Available online at www.sciencedirect.com
ScienceDirect
Mutation Research 649 (2008) 79–90
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
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Sister-chromatid exchange, chromosomal aberrations and delays in cell-cycle kinetics in human lymphocytes induced by dental composite resin eluates

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Received 15 January 2007; received in revised form 8 June 2007; accepted 7 August 2007
Available online 12 September 2007

Abstract

We have investigated eluates derived from commercially available composite resin-based materials used for direct (Tetric Ceram/Ivoclar-Vivadent, Simile/Pentron, Filtek Z-250/3M ESPE) and indirect (Adoro/Ivoclar-Vivadent and Conquest Sculpture/Pentron) dental restorations, with respect to their genotoxic effects on human peripheral lymphocytes. Primary lymphocyte cultures obtained from blood samples of three healthy donors were exposed to eluates of freshly cured specimens of all the materials tested. Metaphases were induced with phytohaemagglutinin, collected after a 72-h treatment using colchicine and stained with the Fluorescence Plus Giemsa (FPG) procedure. Preparations were scored for sister-chromatid exchange (SCE) and chromosomal aberrations (CAs). The proliferation rate index (PRI) and the mitotic index (MI) were also calculated.

Our results show that eluates derived from the three direct composites (Filtek Z-250, Simile and Tetric Ceram) increased the frequencies of SCE and CAs and markedly reduced PRI and MI. Tetric Ceram's eluate, being the most genotoxic of all eluates tested, increased the frequencies of SCE up to 24.40 per cell (control, 9.87 per cell) and of CAs up to 424 per 100 metaphases scored (control, 5). Moreover, it caused a pronounced decrease of the PRI down to 1.31 (control, 2.44) and of the MI down to 9.8‰ (control, 19.2‰). In contrast, eluates derived from the laboratory-processed composites (Adoro and Conquest Sculpture) induced much less cytogenetic damage. Overall, the degree of genotoxicity and cytotoxicity decreased as follows: Tetric Ceram > Filtek Z-250 > Simile > Adoro = Conquest Sculpture.

These results indicate that composite resins used for direct and indirect dental restorations differ extensively in their cytotoxic and genotoxic potential and in their ability to affect chromosomal integrity, cell-cycle progression, DNA replication and repair. This underlines the impact of improved polymerization with respect to their biological behavior.

Keywords: Composite resins; Dental restoration; Sister-chromatid exchange (SCE); Chromosomal aberrations (CAs); Proliferation rate index (PRI); Mitotic index (MI)

1. Introduction

Dental composite resin-based materials have been widely studied for cytotoxicity in various cell culture systems [1,2]. Their cytotoxic effects reported thus far have been attributed to the release of residual

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doi:10.1016/j.mrgentox.2007.08.009

monomers or other substances derived either from incomplete polymerization or resin degradation [3,4]. More than 30 different compounds have been identified as being released from the polymerized dental composites, including major monomers, co-monomers, additives and reaction products, all with different cytotoxic potential [5]. The released components may diffuse through the dentine into the pulp space or, alternatively, they may either come in contact with the gingiva, or be swallowed via the saliva, taken up by the circulating blood and excreted with the urine [6].

There are a few studies dealing with the possible mutagenicity and genotoxicity of various single components released from polymerized composite resins. TEGDMA, bisphenol A and HEMA have been found to increase the number of micronuclei at sub-toxic concentrations and promote degradation of DNA from salivary gland tissue and lymphocytes, as shown by a comet assay [7–9]. TEGDMA was also reported to induce extensive deletions of sequences in the hypoxanthine-guanine phosphoribosyl-transferase (*Hprt*) gene in V79 Chinese hamster lung fibroblasts [10]. Other substances released from composite resins, such as the photo-initiator camphoroquinone, have been found to promote oxidative DNA damage via the generation of reactive oxygen species (ROS) [11].

