Investigation of Cytotoxicity of Dental Light-Curing Composite Materials

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Abstract

Light-curing composite restorative materials are widely used by dentists of various specialties in the esthetic restoration because of their optimal mechanical properties and predictable desired aesthetics. However, their use requires special attention to the safety of the components used. In recent decades, polymer composites have been developed with the objective of reducing cytotoxicity and shrinkage during polymerization and improving aesthetics. The biocompatibility of all polymeric materials is an issue that requires special focus on the chemical composition of biopolymers, which consist of an organic resin matrix and ceramic reinforcing filler particles bonded with a silane. The special priority in biocompatibility studies has focused mainly on the organic resin polymer matrix. And among the components of composite resins, the polymer matrix is the only unstable one, mainly because of the unbound monomers that can be released and remain as free monomers during the polymerization step.

The aim of this study was to evaluate the effectiveness of preheating composite restorative materials on composite conversion and their cytotoxic properties.

The use of composite restorative materials preheating in dental practice allows dentists to reduce the cytotoxicity of composite materials and improve their degree of conversion, since there is a direct relationship between increasing the conversion of the material and reducing its cytotoxicity.

Preheating has a positive effect on composite, resulting in reducing their cytotoxic properties and optimizing the biocompatibility of the polymer matrix with hard tissues. The conversion of the material is improved, since there is a direct correlation between the increase in the conversion of the material and the reduction of its cytotoxicity.

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Introduction

Treating hard tissue pathology is a prevalent and widespread procedure in operative dentistry. To restore lost tissue volume, direct and indirect fillings are the most commonly used methods. Currently, light-curing composite dental materials are being increasingly used by practicing dentists in adhesive restorative techniques.[1] These modern composite materials yield high clinical and aesthetic results immediately after application.[2,3]

Dual-curing composites comprise initiators and accelerators that permit light-induced activation, leading to chemical curing. During the polymerization process, a polymer network forms via a matrix of double carbon bonds (C=C) connected with single covalent bonds (C-C). The extent of monomer-to-polymer transformation is crucial for the filling's strength, as it correlates to the material's physical and mechanical features (microhardness). The organic matrix, inorganic filler, silane, and photoinitiator mixture is a common composition

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for this type of material. Within this mixture, bisphenol A-glycidyl methacrylate (Bis-GMA) monomer is often utilized due to its large molecular size and rigid structure, resulting in high viscosity and less polymerization shrinkage.

To improve the degree of conversion, low-viscosity monomers such as triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) are incorporated into the organic matrix, since bisphenol A glycidyl methacrylate has a high viscosity that can limit the achievement of an optimal conversion rate during polymerization. The conversion of the composite is one of the most important factors determining the physical and mechanical properties of the material and the durability of the future restoration. In the polymerization process, monomer particles are integrated into a single chain. In an "ideal material," all monomers should be transformed into a polymer.[4-6] The presence of unreacted monomer may decrease the mechanical strength and dimensional stability of restorations. Furthermore, these monomers, upon release into saliva, could promote allergic reactions, bacterial growth around restorations, or lead to caries complications. Additionally, they could have a presumed cytotoxic effect.[5,7,8]

Studies have indicated that uncured monomers could result in leaching, which may increase adhesion and the growth of cariogenic particles.[9-11] Consequently, this factor could contribute to resin degradation, leading to the appearance of microleakage and penetration of microbial flora, further increasing the risk of recurrent caries.[12-17]

Materials and methods

- Preparation and grouping of samples

The present study included only light-cured composites used in operative and prosthetic dentistry as restorative materials and cements for the luting of fixed structures. The selection criteria for the materials considered include their filler particle size and the composite manufacturer. Table 1 presents 30 specimens with a thickness of 1mm and diameter of 2.5mm, as prepared for the study.

The prepared specimens were categorized into three groups.

Group 1 (A) included samples made of a light-cured microhybrid composite Esthet X (Dentsply Sirona, USA);

Group 2 (B) included samples made of a light-cured microfilled composite material Enamel Plus HRi (Micerium, Italy).

Group 3 (C) included samples made of a light-curing microhybrid composite Unirest (Stomadent, Russia).

