DENTAL MATERIALS 33 (2017) 788–795

Available online at www.sciencedirect.com

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journal homepage: www.intl.elsevierhealth.com/journals/dema

Genotoxic potential of dental bulk-fill resin composites

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ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form
17 April 2017
Accepted 18 April 2017

Keywords:
Resin composite
Bulk-fill
Biocompatibility
Genotoxicity
Microhardness

ABSTRACT

Objective. To investigate both genotoxicity and hardening of bulk-fill composite materials applied in 4-mm layer thickness and photo-activated for different exposure times.

Methods. Three flowable bulk-fill materials and one conventional flowable composite were filled in molds (height: 4 mm) and irradiated for 20 or 30 s. The top (0 mm) and bottom (4 mm) specimen surface were mechanically scraped, and eluates (0.01 g composite in 1.5 ml RPMI 1640 cell culture media) prepared for each material, surface level and irradiation time. Genotoxicity was assessed in human leukocytes using both the alkaline comet assay and cytokinesis-blocked micronucleus assay, and Knoop hardness (KHN) was measured at the top and bottom specimen surface (n = 8).

Results. At both irradiation times, none of the bulk-fill composites significantly affected comet assay parameters used in primary DNA damage assessment or induced significant formation of any of the scored chromatin abnormalities (number of micronuclei, nuclear buds, nucleoplasmic bridges), whether eluates were obtained from the top or bottom surface. Furthermore, no decrease in KHN from the top to the bottom surface of the bulk-fill materials was observed. On the other hand, the conventional composite irradiated for 20 s showed at 4-mm depth a significant increase in the percentage of DNA that migrated in the tail and a significant increase in the number of nuclear buds, as well as a significant decrease in KHN relative to the top surface.

Significance. Bulk-fill resin composites, in contrast to conventional composite, applied in 4-mm thickness and photo-activated for at least 20 s do not induce relevant genotoxic effects or mechanical instability.

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http://dx.doi.org/10.1016/j.dental.2017.04.011

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1. Introduction

Light-activated resin composites are nowadays the most frequently used direct restorative materials in dentistry [1]. Despite their widespread use and reliable evidence of their clinical long-term success [2], concerns exist about possible intrinsic toxicity of resin-based composite materials [3,4].

The effects of masticatory forces and chemical degradation can cause composite restorations to release harmful substances into the pulp or saliva, which may thereby pass into the bloodstream. Nearly all components of dental resin composites can be eluted in the oral cavity, but the elution of resin monomers is of particular interest due to their potential cytotoxic and genotoxic effects [5–7]. It has been shown that the release of residual unreacted monomers inversely correlates with the degree of monomer to polymer conversion [8]. In order to increase the degree of conversion of resin-based composite materials, low molecular weight monomers, such as 2-hydroxyethyl methacrylate (HEMA) and triethylene glycol dimethacrylate (TEGDMA) are usually utilized as diluent monomers [9,10]. However, these monomers also reduce the levels of glutathione, a natural radical scavenger that protects cell structures from damage caused by reactive oxygen species. These effects can cause oxidative stress and DNA strand breakage [11,12]. In addition to HEMA and TEGDMA, bisphenol-A-glycidyldimethacrylate (Bis-GMA), an often-used base monomer in composite materials, has also demonstrated dose-dependent genotoxicity by increasing the number of micronuclei and DNA strand breaks [13,14].

In recent years, a new category of composite materials, so-called bulk-fill resin composites, have been developed in order to simplify and expedite the restorative process. According to manufacturers' claims, these materials can be properly photo-polymerized even when applied in thick layers up to 4–5 mm, and maintain low polymerization stress at the same time. To this end, novel proprietary resins, unique fillers, special polymerization modulators, and optimized photoinitiators were formulated. While studies substantiated reduced polymerization stress formation [15–17] and increased curing depths of bulk-fill composite materials compared with conventional resin composites [18,19], a decline in micromechanical properties of bulk-fill resin composites at 4-mm depth and beyond has also been reported [20,21]. At such composite layer thickness, curing light penetration might be hindered, thus reducing the degree of monomer to polymer conversion and increasing the release of unconverted monomers, which might compromise biocompatibility. Indeed, a recent study revealed for some bulk-fill resin composites cytotoxic effects not compatible with the ISO cutoff of 70% cell viability when the materials were applied in 4-mm layer thickness and photo-activated for 20 s [22]. The genotoxic potential of bulk-fill composite materials, as well as its dependence on light exposure time, is as yet unknown. Due to their higher resin content and more persistent mass leaching compared with conventional hybrid composite materials [23], low-viscosity flowable (bulk-fill) composites might be particularly relevant for genotoxicity testing.

The comet assay was previously established as an initial indicator of general, non-specific DNA damage/genotoxicity [24], enabling detection of a wide range of primary DNA damage such as single and double strand breaks, alkylation, and oxidatively damaged DNA bases. To quantify DNA damage by means of the comet assay, the parameters tail length (μm) and tail intensity (% DNA) are most frequently used. Tail length determines the length of DNA migration and is directly related to DNA fragment size and the extent of DNA damage, whereas tail intensity denotes the number of DNA fragments, which directly indicates the proportion of the genome affected by the damage [25].

In recent years, the micronucleus has been accepted as the predominant biomarker in genotoxicity evaluation [26]. The micronucleus is formed in cells exposed to a genotoxic agent as the consequence of induced DNA strand breaks that will result in chromosome aberration, or damage to mitotic spindle proteins, which leads to the lag of chromosomes and unsegregation. In addition to the micronucleus, other aberrant chromatin structures such as nuclear buds and nucleoplasmic bridges should be considered when evaluating genotoxic potential, because they represent visualization of chromosomal re-arrangements and premature telomere shortening [27].

The aim of the present study was to investigate the in vitro genotoxicity of low-viscosity bulk-fill resin composites applied in 4-mm layer thickness and photo-activated for different exposure times. In addition, microhardness, as an indirect measure of the degree of conversion [28,29], was assessed at both the top and bottom surface of the composite specimens in order to allow an estimation of the extent of resin polymerization.

2. Materials and methods

2.1. Specimen preparation

Three flowable bulk-fill composite materials [SDR (Dentsply DeTrey, Konstanz, Germany), Venus Bulk Fill (Heraeus Kulzer, Hanau, Germany), x-tra base (VOCO, Cuxhaven, Germany)] and one conventional flowable resin composite [Tetric EvoFlow (Ivoclar Vivadent, Schaan, Liechtenstein)] were used. Details of the test materials are presented in Table 1. The composite materials were filled into cylindrical Teflon molds (height: 4 mm, diameter: 10 mm) placed on a glass plate and Mylar strip. The applied composite materials were covered with another Mylar strip and 1 mm thick glass plate, and pressed to the height of the mold. Photo-activation was performed for either 20 or 30 s with a LED light-curing unit (Bluephase G2; Ivoclar Vivadent) by placing the curing light tip in contact with the glass plate covering the top surface of the specimen. Output irradiance of the light source (1170 mW/cm^2) was measured by using a calibrated FieldMaxII-TO power meter and PM2 thermopile sensor (Coherent, Santa Clara, CA, USA), and verified periodically during the experiments. After photo-activation, the composite specimens were stored for 24 h in the dark at 37 °C.

2.2. Genotoxicity testing

2.2.1. Preparation of eluates

Both the top and bottom surface of the composite specimens were used to prepare eluates of each material tested. Each

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