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Cytotoxicity and DNA double-strand breaks in human gingival fibroblasts exposed to eluates of dental composites

Yang Yang[a,b], Franz-Xaver Reichl[a,b], Jianwei Shi[c], Xiuli He[a,b], Reinhard Hickel[a], Christof Högg[a,b,*]

[a] Department of Conservative Dentistry and Periodontology, University Hospital, LMU Munich, Germany
[b] Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University of Munich, Nußbaumstr. 26, 80336 Munich, Germany
[c] Department of Orthodontics, Ludwig-Maximilians-University of Munich, Goethestr. 70, 80336 Munich, Germany

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ABSTRACT

Objective. Previously, single composite components were used to study cytotoxicity and induction of DNA double-strand breaks (DNA-DSBs) of dental composite resins. In the present study, cytotoxicity and induction of DNA-DSBs in human gingival fibroblasts (HGFs) were investigated with dental composite eluates consisting of multiple components. The eluates were qualified and quantified.

Methods. The composites Esthet.X® HD, Venus®, X-tra fil®, CLEARFIL™ AP-X, Admira® Fusion and QuiXfil® were polymerized and immersed into Dulbecco's modified Eagle's medium (DMEM) for 72 h. Subsequently, HGFs were incubated with the corresponding composite eluates. The cell viability of HGFs was obtained from an XTT assay. DNA-DSBs were determined using a γ-H2AX assay. The qualification and quantification of eluates were performed by gas chromatography/mass spectrometry (GC/MS).

Results. HGFs exposed to the eluates of all investigated composites showed no significant loss of cell viability, compared to negative control. Significant DNA-DSBs induction could be found in HGFs exposed to the eluates of Esthet.X® HD (0.43 ± 0.05 foci/cell) and Venus® (0.39 ± 0.04 foci/cell), compared to control (0.22 ± 0.03 foci/cell). A total of 12 substances were detected from the investigated composite eluates. Five of them were methacrylates: tetraethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA), ethyleneglycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TMPTMA). The highest concentration of HEMA (110.5 μM), HPMA (86.08 μM) and TMPTMA (4.50 μM) was detected in the eluates of QuiXfil®. The highest concentration of TEGDMA was 1080 μM in Venus® eluates and the highest concentration of EGDMA was 3.18 μM in Esthet.X® HD eluates.

Significance. Significant DNA-DSBs induction can be found in HGFs exposed to the eluates of Esthet.X® HD and Venus®. The interactive effects among released (co)monomers and

* *Corresponding author at*: Department of Conservative Dentistry and Periodontology, University Hospital, Ludwig-Maximilians-University of Munich, Goethestr. 70, 80336 Munich, Germany. Fax: +49 89 7095 73817.
E-mail address: christof.hoegg@lrz.uni-muenchen.de (C. Högg).

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additives may influence the cytotoxicity and induction of DNA-DSBs, compared to exposure with single composite component.

1. Introduction

Light-cured composite resins consist of (co)monomers and additives like photoinitiators, coinitiators, photostabilizers, inhibitors and inorganic fillers [1]. The polymerization of dental composites is incomplete [2]. Previous studies revealed that (co)monomers and additives can be eluted from dental composites [2–5]. The degree of conversion (DC) depends on many factors such as the light density, curing time and distance between light source and dental composite, as well as the composition and shade of the dental material [6]. The lower the DC of a composite the more composite components can be eluted [7]. (Co)monomers and additives may penetrate to pulp via dentinal tubules, then affect the activity of dental pulp cells or enter the intestine by swallowing, subsequently reaching the circulatory system and organs [8–10]. Additionally, the (co)monomers (methacrylates) can cause allergic reactions such as asthma and contact dermatitis [11].

Geurtsen et al. investigated 35 dental resin composite monomers and additives in human primary fibroblast cultures, in which, the cytotoxicity of (co)monomers and additives was revealed [12]. The mutagenicity, embryo toxicity and teratogenicity caused by released (co)monomers were also reported [13]. Moreover, it was shown that TEGDMA and HEMA can be metabolized to epoxy compound 2,3-epoxy-2-methylpropionic acid (EMPA) [14], and the formation of another epoxide, 2,3-epoxy-2-methylpropionicacid-methylester (EMPME), was postulated [13]. The formation of epoxide in human oral cells (for example, human gingival fibroblasts (HGFs) and human pulp fibroblasts) has been demonstrated [15]. In our previous study, EMPME and EMPA were not only found to induce cytotoxicity, but also to induce higher rates of DNA double-strand breaks (DNA-DSBs) in HGFs, compared to their metabolic precursors, TEGDMA and HEMA [16,17]. DNA-DSBs are considered as the most toxic type of DNA lesion [18].

To date, studies on cytotoxicity and DNA-DSBs concerning dental composite resins have dealt with the effects of single composite components [16,18,19]. However, less data for cytotoxicity and no data for induction of DSBs are available for composite eluates consisting of multiple components. Experiments with qualified and quantified eluates may reflect a situation closer to physiology, compared to single-component experiments. Therefore, in the present study, cytotoxicity and induction of DNA-DSBs in HGFs were investigated with dental composite eluates. The multiple composition of eluates was qualified and quantified.

In the null hypothesis, it is assumed that composite eluates do not induce cytotoxicity and DNA-DSBs in HGFs.

2. Methods

The investigated composites including manufacturers' data are listed in Table 1. The six types of investigated composites

Table 1 – Investigated dental materials, manufacturers, LOT numbers, types, and polymerization times; composition of each material based on manufacturer's data.

Product name	Type	Manufacturer	LOT	Composition of materials based on manufacturer's data	Polymerization time
Esthet.X® HD	Micro-hybrid	Dentsply, Caulk, USA	160523	Bisphenol A-glycidyl methacrylate (Bis-GMA), ethoxylated bisphenol-A dimethacrylate (BisEMA), TEGDMA, CQ, photoinitiator, stabilizer, pigments	20 s
Venus®	Micro-hybrid	Heraeus Kulzer, Hanau, Germany	010504A	Bis-GMA, TEGDMA and contains 58.7% filler (by volume), such as Barium Aluminium Fluoride glass; Highly dispersive Silicon Dioxide	20 s
X-tra fil®	Multi-hybrid	VOCO GmbH, Cuxhaven, Germany	010106	Bis-GMA, urethane dimethacrylate (UDMA), TEGDMA	10 s
CLEARFIL™ AP-X	Micro-hybrid	Kuraray Europe GmbH, Hattersheim am Main, Germany	A50079	Bis-GMA, TEGDMA; silanated barium glass filler, silanated silica filler, silanated colloidal silica	20 s
Admira® Fusion	Nano-hybrid Ormocer®	VOCO GmbH, Cuxhaven, Germany	1648518	ORMOCER®	20 s
QuiXfil®	Micro-hybrid	DENTSPLY DeTrey GmbH, Konstanz, Germany	1605000136	UDMA, TEGDMA, Di- and trimethacrylate resins, carboxylic acid modified dimethacrylate resin, BHT, silanated strontium aluminium sodium fluoride phosphate silicate glass	10 s

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