Composite material	Manufacturer	Composition of the monomer matrix	Filler	Material group
Esthet X HD	Dentsply Sirona (USA)	Bis-GMA, Bis-EMA accession product, triethylene glycol dimethacrylate, camphoroquinone (CQ), photoinitiator, stabilizer, pigments	Barium fluoroborosilicate crystals with an average particle size of less than 1 μm and silicon nanofiller (particle size 0.04 μm)	Light-cured microfilled composite restorative material
Enamel plus HRi	Micerium (Italy)	Diurethandimethacrylate, Iso-propylidene-bis (2(3)-hydroxy-3(2)-4(phenoxy) propyl)-bis(methacrylate)(Bis-GMA); 1,4 - Butanedioldimethacrylate	75% by weight (53% by volume). Glass filler: average particle size 0.7 μm; Highly dispersed silica: average particle size 0.04 μm	Light-cured microhybrid composite filling material
Unirest	Stomadent (Rissia)	Urethane methacrylate, bisphenol A glycidyl methacrylate (Bis GMA), triethylene glycolldimethacrylate (TGM), butylated hydroxytoluene, camphoroquinone, trimethacrylate triethanolamine (TMATEA), fluorescent pigment, barium aluminoborosilicate glass PM-3, glass filler GM32087, glacial acetic acid, aluminum oxide, silane A-174, iron oxide pigments: Red 7067, Yellow 7055, Hema Phoshate, acetone	Hybrid with an average filler particle size of 0.7 microns, glass filler, organic matrix filling degree of 78±1%.	Light-cured microhybrid composite filling material

Table 1. Characteristics of the investigated light-cured composite restorative materials.

It is noteworthy that each group was divided into two subgroups based on the composite's pre-polymerization preparation.

The specimens in the first subgroup (1A, 1B, 1C) consisted of composites polymerized without thermal pre-polymerization (NH-non-heated).

In the second subgroup (H-heated) (2A, 2B, 2C), the specimens were prepared after pre-polymerization heating of the composite in a special furnace (Micerium; Avegno, Italy) with two temperature modes (first mode - heating to 39C, second mode - heating to 55C). The furnace provided two temperature regimes, with the second mode being heating up to 55°C, which was used in our study (55C).

Polymerization of the material samples was performed using an LED lamp with a wavelength of 430-480 nm and a light intensity of 900 mV/cm2 (Premium Plus, China).

Sterilization of the samples was conducted through exposure to UV irradiation for 24 hours. Subsequent work did not detect any contamination by fungi, bacteria, or other pathogens.

- Cell culture preparation

To assess the cytotoxic effects of the samples, primary cell culture of stromal cells isolated from the human oral floor mucosa during biopsy was used.

DMEM/F12 growth medium (Paneco, Russia) was utilized in the experiment, supplemented with fetal calf serum (Capricorn Scientific, Germany) up to 10% and penicillin-streptomycin (Paneco, Russia) up to 1%. The cells were detached from the culture substrate using a trypsin-EDTA solution (Paneco, Russia) and cell quantity and viability were estimated using a Luna-dual automated counter (Logos Biosystem, USA). The sample was inoculated into the wells of a 48-well culture plate (1 sample in 1 well). The cell suspension was plated at a density of 30 thousand live cells per well. After 48 hours, in vitro evaluation of cytotoxic properties of the tested samples was performed.

- Assessment of cell viability by MTT-test method in vitro.

MTT (Sigma-Aldrich, USA) was added to the wells containing seeded samples in growth medium, reaching a final concentration of 1.5 mg/mL. The samples were left under standard culture conditions for 4 hours. During this incubation period, succinate dehydrogenase reduced light yellow MTT tetrazolium (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) in mitochondria of living cells to form insoluble dark-colored formazan. Formazan crystals were visually observed under light microscopy to monitor the reaction development. The MTT medium was removed, and 100 µl of solvent (DMSO, PanEco) was added to the wells for dissolving precipitated formazan crystals. The solution was then incubated for an hour at 37°C.
After incubation of cells with the material, further visual assessment of cell viability was performed following a direct cell contact test using a ZOE™ fluorescence microscope (Bio-Rad, USA).
OD540 optical density was measured using the CLarioStar device (BMG Labtech, USA).

