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Effects of HEMA on human gingival fibroblasts/*Streptococcus mitis* strains co-cultured in vitro

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Recently dental implants have been modified in order to obtain an optimal integration between host tissues and implant material, besides the easy handle, sealing capability and better aesthetic performance. In addition, one of the principal aims is the preparation of biomaterials not available for bacterial biofilm adhesion and colonization. Thus it is necessary to obtain standardized methods and protocols in order to better evaluate the efficiency of biomaterials used in dentistry to repair and replacement of previously damaged soft and hard tissue and/or organ by the study of biocompatibility.

Thus, the objective of this research has been the evaluation of the biological and molecular modifications occurring in human gingival fibroblasts/*Streptococcus mitis* co-cultures "in vitro", in the presence of HEMA (2-Hydroxyethyl methacrylate), a monomer released by resinous dental materials, in order to reproduce the real situation existing in the oral cavity and to understand cellular and tissue reactions which can occur during interaction/integration between biomaterials/host tissue/microbial environment.

Trypan blue exclusion test evidences a 50% of dead cells in HEMA treated cells when compared to untreated and cocultured ones.

In parallel the bacterial viability, evaluated by Live/Dead kit, is not modified in presence of HEMA and/or cells with respect to the controls.

The effects of HEMA and bacteria alone and in combination on the adhesion cells protein Pro-collagen I expression, involved in structural adhesion, carried out by means of immunofluorescence, evidences a physiological Pro-collagen I expression only in untreated samples, disappearing in the other experimental points. Surprisingly and unlike what has been revealed by trypan blue test, co-cultured samples treated with HEMA show a greater number of apoptotic nuclei when compared with HEMA treated ones. These data suggest that HEMA cytotoxic effect is more evident on eukaryotic cells than on prokaryotic ones and that it seems to be increased by bacteria presence.

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