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Cytotoxic effects of halogen- and light-emitting diode-cured compomers on human pulp fibroblasts

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Objective. The aim of this study was to determine the cytotoxic effects of three different compomers (Dyract AP, Compoglass, and Hytac) cured using a halogen light-curing unit (LCU) and a light-emitting diode (LED) LCU on human pulp fibroblasts.

Methods. Specimens of three compomers were added to human pulp fibroblast cultures. Cytotoxicity was evaluated over 96 h using the agar overlay method.

Results. All three compomers tested were found to be moderately cytotoxic to human pulp fibroblasts, regardless of whether they were cured using halogen or LED LCUs. The decolorization zone of Hytac was significantly larger than those of the other compomers tested ($P < 0.05$). Dyract AP and Compoglass specimens showed greater decolorization when cured with LED than with halogen LCUs ($P < 0.05$).

Conclusion. Compomers are potentially toxic to human pulp fibroblasts, and the type of curing unit may affect compomer toxicity.

Introduction

Compomers represent a new category of filling material considered to be one of the most recent improvements in paediatric dentistry. Compomers were developed with the aim of combining the positive properties of light-cured composites with those of glass ionomer cements. Compomers are easy and safe to apply, and provide good aesthetic and mechanical properties, bonding to dental hard tissue, fluoride release, radiopacity, and biocompatibility[1], making them a useful alternative to amalgam in paediatric dentistry.

In general, two mechanisms are involved in setting compomer materials. Like standard light-cured resin composite, compomers rely on the visible light-initiated polymerization of free radicals, and like glass ionomer cements, they require an acid–base reaction[2].

Residual monomer has been shown to leach from polymerized resin-based materials during both the initial setting period and as the resin is degraded over time. In the first case, the leaching process is related to the degree of monomer–polymer conversion[3], making adequate polymerization a crucial factor in both the physical[4] and cytotoxic properties of resin-based restorative materials[5].

For many years, halogen light-curing units (LCUs) were preferred as the most practical method for polymerizing light-cured resin. Despite its relatively low costs, however, halogen technology has certain inherent drawbacks[6]. Halogen bulbs have a limited effective lifetime of approximately 100 h[7], and the high operating temperatures and large quantities of heat produced during the operating cycles cause the LCU bulbs, reflectors, and filters to degrade over time, thereby reducing curing efficiency[8].

Recently, manufacturers have turned their attention to other light sources such as lasers, plasma arc units, and light-emitting diodes (LEDs) to polymerize resin-based materials. However, both lasers and plasma arc units are more complex and more costly than halogen units, and lasers require additional stringent safety precautions[9]. In contrast, LEDs have a lifetime of over 10 000 h, with relatively little

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degradation[10]. They require little power to operate, are resistant to shock and vibration, and require no filters to produce blue light[11]. All these positive properties make them an excellent alternative to conventional halogen lamps.

Although the effects of LED polymerization on the physico-mechanical and cytotoxic properties of resin composites have been investigated[12,13], little research has been conducted on the effects of LED polymerization on the physico-mechanical and cytotoxic properties of compomers. The aim of this study was to evaluate the cytotoxic effects of three different compomers (Dyract AP, Compoglass, Hytac) cured with either halogen or LED units on human pulp fibroblasts.

Materials and methods

Materials

Three different compomer materials were used in this study: (i) Dyract AP (Caulk, Dentsply, Milford, DE, USA); (ii) Compoglass (Vivadent, Schaan, Liechtenstein); and (iii) Hytac (ESPE, Dental Medizin GmbH & Co., Seefeld, Germany) (Table 1). The same shade (A2) was used for each compomer. Compomer specimens were polymerized using either a halogen (Optilux 401, Demetron, Kerr, Danbury, CT, USA; light intensity of 600 mW/cm^2) or LED (FreeLight Elipar 1, 3M ESPE, Seefeld, Germany; light intensity of 400 mW/cm^2) LCU.

