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ORIGINAL ARTICLE

Iodonium salt incorporation in dental adhesives and its relation with degree of conversion, ultimate tensile strength, cell viability, and oxidative stress

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Abstract

Objective The aim of this study was to evaluate the degree of conversion, ultimate tensile strength, cell viability, and oxidative stress of two different ternary initiation systems, using two photoinitiation polymerization times.

Methods The groups investigated were camphorquinone (CQ); CQ and diphenyleneiodonium hexafluorophosphate (DPI); CQ and ethyl 4-dimethylamine benzoate (EDAB); and CQ, EDAB, and DPI, with EDAB in high and low concentration. To assess the degree of conversion (DC) and the ultimate tensile strength (UTS), a real-time Fourier transform infrared spectroscopy and a universal test machine Emic DL-500 were used, respectively. Cell viability and oxidative stress were evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), superoxide dismutase (SOD), total sulfhydryl (SH) content, and thiobarbituric acid reactive species (TBARS) formation assays.

Results Slight lower cell viability was shown when DPI was associated with high concentrations of EDAB; this reduction seemed to be attenuated when lower concentrations of EDAB were used. When EDAB and DPI were associated, no oxidative damage was shown. The degree of conversion was increased in the ternary systems (CQ + EDAB lower concentration + DPI) group, which did not affect the UTS, cytotoxicity, and oxidative stress parameters. The polymerization time did not affect cell viability, total SH, and TBARS; however, a slight increase was shown in SOD levels.

Clinical relevance Our study emphasizes the relevance of incorporating the third element—iodonium salt—in a binary adhesive systems composed exclusively of CQ and EDAB.

Keywords Diphenyleneiodonium · Ultimate tensile strength · Degree of conversion · Cell viability · Oxidative stress · Polymerization

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Introduction

Low-viscosity resins are commonly used in dentistry as bonding agents that connect resin dental materials to the dental substrate. The deficiency or loss of this union can influence directly in restoration longevity [1, 2]. However, despite the advances in the area of dental adhesive development, they have not yet reached the ideal condition [3]. In this context, several researchers have studied in order to circumvent aspects that affect restoration quality and longevity [2].

Thereby, the incorporation of different components to adhesive systems has been common. Tertiary amines, such as ethyl 4-dimethylamino benzoate (EDAB), have improved the quality of adhesive systems, since they are effective proton donor for photoinitiators, thus eliminating the chain effect caused by camphorquinone (CQ) excitability [4]. Furthermore, the presence of amines in the adhesive system may attenuate the inhibitory effect of O_2 [1]. Whereas, the presence of iodonium salt as co-initiator of adhesive system has been considered interesting, it requires further research. Derived iodonium salt should be analyzed with caution, since they can have specific behaviors. The diphenyliodonium chloride is a mutagen component, able to promote DNA double-strand breaks, apoptosis, and cell necrosis [5]. However, the diphenyleneiodonium hexafluorophosphate (DPIHFP) as co-initiator of adhesive system has been considered effective since even at low concentrations, it shows significant effect on the conversion of monomers [6].

It is important to be mindful to the mechanical and biological properties of materials incorporated to dental adhesives. The mechanical characterization of dental materials is important to understand the performance of these materials in the clinical practice. Through laboratory tests that investigate the degree of conversion of dental adhesives, the literature may suggest adaptations in the clinical practice, such as the increase in the polymerization time to reduce permeability [7]. In this context, 2-hydroxyethyl methacrylate (HEMA), a usual dental adhesive component, was investigated and did not seem to influence the bond strength to dentin, degree of conversion, and resistance to degradation in ethanol. However, a complete experimental design is necessary in order to evaluate the biological properties of such dental materials. It is well known that polymerization of resinous dental materials is incomplete in clinical conditions and several components can be leached from the polymerized material [8] and remain in contact with the oral mucosa. This can cause many adverse reactions such as irritation, epithelial proliferation, and lichenoid reaction [9]. These clinical implications are associated with HEMA [10], triethylene glycol dimethacrylate (TEGDMA) [8–10], and other components, commonly identified as dissolvable products of resin materials [10], which have been identified as cytotoxic by various methodologies. These components have also shown to induce genotoxicity, indicating modifications in basic cell structure, as well as alteration in the cell membrane integrity and cell function, such as enzymatic activity and macromolecule synthesis [11].

Oxidative stress has recurrently been shown to induce alteration in cell viability [12–14]. During normal cell function, cells produce reactive oxygen species (ROS) and nitrogen (RNS) especially in the mitochondria, cell membranes, and cytoplasm [15]. ROS and RNS are the most common forms of free radicals, which are atoms or compounds containing one or more unpaired electrons in their external orbits [16]. Although most ROS are generated through endogenous production [17], exogenous sources such as ultraviolet radiation [18] and chemical compounds may also be responsible for triggering the formation of free radicals [17]. In the mammal's organism, the antioxidant system is machinery composed mainly by two pathways, the enzymatic and non-enzymatic [15]. The enzymatic antioxidant system includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [19]. The non-enzymatic consists of some endogenous substances and dietary origin, such as vitamin C (ascorbic acid), vitamin E (alpha-tocopherol), glutathione (GSH), and some flavonoids and carotenoids [15, 19]. However, when imbalances occur, between the production of ROS or RNS and the removal of free radicals by the antioxidant system, the oxidative stress (OS) arises [20].

