2-Hydroxyethyl Methacrylate-mediated Changes in the Expression of Genes Involved in Apoptosis and Proliferation in Human Gingival Fibroblasts: an *in vitro* Cytotoxicity Study

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2-Hydroxyethyl methacrylate (HEMA), a component of dentine-bonding materials, flows into the network of the dentine organic matrix due to its low molecular weight and high water affinity. This monomer could also affect odontoblast vitality and diffuse into the gingiva and pulp. It can react with DNA nucleophilic centers *via* Michael addition. A large number of micronuclei, indicating chromosomal aberrations *in vitro* and therefore DNA damage, is induced in mammalian cell cultures by a relatively high concentration (5 mM) of HEMA. It had been thought that the release of monomers and other components from tooth filling systems was greatest 24-48 h after placement in the oral cavity. However, monomer release goes on for weeks or months, albeit often at reduced levels.

The present study was aimed at investigating the effects of a relatively low HEMA concentration, at different incubation times, on the expression of several genes regulating apoptosis and proliferation in human gingival fibroblasts (HGFs), in order to contribute to the evaluation of resin-based material biocompatibility and toxicity.

Real-time reverse transcription-polymerase chain reaction and Annexin-V/Propidium Iodide detection of apoptotic and necrotic cells in flow cytometry were performed on cultured HGFs exposed to 3 mM HEMA for 0, 24 or 96 h, in 3 independent experiments.

24-h HEMA treatment significantly enhances the gene expression of nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1), v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA), inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta (IKBKB), X-linked inhibitor of apoptosis (XIAP), signal transducer and activator of transcription 3 (STAT3), aurora kinase A (AURKA) and protein tyrosine kinase 6 (PTK6) and significantly decreases caspase-3 and receptor (TNFRSF)-interacting serine-threonine kinase 1 (RIPK1) mRNA levels compared to control.

96-h HEMA exposure significantly increases caspase-3 and RIPK1 gene expression and significantly decreases NFKB1, RELA, IKBKB, XIAP, STAT3, AURKA and PTK6 mRNA levels compared to control.

NFKB and STAT3 regulate the expression of anti-apoptotic gene products. 24-h HEMA treatment could inhibit apoptosis by increasing NFKB complex-dependent transcription of XIAP gene, decreasing caspase-3 and RIPK1 mRNA levels and enhancing the gene expression of STAT3, which functions as an oncogene. RIPK1 gene encodes a kinase involved in mediating apoptosis and necroptosis. Moreover, 24-h HEMA incubation might enhance cell survival and proliferation by increasing AURKA and PTK6 gene transcription levels. In fact, AURKA gene encodes a protein, involved in cell-cycle regulation, whose target is p53 pathway. AURKA protein mediates a pro-proliferative signal. PTK6 gene encodes an intracellular tyrosine kinase that modulates cell proliferation *via* Akt. Therefore, 24-h exposed HGFs seem to be able to react to HEMA stress by inducing cell proliferation and resistance to apoptosis.

96-h HEMA treatment could increase apoptosis by reducing NFKB complex-dependent transcription of XIAP gene, enhancing caspase-3 and RIPK1 mRNA levels and decreasing STAT3 gene expression. Furthermore, 96-h HEMA incubation might decrease cell survival and proliferation by reducing AURKA and PTK6 mRNA levels.

These data are confirmed by Annexin-V/PI staining, which highlights that 96-h HEMA treatment enhances the percentage of early apoptotic cells.

In short, after 24-h HEMA exposure HGFs appear to react to monomer-induced damage, promoting cell proliferation and resistance to apoptosis. On the other hand, the negative effects of HEMA could turn chronic after long incubation: after 96-h treatment, cells seem no more able to react, showing an increased apoptosis, a hardline defense to prevent perpetuation of DNA damage. Therefore, a complete polymerization of tooth filling materials might contribute to assure their biocompatibility.