Specimen fabrication

Compomer specimens were prepared in Teflon moulds with a diameter of 5 mm and a depth of 2 mm under aseptic conditions. Moulds were filled with the compomer material, covered with polyester film, and pressed with a glass plate. Excess flash was trimmed away with a sterile scalpel. A total of 72 specimens were prepared and distributed among six groups by compomer and LCU, as follows: group 1: Compoglass–halogen LCU (CH); group 2: Compoglass–LED LCU (CL); group 3: Dyract–halogen LCU (DH); group 4: Dyract–LED LCU (DL); group 5: Hytac–halogen LCU (HH); group 6: Hytac–LED LCU (HL). All specimens were light cured for 40 s with the light tip approximately 1 mm away from the specimen.

Cell culture

To evaluate cytotoxicity, human primary pulp fibroblast cultures were used. The volunteers were acquainted with the purpose of the study, and they gave an informed consent for participation in the study. Human dental pulp fibroblasts were cultured using an explant technique, as described previously by Chang *et al.*[14], whereby impacted human mandibular third molars were extracted and sectioned horizontally below the cementoenamel junction with a Number 330 bur in a high-speed water spray. Pulp tissue was removed aseptically, rinsed with Dulbecco's modified eagle's medium (DMEM) (Sigma, St Louis, MO, USA), placed in a 35 mm Petri dish (Greiner Bio-one, Frickenhausen, Germany), minced into small fragments with a number 15 blade, and grown in DMEM supplemented with 10% fetal bovine serum (Biochrom, Rehovet, Israel) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL fungizone). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Confluent fibroblast cells were detached with 0.25% trypsin and 0.05% ethylenediamine tetra-acetic acid for 5 min, and aliquots of separated fibroblast cells were subcultured. Fibroblast cell cultures between the third and eighth passages were used in this study.

Table 1. Manufacturer and monomer contents of materials.

Product	Manufacturer	Monomer Contents
Compoglass	Vivadent, Schaan, Liechtenstein	UDMA (%5–10) (70 mg/g), Bis-GMA (%1–5) (30 mg/g), TEGDMA (40 mg/g), HEMA, CADCADM (60 mg/g)
Dyract AP	Caulk, Dentsply, Milford, DE, USA	UDMA, TEGDMA (%25), PENTA,
Hytac	ESPE, Dental Medizin GmbH & Co. Seefeld, Germany	UDMA (%60–100)

Table 2. Decolorization index (ISO 10993-5).

0	1	2	3	4	5
No decolorization detectable	Decolorization only under the specimen	Zone not greater than 5 mm from the specimen	Zone not greater than 10 mm from the specimen.	Zone greater than 10 mm from the specimen	Total culture is decolorized

Table 3. Cell lysis index (ISO 10993-5).

0	1	2	3	4	5
No cell lysis detectable	Less than 20% cell lysis	20% to 40% cell lysis	> 40% to < 60% cell lysis	60% to 80% cell lysis	More than 80% cell lysis

Agar overlay method

A suspension of human pulp fibroblast cells was prepared at a concentration of 2.5×10^5 cells/mL and seeded in 60 mm tissue culture dishes (5 mL per dish) incubated at 37 °C in an atmosphere of 95% air and 5% CO_2 for 24 h. After 24 h, the culture medium was replaced with 5 mL of a freshly prepared agar/nutrient medium containing DMEM, 10% fetal calf serum, and 2% agarose (FMC BioProducts, Vallensbaek Strand, Denmark). Following solidification of this mixture, test specimens were placed on the agar surface, along with a PVC disk as a positive control and typical soda lime glass as a negative control. Specimens were incubated for 24, 48, 72, or 96 h, with three replicates per time period per group. Following incubation, the specimens and agarose mixtures were removed, and the fibroblast cells were fixed with 5 mL of 10% formaline for 30 min. Following removal of formaline, 2 mL crystal violet (Merck, Darmstadt, Germany) was added to each dish, which was kept at room temperature for 30 min. Excess dye was washed, and specimens were allowed to dry at room temperature. Cultures were examined under a microscope (Eclipse 150, Nikon, Tokyo, Japan) by an experienced, blind examiner (Fig. 1).

Decolorization zone, cell lysis, and response indices are given in Tables 2–4, respectively. Decolorized zones and cell lysis around and/or under the specimens were evaluated according to ISO 10993-5[15]. Decolorized zones were measured with a caliper (Mitutoyo, Kanagawa, Japan). Cell lysis, defined as loss of cell membrane integrity, was visually evaluated under light microscopy. Response indices were obtained by averaging the decolorization zone and lysis indices of the three replicates, and calculating decolorization index/lysis index.

