Biological properties of a novel bioactive poly(ε-caprolactone) matrice for tissue engineering.

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Introduction: Bioabsorbable polymers have been played an important role in tissue engineering and biomaterial science. 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 synthesized in our laboratory seems to be an interesting product for application in bone prosthesis and implants, drug delivery and others medical applications. In this study, the objective was the *in vitro* and *in vivo* evaluation of biocompatibility by the comparison of the novel PCL composite and the commercial material.

Materials and Methods: To evaluate the biocompatibility and safety of the PCL tests were performed following the conditions established by FDA, OECD and ISO 10.993 guidelines.
For the in vitro studies the PCL powder and PCL/Sigma® pellet were sterilized by gamma irradiation and immersed in RPMI-1640 medium at 37°C for 48 hours for extract preparation. The extracts were used to carry out the cytotoxicity, genotoxicity and immunotoxicity tests. Cytotoxicity test was based on the quantitative assessment of surviving viable cells (CHO k1 cell line) upon exposure to the samples extracts, by incubation with the supravital dye tetrazolium compound MTS. Genotoxic effects were evaluated by measuring the micronucleus frequency (MN) using CHO cells with S-9 mix and without S-9 mix. Immunotoxicity test were performed using enzyme immunoassay system for the PGE_2 extraction from cell cultures exposed to the samples extracts.
In the *in vivo* assays were determined the acute toxicity and systemic toxicity in rat and rabbit animal models. In the irritation test the scores were measured at 0, 24, 48, 72h and 7 days after the material direct contact in rabbits skin. Systemic toxicity (acute and subacute categories) was evaluated by subcutaneous administration (dosage groups 0, 50, 5000, and 2000 mg/kg) of extracts of the polymers in rats and observed daily during two weeks.

Results and Discussion:
The process for the synthesis of poly--caprolactone (PCL) to be used as a matrix material has been developed in our laboratory. PCL (Sigma® - Mn50000) was used as reference material to be compared with the synthesised polymer. The cell viability determination is showed in figure 1. The tested samples did not presented any cytotoxic effect similarly to the negative control ($IC_{50\%} > 100$) showing their harmless character. In the genotoxic assay, the micronucleus frequency effect was not altered when compared with the negative controls. Similarly the immunotoxic effects were not detected in the PGE_2 assay.
Systemic toxicity parameters as diarrhoea, emesis, weight loss, and altered motor activity did not appeared in the first 24h nor in the next 14 days. Irritation parameters (erythema and oedema) were absent proving that PCL was well tolerated with no inflammation around the local skin application. The scores are showed in table 1.

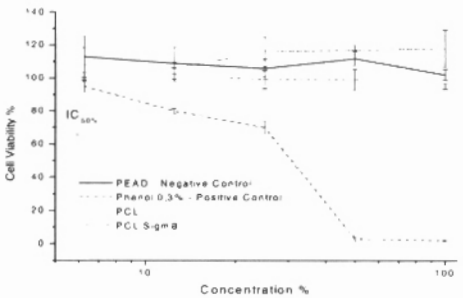

Figure 1. Cytotoxicity Test

Table 1 Irritation Test

Group	Control	PCL	PCL Sigma®
Index *	0	0.1	0.2
Classification	No irritant	No irritant	No irritant

* Index Evaluation: 0.00 – 0.99 No irritation; 1.00 – 1.99 Slight irritation; 2.00 – 2.99 Mild irritation; 3.00 – 5.99 Moderate irritation; 6.00 – 8.00 Severe irritation

Conclusions/Summary: The behavior of the PCL novel matrice compared to the commercial PCL (Sigma®) in the *in vitro* and *in vivo* tests showed to be similar. The 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. There was no toxicity activity neither differences between PCL e PCL/Sigma® on the parameters evaluated.
In the near future the development of prototypes for use in bone reconstructive surgery will be conducted.

References:
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