- Statistical data processing

Statistical analysis was conducted with ANOVA multiple comparison in GraphPad Prism 8.
The Kruskal-Wallis test was utilized to assess normality of samples, followed by Tukey's test as a post-hoc analysis. The findings are displayed in Table 2 and Figure 1.

Results

For materials in the second subgroup (pre-polymerization thermal preparation), high positive results were obtained after assessment of stromal cell viability using the MTT test method in a well culture plate. Following cell incubation with the composite material, a fluorescence microscope ZOE™ (Bio-Rad, USA) was used for further visual assessment of direct cell contact (Figures 2 and 3).

According to the manufacturer's specifications, fluorescence microscopy enables detailed examination of dynamic processes at the molecular and cellular levels. It facilitates not only assessment of cellular structures' biological integrity but also the analysis of cells that have made contact with macro-objects, regardless of the samples' optical density. This technique produces highly precise data and images of the studied samples.

The OD540 optical densities for the composite samples after MTT test are shown in Table 2 and Figure 1. Table 3 shows the visualization of the tablet wells for the different composite groups included in the present study.

	Tested composite samples groups					
Control	1 A	1B	1C	2A	2B	2C
0,299	0,28	0,283	0,222	0,29	0,231	0,303
0,32	0,277	0,265	0,207	0,335	0,298	0,37
0,276	0,215	0,294	0,236	0,285	0,261	0,294

Table 2. Optical densities of OD540 for samples, after MTT test.

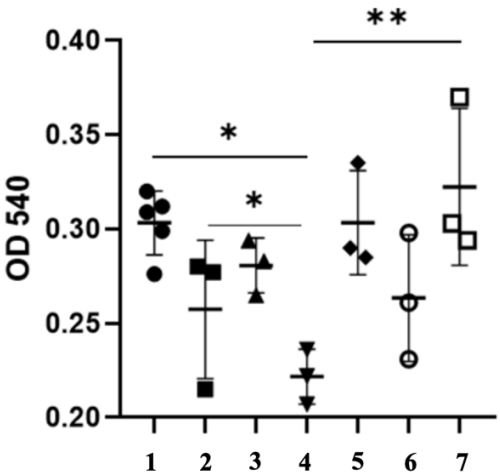

Figure 1. Sample arrangement on the plate and visual interpretation of the MTT test, bottom row - control wells. 1 – Control, 2 – Non-heated (NH) Esthet X HD, 3 - Non-heated (NH) Enamel plus

HRi, 4 – Non-heated (NH) Unirest, 5 – Heated (H) Esthet X HD, 6 - Heated (H) Enamel plus HRi, 7 - Heated (H) Unirest.

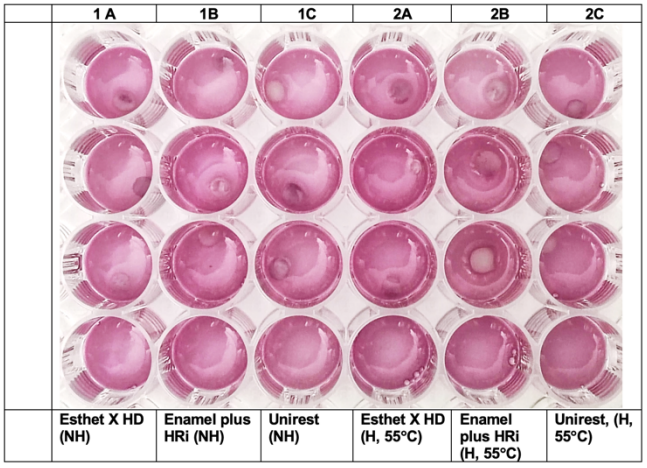

Table 3. Visualization of the tablet wells for the different composite groups.