There is quite limited knowledge concerning the genotoxic and mutagenic effects of the many compounds released in eluates of commercially available, widely used composite resins and dental adhesives [12,13]. Monitoring the genotoxicity of these eluates would provide a better understanding of their interaction with the oral tissues and the potential to cause genotoxic effects *in vivo*.

Recently, we have observed that eluates from specific direct composite resins show extensive time- and concentration-dependent cytotoxicity to murine WEHI 13 var fibroblasts, accompanied by induction of DNA fragmentation, as shown by agarose gel electrophoresis [14]. This prompted us to extend these observations in the present study, which was aimed at testing the effect of five widely used composite resins for direct and indirect dental restorations with respect to induction of sister-chromatid exchange (SCE), chromosomal aberrations (CAs), and effects on cell-cycle kinetics and mitotic indices of human peripheral lymphocytes. SCE have been proposed as a very sensitive indicator of DNA damage and/or subsequent DNA repair. In combination with the evaluation of chromosome aberrations, SCE can provide insight in the cytogenetic damage induced by various genotoxic agents at very low concentrations. Moreover, the determination of proliferation rates and mitotic indices in lymphocyte cultures has been proved to be a very useful and sensitive indicator of the cytostatic and cytotoxic action of various environmental hazards or therapeutic agents [15,16]. Our test hypothesis was that laboratory-polymerized (indirect) composite resins would be less cytotoxic and genotoxic compared with conventional direct composites.

2. Materials and methods

2.1. Materials and sample preparation

Two hybrid composites (Tetric Ceram/Ivoclar-Vivadent, Schaan, Lichtenstein and Filtek Z-250/3M ESPE, Seefeld, Germany), one nano-hybrid (Simile/Pentron, Wallingford, USA) and two second-generation laboratory composites for indirect restorations (Adoro/Ivoclar-Vivadent, Schaan, Lichtenstein and Conquest Sculpture/Pentron Wallingford, CT, USA) were tested (Table 1). Specimens of each biomaterial were prepared aseptically with a prefabricated mould as disks of 5-mm diameter and 2-mm thickness, with a surface area of 0.7 cm^2. The first three direct composites were cured for 40 s on one side, using a light-emission diode (LED) curing unit at 650 mW/cm^2 (bluephase, Ivoclar-Vivadent, Lichtenstein). Adoro and Sculpture were pre-cured for 20 s with the same LED-curing unit and then post-cured by the proprietary laboratory curing units proposed by the manufactures (Table 1). Specimens of all materials were not covered with polyester film during curing. However, the upper surface of the specimens (the one exposed to the oxygen during curing) was routinely polished using Sof-Lex discs (3M, ESPE).

Eluates were prepared by placing freshly cured specimens of each material in numerically equal volumes of extraction medium in Falcon micro-centrifuge tubes (Corning Inc., NY, USA). In the first two experiments (each with lymphocytes from a different donor) the specimens were extracted for 48 h at 37 °C. In addition, the third experiment (third donor) was carried out with two additional extraction periods of 24 and 72 h at 37 °C, in order to determine whether the duration of extraction can substantially affect the cytotoxicity of the eluates. Melphalan, a well-known alkylating agent, was used as a positive control at three different concentrations (0.5, 1.0 and 1.5 μM). RPMI 1640 cell culture medium, with 100 IU/ml penicillin and 100 μg/ml streptomycin was used for the extraction of the specimens (Biochrom, Berlin, Germany). Fetal calf serum (FCS) (Biochrom) was not added prior to exposure to the cells. The ratio between the surface area of the specimens and volume of extraction medium was 0.7 cm^2/ml and was at the lowest levels of the ISO 10993/12 requirements [17]. RPMI 1640 medium without composite resin–eluate was incubated under the same conditions as a negative control.

2.2. Cell culture

Primary cultures of lymphocytes were set up by adding 11 drops of heparinized whole blood derived from three normal