Mineralized Tissue Formation by Human Osteoblasts in Contact with Biodegradable Polymers

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Statement of Purpose: A variety of materials have been used for replacement and repair of damaged or traumatized bone tissues.[1] These materials include metals, ceramics, polymers (natural and synthetic) and their combinations. Metals and ceramics have two major disadvantages for tissue engineering applications: their lack of degradability in a biological environment and limited processability. In contrast, polymers have great design flexibility because the composition and structure can be tailored to the specific needs. Biodegradability can be imparted into polymers through molecular design. Some polymers contain chemical bonds that undergo hydrolysis upon exposure to the body's aqueous environment, and some others can degrade by cellular or enzymatic pathways. With the progress of tissue engineering, cell surface-material interaction is of great relevance, and cell adhesion is essential to regulation of differentiation, growth and viability.[2]
For these reasons, polymeric materials have received considerable attention and are widely studied for bone tissue engineering applications. Thus, the purpose of this investigation was to evaluate the effect of three different polymers in the process of osteogenesis induced by human alveolar bone.

Methods: Human alveolar bone fragments (explants) were obtained from healthy donors, using the research protocols approved by the local Research Ethics Committee. Osteoblastic cells were obtained from these explants by enzymatic digestion using collagenase type II. These cells were cultured in α-minimum essential medium, supplemented with 10% fetal bovine serum, 50 μg/mL gentamicin, 0.3 μg/mL fungizone, 10^{-7} M dexamethasone, 5 μg/mL ascorbic acid, and 7 mM β-glycerophosphate. Subconfluent cells in primary culture were harvested and subcultured in 24-well culture plates on poly(L-lactic acid)/PLLA, poly(lactic-co-glycolic acid)/PLGA and Biodegradable polyurethane /BPU discs at a cell density of 2×10^4 cells *per* disc. Cells subcultured in wells without polymer discs were used as a control of culture conditions. During the culture period, cells were incubated at 37ºC in a humidified atmosphere of 5% CO_2 and 95% air. The medium was changed every 3 or 4 days. The evaluation of biochemical parameters were performed after 7, 10 and 14 days of culture. To evaluate cell proliferation and viability, cells were cultured and counted using a haemocytometer. Viable and non-viable cells were detected by trypan blue, which dyed in blue non-viable cells. Cell proliferation was expressed as the number of cells x 10^4/well, and cell viability was expressed as a percentage of the viable cells. Alkaline phosphatase (ALP) activity was assayed as the release of thymolphthalein from thymolphthalein monophosphate using a commercial kit (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil). Results were calculated and data were expressed as ALP activity normalized by the number of cells.
Bone-like nodule formation was evaluated after culturing for 14 days. The attached cells were fixed in 10% formalin and stained with Alizarin red S, which stains areas rich in calcium. Data of *in vitro* cytocompatibility presented in this work are the representative results of 2 separate experiments in cell cultures established from 2 different donors. Thirty-five samples of each surface were used for each separate experiment, with 5 discs (quintuplicates; n=5) for each polymer, for each assay and period evaluated. Comparisons were done using the nonparametric Kruskall-Wallis test, for independent samples at 5% significance level, and the results were presented as mean ± standard deviation.

Results: it was observed that osteoblastic cells in contact with the polymer discs had a significant decrease in their proliferation rate when compared to the control group. In spite of that, cell viability was above 80% in all groups except in the cells in contact with BPU after 14 days, suggesting that the materials were not toxic to the cells. Evaluation of ALP showed a significant increase in its activity in the cells in contact with the polymers, especially after ten days in contact with PLLA and PLGA ($P<0.05$) and seven days in contact with BPU ($p<0.01$). The experiment to evaluate the presence and quantification of mineralization nodules showed that there was no significant difference among the studied groups.

Conclusions: it is concluded that the polymers evaluated in this study are biocompatible with osteoblastic cells and do not impair their growth and function to form mineralized tissue. These results suggest that these polymers may have the potential to be used for bone tissue engineering.

References:

1. Liu X. Ann Biomed Engineering. 2004; 32: 477-486.
2. Guarino V. Biomaterials. 2008;29:3662-70.