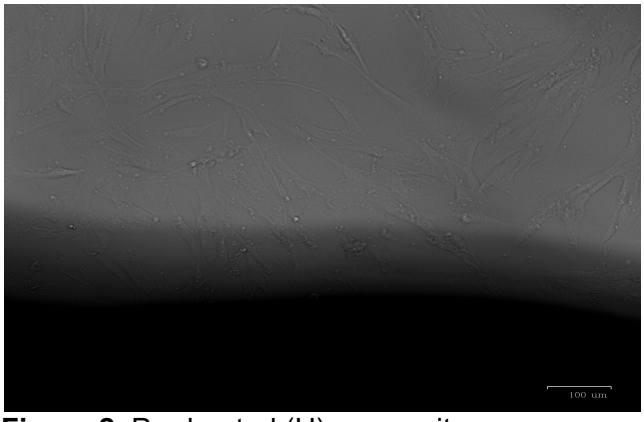

Figure 2. Pre-heated (H) composite.

The phase-contrast microscopy image shows active and abundant cell culture repopulation of a light-cured composite material that has undergone pre-polymerization heating.

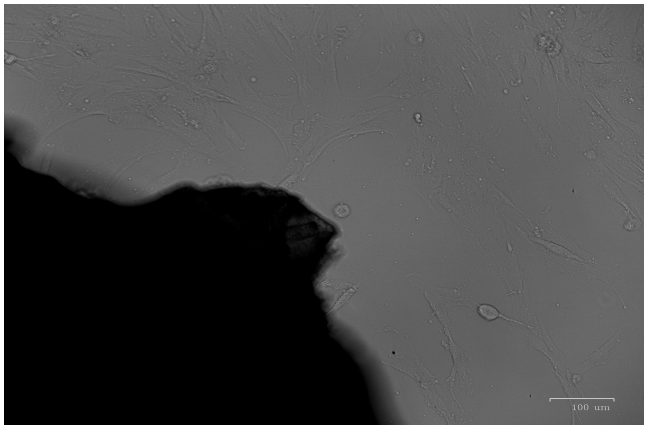

Figure 3. Non-heated (NH) composite.

The phase-contrast microscopy image shows low cell culture occupancy of the light composite material without prior pre-polymerization heat treatment.

Discussion

Based on the findings of the preheating effect on the cytotoxicity of composite materials in the MTT test, it can be confidently stated that this method of processing the light-curing dental material improves the biological properties of the filling material and optimizes its biocompatibility with dental tissues. In contrast, samples that were not preheated (1A subgroup) exhibited higher levels of cytotoxicity, which were statistically insignificant compared to the control group (15%, $p<0.05$). Samples from the 1C subgroup, which were not preheated, displayed higher cytotoxicity compared to the control group (27%, $p<0.05$). However, the difference was statistically insignificant. These samples can be categorized as "1 - mild toxicity" according to the russian cytotoxicity scale of GOST standard ISO 10993-5-2009 "National Standard of the Russian Federation for the biological assessment of medical devices using in vitro methods. Technical terms used in this standard will be explained on their first usage".

Upon analyzing phase-contrast microscopy data under a fluorescent microscope, direct cellular contact with the material that underwent preliminary thermal pre-polymerization treatment was observed. The composite material had an active, dynamic and a high population of cells noted from the used cultures. A clear reduction of confluence was observed in the places of direct contact between stromal cells, which were isolated from the biopsy of the mucous membrane of the floor of the human mouth and the samples of composite filling material that had not undergone the preliminary thermal pre-polymerization treatment.

The findings of this study are reinforced by prior research,[18] which indicates that there is a clear correlation between the degree of conversion of the composite and its cytotoxicity. The preheating of the composite filling material achieves high conversion, resulting in improved physicochemical properties and reduced cytotoxicity.

Conclusions

Consequently, preheating of composite dental materials prior to polymerization has a positive effect on reducing the cytotoxic properties, thus optimizing the biocompatibility of the polymer material with dental tissues. Preheating composite in dental practice can decrease the cytotoxicity of the material and simultaneously increase its conversion rate. The conversion rate and cytotoxicity of the material are related to each other. Further investigation of the composites cytotoxicity depending on different methods of their polymerization conversion is an important and relevant issue that we will address in our next scientific studies.

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Declaration of Interest

The authors have no conflicts of interest.

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