Poly(ε-Caprolactone) Based Biomaterial: Pre-clinical Evaluation

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Introduction: Bioabsorbable polymers have been played an important role in tissue engineering and biomaterial science. The presence of bioabsorbable materials in orthopaedics has grown significantly over the past two decades with applications in fracture fixation, bone replacement, cartilage repair, meniscal repair, fixation of ligaments, and drug delivery. The poly(ε-caprolactone) (PCL) is one of the aliphatic biodegradable polyester which is considered highly biocompatible. In this sense, the approbation of biodegradable sutures by the FDA in the early 60's, medical products based on polycaprolactone, homopolymers and copolymers, has stimulated much research related to PCL matrices for the development of scaffolds to be use in tissue engineering. The novel bioabsorbable PCL composite synthesized in our laboratory seems to be an interesting product for application in bone prosthesis and implants. In this study, the objective was the pre-clinical *in vitro* and *in vivo* evaluation of PCL composite.

Materials and Methods: To evaluate the biocompatibility and safety of the PCL composite tests were performed following the conditions established by FDA, OECD and ISO 10.993 guidelines. The PCL composite pellets and cylinders were sterilized by gamma irradiation. For the *in vitro* studies samples were used to carry out the hemocompatibility assay. Hemocompatibility test were performed according to Part 4 of ISO 10.993 – *Selection of tests for interactions with blood*. Assays for hemolysis, plasmatic coagulation and thrombocyte activation in bovine blood were performed. The in vitro coagulation times, including prothrombin time (PT) were determined. Samples was placed carefully in tubes and incubated at 37±1 °C. Afterward, 100 µl of plasma was layered atop the substrate, and supplemented with 0.9% NaCl-thromboplastin (Factor III; 200 µl TP CLOT). The time taken for the onset of fibrin (clot) formation was detected using a stopwatch. The tests were repeated three times for each sample. For thrombocyte activation was assessed through material-induced changes to morphology of adhered platelets (loss of round shape, and formation of pseudopodia), visualized using SEM. In preparation for SEM, the samples exposed were fixed with 2.5% glutharaldeide, dehydrated with ethanol absolute at different concentrations: 50%, 75%, and 95 % for 5, 10, and 15 min respectively. In the *in vivo* implantation assays evaluated the osseointegration activity in rabbit animal models, according to Part 6 of ISO 10.993 – *Tests for Local Effects After Implantation*. The purpose of this study was to analyze the interface between the implant and the bone medullary canal by histological analysis. Rabbits (Oryctolagus cuniculus, New Zealand, albinus) were selected and assigned to study. The medullary canals of the tibiae, after reaming, were filled bilaterally and implanted with cylinders derived from PCL composite. Euthanasia was performed at the established experimental period, i.e., 20 days postoperatively, and the pieces were removed and submitted to standard histological processing. The histological analysis was carried out in optical microscopy and the biological sequence involved in bone tissue healing was assessed.

Results and Discussion: Osseointegration which is the process of bone healing and the formation of new bone is the clinical goal of implant surgery. As soon as the implant is fixed into a body, number of biological reactions occurs in various stages. Initially, there will be an adsorption of water molecules and proteins and then one of the following processes will take place like showed in Figure 1: a formation of new bone cells on the implant surface, bone cells proliferation and differentiation. When this sequence of events occurs, then the implant is said to be well accepted by the body in which it is inserted. The hemocompatibility showing harmless character.

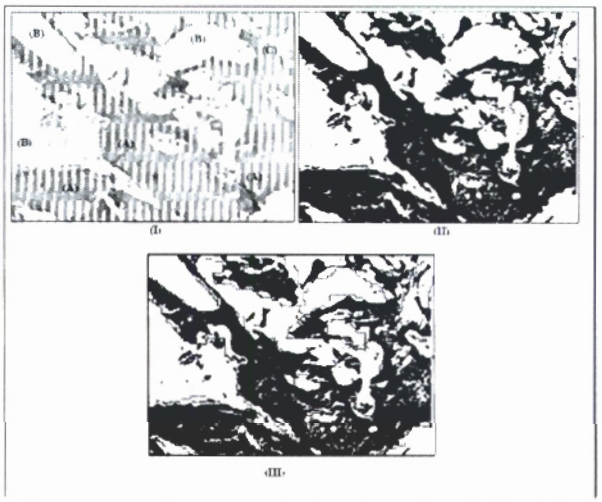

Figure 1. PCL composite implants in rabbits (A) Formation of new bone cells on the implant surface

Conclusions / Summary: With new and promising polymers becoming increasingly available, pre-clinical *in vitro* and *in vivo* biocompatibility testing methods are available and necessary to evaluate these materials for potential clinical applicability. On the parameters evaluated, results revealed the safety and the biocompatible nature of the tested material, indicating their potential use as biomaterial for new medical device development and applications.

Acknowledgement: To the Brazilian Science Council – CNPq and FAPESP.

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