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Cytotoxicity and genotoxicity of a low-shrinkage monomer and monoacylphosphine oxide photoinitiator: Comparative analyses of individual toxicity and combination effects in mixtures

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ABSTRACT

Objective. To compare cytotoxicity and genotoxicity of novel urethane-based monomer FIT-852 and monoacylphosphine oxide photoinitiator (Lucirin TPO) with conventional Bisphenol A-glycidyl-methacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) monomers and camphorquinone (CQ)/amine photoinitiator system, respectively. Moreover, we quantified and analyzed the combinatorial effects of individual substances in resin-based mixtures concerning the nature of the combinatorial effects.

Methods. Cytotoxic and genotoxic effects of BisGMA, FIT, TEGDMA, CQ, DMAEMA and TPO and their combined toxicity in four clinically relevant mixtures (FIT/TPO, FIT/CQ, BisGMA/TPO, BisGMA/CQ) were tested on human fetal lung fibroblasts MRC-5 using MTT and Comet assays. We assessed combination effects of monomers and photoinitiators on overall toxicity from the measured concentration-effect relationships. Combination index (CI) was calculated on the basis of the median-effect equation derived from the mass-action law principle.

Results. Individual substances showed decreasing cytotoxic effects in the following order: BisGMA>TPO>FIT>CQ>DMAEMA>TEGDMA. Experimental mixtures showed decreasing cytotoxic effects in the order BisGMA/TPO>BisGMA/CQ>FIT/CQ>FIT/TPO. FIT-based mixtures exhibited antagonistic cytotoxic effects between components while BisGMA-based mixtures demonstrated synergistic effects at ED_{50}. TPO amplified both antagonistic and synergistic cytotoxic effects in mixtures. Pure substances showed genotoxicity in the following order: TPO>BisGMA>FIT>CQ>TEGDMA. We did not detect the genotoxic potential of DMAEMA. The rank of genotoxic concentrations of the mixtures was: BisGMA/TPO>BisGMA/CQ>FIT/CQ>FIT/TPO.

Significance. Lower cytotoxicity and genotoxicity of FIT than BisGMA suggests its greater biocompatibility. Conversely, photoinitiator TPO was significantly more cytotoxic and genotoxic

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than both CQ and DMAEMA. CI values showed that components of FIT-based mixtures exhibit an antagonistic cytotoxic effect, while compontents of BisGMA-based mixtures show synergism.

1. Introduction

Residual monomers and other unbound substances from resin-based composites (RBCs) may reduce biocompatibility of RBCs *via* direct contact with oral tissues. Unbound components of composites diffuse through dentin into the pulp or elute into the oral cavity [1,2]. These substances may also be systemically distributed throughout the organism, either being taken up by circulating blood or swallowed by saliva, consequently causing adverse systemic effects [3,4].

Several studies reported on the cytotoxicity, mutagenicity, estrogenicity, allergic reactions and systemic toxicity of RBC components [4–6]. Other studies have shown adverse effects of monomers on cellular homeostasis even in sub-toxic concentrations [7,8]. Investigations have also revealed that monomers and photoinitiators cause cellular redox imbalance increasing the levels of reactive oxygen species (ROS) and subsequent cell death *via* apoptosis as well as DNA damage [9–12].

Investigation on the biological effects of resin-based materials usually includes several most commonly used substances (monomers, oligomers, photoinitiators) acting separately [5,12–15], or eluates of commercial materials whose exact compositions remain undisclosed by the manufacturers [16,17]. It is important to emphasize that two or more substances may exert significant cytotoxic and genotoxic effects alone, but in combination they may interact and produce toxic effects different from those expected from their sole actions [18,19]. This necessitates the investigation of potentially combined biological effects of base monomers, co-monomers and photoinitiators. It is particularly important to discover synergistic toxic effects of mixed substances, producing higher biological effects than those coming from pure addition of their separate actions. Combined cytotoxic effects of different monomers and photoinitiators have not been studied extensively in the literature [18–20]. Authors have mostly claimed synergistic effects by simple comparison of monomer mixtures. However, the synergism cannot be claimed by simple arithmetic addition of effects [21]. The synergistic toxic effect of mixture of components occurs as a combination of effects' magnitudes and their concentration dependences, the later obeying the mass-action low principle [21]. These are both accounted in the so-called combination index (CI) which can be calculated when concentration-effect dependences of mixtures and their components are separately determined. Then, combinatorial toxic effect (synergy, addition, or antagonism) can be evaluated from the CI, not only for the concentrations of components in the investigated mixture, but for the range of component's concentrations providing in this way general conclusions for the study. One should note that combinatorial effects may present different nature with different concentrations of components in the mixture; antagonism may occur with *e.g.* low-concentration of one component, but strong synergism when concentration of this component is increased.

Recent improvements of the organic matrix of RBCs include the development of new methacrylate monomers and photoinitiators. Polymerization shrinkage and potential risks associated with the toxicity of glycidyl dimethacrylate (BisGMA) [22] and estrogenic effects of its precursor bisphenol-A [23], led to the development of bisphenol-A alternatives. Derivatives of urethane dimethacrylate (UDMA) and long-chain monomers with high molecular weight have already been used in several commercial low-shrinkage RBCs. Phosphine oxide photoinitiators are aimed at overcoming the yellowing effect of camphorquinone (CQ) and maintaining or improving polymerization efficiency without additional co-initiators [24,25].

Previous studies have reported comparable or better conversion, lower shrinkage [26,27] and better color stability [28] of commercial or experimental low-shrinkage RBCs compared to conventional ones. FIT-based experimental RBCs showed a higher degree of conversion and significantly lower shrinkage but lower Vickers hardness, flexural strength and modulus [26] and lower color stability [29] than BisGMA-based experimental RBCs. Lucirin TPO (TPO) photoinitiator exhibited better results over CQ/amine system in terms of polymerization efficiency [24–26] and color stability [29,30].

Scarce literature data are available on toxicity of low-shrinkage monomers and novel photoinitiators. Van Landuyt et al. [31] have reported stronger cytotoxic effect for TPO-containing than CQ/amine-containing adhesive. Eluates from two low-shrinkage RBCs, GC Kalore and Bisco Reflexion, have shown reduced cell viability [17]. It is difficult to ascertain the toxic potential of certain components in multi-component commercial RBCs due to the often undisclosed composition or patent protection, especially in the case of novel, low-shrinkage monomers. Therefore, experimental mixtures seem to be a more appropriate model for biocompatibility testing.

In the present work, we aimed to compare cytotoxicity and genotoxicity of the novel, urethane-based, low-shrinkage monomer, FIT-852 (Code: FIT, Esstech Inc., Essington, PA, USA) and the alternative photoinitiator TPO to the most frequently used cross-linking monomer BisGMA, co-monomer triethylene glycol dimethacrylate (TEGDMA) and photoinitiator system CQ/amine. We used MTT and Comet assays to test cytotoxicity and genotoxicity, respectively, both being performed on fetal lung MRC-5 fibroblasts. Even though there are cell lines that could be considered as more convenient to evaluate dental materials, MRC-5 fibroblasts have already been used in several similar studies [32,33]. This study focused on individual substances and clinically relevant mixtures. Moreover, we aimed to assess and quantify the combinatorial toxic effects of components present in these experimental mix-