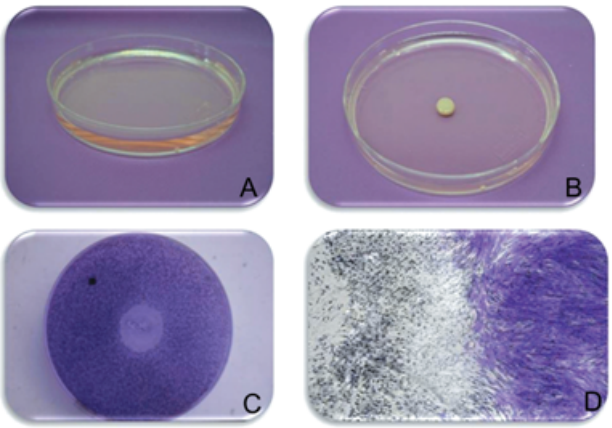

Fig. 1. Agar overlay method. (A) Seeded human pulp fibroblast cells. (B) Test specimen placement on agar. (C) Detection of decolorization zone after staining with crystal violet. (D) Microscopic evaluation of cell lysis (×100).

Table 4. Cell response = Decolorization index/lysis index (ISO 10993-5).

Scale	Cell response	Interpretation
0	0/0	Non-cytotoxic
1	1/1	Mildly cytotoxic
2	2/2 to 3/3	Moderately cytotoxic
3	4/4 to 5/5	Severely cytotoxic

Statistical methods

Two-way analysis of variance was used to analyse decolorized zone measurements, and Duncan's

Table 5. Mean decolorized zones and statistically differences of test groups ($P < 0.05$).

Test group	24 h	48 h	72 h	96 h
1 (CH)	$0.05^{a\ C}$	$0.38^{b\ C}$	$3.26^{b\ B}$	$5.23^{b\ A}$
2 (CL)	$0.22^{a\ B}$	$1.00^{a\ B}$	$5.53^{a\ A}$	$6.62^{a\ A}$
3 (DH)	$0.04^{a\ B}$	$1.18^{a\ B}$	$2.91^{b\ A}$	$4.02^{b\ A}$
4 (DL)	$0.04^{a\ B}$	$0.88^{a\ B}$	$4.22^{a\ A}$	$4.89^{a\ A}$
5 (HH)	$0.71^{b\ C}$	$1.43^{b\ C}$	$5.19^{b\ B}$	$8.84^{a\ A}$
6 (HL)	$1.93^{a\ B}$	$2.63^{a\ B}$	$8.82^{a\ A}$	$9.29^{a\ A}$

(a–c) Horizontal comparisons.
(A–C) Vertical comparisons.

test was used to determine differences in measurements by compomer and by LCU, with a value of $P < 0.05$ considered to be statistically significant.

Results

Results showed that at the end of the trial, all test materials were moderately cytotoxic to primary human pulp fibroblasts according to ISO 10993-5[15]. The level of cytotoxicity varied according to compomer material, incubation period, and LCU type.

Table 5 shows the mean decolorized zone measurements for each of the test groups. Measurements increased from 24 h to 96 h, with measurements for 72 h and 96 h significantly higher than those for 24 h and 48 h ($P < 0.05$). Hytac had a significantly larger decolorization zone than Dyract AP and Compoglass for all time periods ($P < 0.05$). At the end of the trial, the decolorization zones of the LED-cured Dyract AP and Compoglass specimens were significantly larger than those of the halogen-cured Dyract AP and Compoglass specimens ($P < 0.05$).

Discussion

This study was designed to fill the gap in research into the effects of LED polymerization on the cytotoxic properties of compomers. Compomers cured with an LED LCU were found to be more cytotoxic than compomers cured with a halogen LCU.

Polymerization of resin-based material is affected by factors related to the material itself (chemical composition, shade, translucency, etc.)[16], and to the radiation source used for polymerization (spectral distribution, intensity, exposure time, position of the light-cure tip, etc.)[17]. In order to eliminate differences related to these factors, this study used compomer specimens of the same shade and depth; the light-cure tip was set at a fixed position of 1 mm, and exposure time was standardized at 40 s for halogen polymerization and 40 s for LED polymerization.

