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BIOCOMPATIBILITY OF THREE POLYMERIC BLENDS

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The main characteristic of biomaterials is their capability to help the body to restore a tissue or function without inducing any significant alteration on it; it is also desirable that they are able to attach to the tissue, induce cell proliferation and to be biodegradable [1, 2]. Biocompatibility tests include both *in vitro* and *in vivo* assays. From the last decade the use of cell cultures has gained more popularity since they throw information about the possible biological responses to the material and they are also a good resource where experiments using laboratory animals are restricted. Several cellular markers such as morphology, adhesion, proliferation, enzymatic activities, among others, can be used to determine the compatibility of a given material within the living organism.

The ASTM recommends the use of cell lines for *in vitro* tests but primary cell cultures are also a good alternative. Lymphocyte cultures have proved to be a very convenient experimental system since they are easy to obtain, handle and proliferate. As these cells can be cultured without being isolated from the other cellular and chemical elements of the blood, they keep most, if not all, of their physiological properties. Furthermore, several biomarkers at different complexity levels can be registered using this system in a 72 h lasting period [3, 4].

Three PHEMA – PHBHV blends were prepared and characterized in our laboratory and, in order to test their biocompatibility and to determine if they can be used in prosthesis and surgical instruments, these materials were subject to cyto and genotoxicity tests using primary human lymphocyte cultures.

Blood samples from 6 adult, healthy donors were cultured and incubated at 37 ºC. After the first 24 h, five sets of cultures were formed to be exposed for the last 48 h of incubation at 37 ºC to the synthetic polymer (PHEMA) and three PHEMA-PHBHV blends: 20:80, 30:70 and 50:50. Rectangular strips of 1.25 cm^2 of these materials previously sterilized in absolute methanol and then rinsed in sterile distilled water were introduced into the culture flasks. The control group remained unexposed. All cultures were re incubated at 37 ºC for 48 h more.

The toxicity of the materials at the cellular level was determined through the proportion of dividing cells in a total of 6,000 cells per set, per donor. The genotoxic potential of the materials, *i.e.*, their ability to harm the DNA molecule, was

determined through single cell electrophoresis or "comet assay". In this test, 300 cell per set of cultures of each donor were analysed.

The results showed that PHEMA alone is cytotoxic since it diminished cell proliferation in a significant manner while blends showed an opposite effect: the more microbial polymer content the higher mitotic index. Considering that lymphocytes belong to the immune system, it is suggested that blends might be acting as antigens promoting T-lymphocyte proliferation but not affecting the cell cycle in itself.

With regard to the genotoxic potential of these polymers, all of them significantly increased the proportion of cells with damage. The average lengths of DNA migration after electrophoresis in the experimental cultures were longer than that of the control though they were relatively short migrations less than 7 μm. PHEMA damages more the hereditary molecule but analysing length data distribution it is observed that the longest migration induced by this polymer did not exceeded 21 μm and its frequency was only 0.29 % . It is considered that the lowest level of genotoxicity includes lengths between 1 and 20 μm [5] and more than 94 % of damaged cells in all sets of cultures except PHEMA, had lengths ranging from 1 to 10 μm. Taking into account that even the control cultures showed an average length of 4 μm and the low frequency of damaged cells with lengths higher than 11 μm in the PHEMA set, we conclude that our blends are not genotoxic and that PHEMA slightly is.

Based on our results, we conclude that the tested materials could be considered as candidates for biomedical applications and new experiments are being performed at present to support this conclusion.

References:

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