”GEORGE EMIL PALADE” UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE, AND TECHNOLOGY OF TÂRGU MUREȘ

SCHOOL OF DOCTORAL STUDIES

DOCTORAL THESIS
ABSTRACT

A study of osteoblasts cells adaptability, behavior and functions on modern orthopedic substrates

PhD candidate: **Andrei-Marian FEIER**

PhD supervisor: **Prof. Univ. Dr. Tiberiu BĂȚAGĂ**

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INTRODUCTION

At the moment, on a global and national level, orthopedic endoprostheses industry is registering an exponential increase in the number of devices implanted annually, being in close correlation with the increased incidence of degenerative pathologies in the field. Of course, the overcrowding of global health systems is expected, but also an increase in the demand for implantable prosthetic materials with improved osteogenic properties. In this context, the need of innovative research and studies that are at the base of obtaining modern orthopedic materials with optimal surface coating and improved mechanical properties becomes imperative.

The first step in this endeavor is an improved understanding of the physical, mechanical and synthesis processes of these materials, from the moment of their manufacture to studying their characteristics *in vitro*. Another extremely important step is the study of the types of implants adapted to confer a high degree of biocompatibility and to analyze their interaction with human tissues and cells.

OBJECTIVES

The present thesis had its primary aim of studying the adaptability, behavior and functions of osteoblast cells seeded in contact with modern orthopedic substrates in order to standardize the steps required for *in vitro* biocompatibility analyses. In order to achieve this objective, other secondary objectives were established, including:

(a) establish a standardized protocol for obtaining mesenchymal stem cells derived from bone tissue and their differentiation towards the osteoblastic line;

(b) confirmation of a standardized storage protocol (cryopreservation) of mesenchymal stem cells for their further use;

(c) development of a standardized protocol regarding an optimal number of osteoblast cells required to be cultured on substrates for biocompatibility analyses.

MATERIALS AND METHODS

Using human bone tissue, but even more so human cells, for research purposes is a morally and ethically responsible activity. For this reason, after establishing the working hypothesis, the first step was to obtain the approval of the Ethics Committee of the University of Medicine, Pharmacy, Sciences and Technology "George Emil Palade" from Târgu-Mureș and the Ethics Committee of the Clinical County Hospital of Mureș. After the favorable response received from the two committees, the studies were outlined as follows:

(a) the first study which assumed the development and adaptation of a standardized protocol for obtaining mesenchymal stem cells derived from bone tissue and their differentiation towards the osteoblasts line together with their storage for long periods (cryopreservation); a step-by-step guide from the operating room to the research laboratory was established for obtaining bone-derived mesenchymal stem cells;

(b) a second experiment, which focused on establishing a standardized protocol regarding an optimal number of osteoblast cells required to be seeded on substrates when used for biocompatibility analyses; osteoblasts were seeded in different numbers ($3x10^4$, $6x10^4$, $1x10^5$) on a control material and their morphology was assessed at different timeframes (1, 3, 7 and 10 days).

(c) the third analysis, based on comparative biocompatibility studies, aimed to study the adaptability, behavior and functions of osteoblast cells that were in contact with modern orthopedic substrates by analyzing alkaline phosphatase (ALP) and total protein (TP) biomarkers and confocal microscopy images (obtained by 3D printing and electrospinning); the modern orthopedic materials studied were as follows: PCC - cell culture polystyrene; Ti-orthopedic titanium; PEI 1% HAp- Polyetherimide reinforced with 1% hydroxyapatite; PEI 15% HAp- Polyetherimide reinforced with 15% hydroxyapatite; PLA 100 μm - polylactic acid with an interporous size of 100 μm; PLA 60 μm - polylactic acid with an interporous size of 60 μm.

RESULTS

The most important finding of our first study was the fact that the harvesting of structured bone residues and the obtaining of primary osteoblast cells derived from human bone tissue is deeply dependent on the following an appropriate protocol of sterile environment and antisepsis, on the femoral neck osteotomy technique but also on the transport of the residual tissue from the operating room to a research lab.

A maximum number of 50.000 osteoblasts are optimal to be seeded for each sample in tests that imply biocompatibility testing. If the materials have a reduced roughness, this number can be reduced to 20.000 osteoblasts/ sample. If the study has a hypothesis of a long-term study of osteoblasts behavior, including the analysis of the mineralization phase, it is recommended to use a number of 20.000 osteoblasts/ sample from the beginning.

The two biomarkers analyzed in the current biocompatibility study had different variations depending on the total contact time with the materials but also depending on the different nature and structure of each material. Results analysis performed after 24 and 72 hours of incubation on the tested materials reveals particular characteristics for each material. The most biocompatible biomaterial in terms of ALP and TP combined with results of confocal microscopy images was PLA 60 μm ($p<.0001$, CI 95%).

CONCLUSIONS

Until now, a series of characteristics that determine to what extent the properties of a material influence cellular activity have not been defined. While cell populations can adapt to changes in their chemical, mechanical, and structural environments, there is currently no model to characterize all of these factors simultaneously. Based on biocompatibility analyses results, researchers in collaboration with clinicians can offer patients a therapeutic hybrid system that consists of synthetic grafts populated with autologous mesenchymal stem cells and their implantation in anatomic areas of interest.