineering and our results on in vitro behavior of adipose-derived stem cells (hASCs) embedded in layer-shaped alginate hydrogels*. These hydrogels were prepared by diffusion of two different cross-linking agents: calcium chloride and calcium gluconate in the cell loaded alginate solution. The results obtained demonstrated that both alginate microenvironments support hASC viability and proliferation. These matrices did not alter the cell morphology and created conditions that were favorable for adipogenic differentiation. Furthermore, an enhancement of all these cellular parameters was found out in the case of alginate hydrogel obtained by using calcium gluconate as reticulating agent, suggesting its promising application in soft tissue engineering.

Chapter 4 is devoted to my *teaching activity* in Cell culture and Tissue Engineering.

The thesis continues with **Part II “Future Perspectives in Research and Academic Fields”** that is structured in “*Research development future plans*” and “*Teaching future plans*” and ends with **Appendices 1-3** comprising 376 references.

For future perspectives, using my expertise in the cell culture and tissue engineering fields I will *develop new approaches to unravel the interactions of newly-developed biomaterials with different cell types* in order to prove if these biomaterials are suitable and efficient for the targeted biomedical application.

To fulfil this objective I shall benefit by the cooperation of partners from academic and industrial institutions that will be involved in biomaterials design and synthesis.