Cytotoxicity testing has become an accepted means of screening dental materials for biocompatibility. Not only does cytotoxicity testing of restorative material reduce the need for animal or human testing, an understanding of how a restorative material's components interact with cell material at the molecular level can help provide an understanding of their interaction *in vivo*[18]. Moreover, *in vitro* methods are simple, reproducible, cost effective, and suitable for use in evaluating the basic biological properties of dental materials[19].

This study used the agar overlay test method in accordance with ISO 10993-5 to examine the effects of different curing techniques on three different compomers. Because dentine acts as a barrier between the compomer material and pulp tissue, it is rare for compomers to come into direct contact with pulp under clinical conditions. In order to reflect the clinical experience, materials such as agar[20], Millipore[20], and dentin sections[21], have been used as barriers. Although dentine sections mimic clinical conditions much better than other materials, dentin barrier tests are expensive and difficult, particularly in terms of obtaining homogenous, standardized dentin sections.

Many cell culture techniques have been applied to assess the cytotoxicity of dental materials, including methods based on cell cultures with established or diploid cell lines, as well as tissue-explant techniques[19]. However, an increasing number of authors have stated that *in vitro* toxicity tests should be performed using the most appropriate cells[22,23]. For this reason, human primary pulp fibroblasts were used in this study.

Aqueous eluates of compomers have previously been shown to induce moderate injury in cultured cells[24]. Monomers such as bisphenol-

A-glycidil methacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), and urethane dimethacrylate (UDMA) have been found to leach from compomers and possibly cause adverse effects[25]. Compomers may also release fluoride ions from glass fillers, especially during the first few days following polymerization[26]. The various substances leached from compomers may be responsible for the cytotoxic effects exhibited by these materials.

One study on resin components found that their cytotoxicity was related to their lipophilicity[27]. Another study found the cytotoxicity of hydrophobic monomers such as Bis-GMA and UDMA to be greater than that of hydrophilic monomers such as 2-hydroxyethyl methacrylate and TEGDMA[28]. This latter finding could explain why our study found Hytac, whose main monomer is UDMA, to be the most cytotoxic of the three compomers tested, regardless of whether a halogen or LED LCU was used for polymerization.

The polymerization of resin-based materials requires sufficient intensity of light and suitable wavelength to activate a light-sensitive material[29]. Inadequately cured resin-based materials can have a cytotoxic effect on pulp tissue by releasing unreacted monomers[30]. It is well documented that energy density (light intensity × exposure duration) of the LCU influences the degree of cure and depth of cure. The higher the energy density, the higher the degree of conversion that was achieved[31]. Although higher-energy density was associated with higher temperature rise[32]; within the limitation of this *in vitro* study, higher-energy density of halogen LCU may lead to lower degree of cytotoxicity.

In comparison to traditional halogen LCUs, the blue LED LCUs used for polymerization emit a narrower spectrum of light, with a peak of around 470 nm. This value matches the absorption peak value of camphorquinone, the photoabsorbing compound most commonly found in the photoinitiator systems of resin-based material[33]. However, in cases where camphorquinone is not the main photoinitiating component, the narrow wavelength spectrum of LED represents a disadvantage, as it cannot efficiently activate photoinitiators other than camphorquinone[34]. The fact that compomers contain both camphorquinone photoinitiators and other co-initiators[25] may be another reason of the higher cytotoxicity found in compomer specimens cured with the LED LCU compared to those cured with the halogen LCU in this study. Similarly, Yap *et al.*[13] found LED-cured composite to be more cytotoxic than composite cured with conventional halogen light

Conclusion

The findings of this study indicate that compomers are potentially toxic to human pulp fibroblasts and that the type of curing unit used in polymerization may affect compomer toxicity. Within the limitations of this study, it may be stated that LED LCUs may not be appropriate for use in the polymerization of compomers. Dental clinicians must be aware of which LCU system meets their demands for optimum polymerization.

What this paper adds

- This study demonstrated that all tested compomer materials were moderately cytotoxic to primary human pulp fibroblasts according to ISO 10993-5.
- The level of cytotoxicity varied according to compomer material, incubation period, and LCU type.

Why this paper is important to paediatric dentists

- Paediatric dentists must be aware of which LCU system meets their demands for optimum polymerization.

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