Recently, resin monomers have been identified as chemical agents able to break the stability of redox balance in cells, resulting in increased levels of ROS, leading to cell death throughout apoptosis [11]. Studies suggest that apoptosis and mutagenicity induced by resin monomers are mediated by oxidative stress [21]. Indeed, high levels of ROS are candidate agents for mediating genotoxicity, since the literature has repeatedly demonstrated the in vitro genotoxicity of TEGDMA and HEMA monomers [11, 21]. Additionally, CQ, one of the most used initiators in modern resin-based curing systems, was able to form ROS and induce cell death [11].

Thus, some aspects of iodonium salt could favor its incorporation in dental adhesives, such as ionic character, which allows water solubility, promoting the polymerization of polar monomers in dental adhesives [22]; ability to increase the rate of polymerization, thereby decreasing the amount of leached materials [23]; and compatibility with epoxy-based materials such as silorane [24]. We hypothesized that groups of photoinitiator ternary systems would present results with better performance when compared to binary system. Furthermore, we believe that the increased activation photo time maintains equal or improves the mechanical and biological properties, in all test groups. Thereby, the aim of this study was to evaluate the degree of conversion (DC), ultimate tensile strength (UTS), cell viability, and oxidative stress of two different ternary initiation systems, using two photoinitiation polymerization times (10 and 20 s).

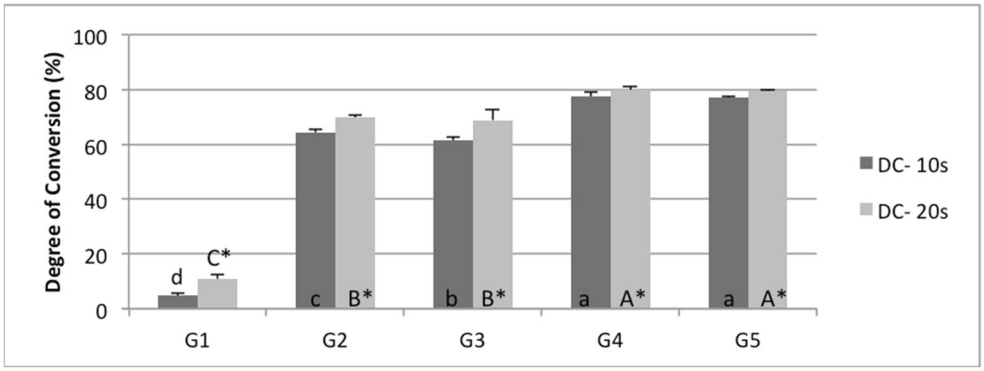

Fig. 1 Degree of conversion (DC) after different polymerization times (10 and 20 s). Lowercase letters indicate differences between groups after 10 s of photoactivation. Uppercase letters indicate differences between groups after 20 s of photoactivation. Data are expressed as the means ± SEM. Asterisks indicate differences between the photoactivation times

Methodology

Cell culture

The mouse fibroblast cells (NIH/3T3) were obtained from the Rio de Janeiro Cell Bank (PABCAM, Federal University of Rio de Janeiro, RJ, Brazil). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), purchased from Gibco (Grand Island, NY, USA). Cells were grown at 37 °C in an atmosphere of 95% humidified air and 5% CO_2. The experiments were performed with cells in the logarithmic phase of growth.

Formulation

A model dental adhesive resin was formulated by mixing 50 wt% bisphenol A glycidyl dimethacrylate (Bis-GMA), 25 wt% triethylene glycol dimethacrylate (TEGDMA), and 25 wt% 2-hydroxyethyl methacrylate (HEMA) which were supplied by Esstech, Inc. (Essington, PA, USA). Photoinitiators and coinitiators, camphorquinone (CQ), diphenyleneiodonium hexafluorophosphate (DPI), and ethyl 4-dimethylamino benzoate (EDAB) are used without further purification. Five initiation systems were investigated: 1 mol% CQ (G1), 1 mol% CQ + 1 mol% DPI (G2), 1 mol% CQ + 1 mol% EDAB (G3), 1 mol% CQ + 2 mol% EDAB + 1 mol% DPI (G4), and 1 mol% CQ + 1 mol% EDAB + 1 mol% DPI (G5).

Degree of C=C conversion

The degree of conversion (DC) of the materials was evaluated in quintuplicate using real-time Fourier transform infrared (RT-FTIR) spectroscopy with a Shimadzu Prestige-21 spectrometer equipped with an diamond attenuated total reflectance device (PIKE Technologies, Madison, WI). The IR Solution software (SHIMADZU, Columbia, MD) was employed in the monitoring scan mode, using the Happ-

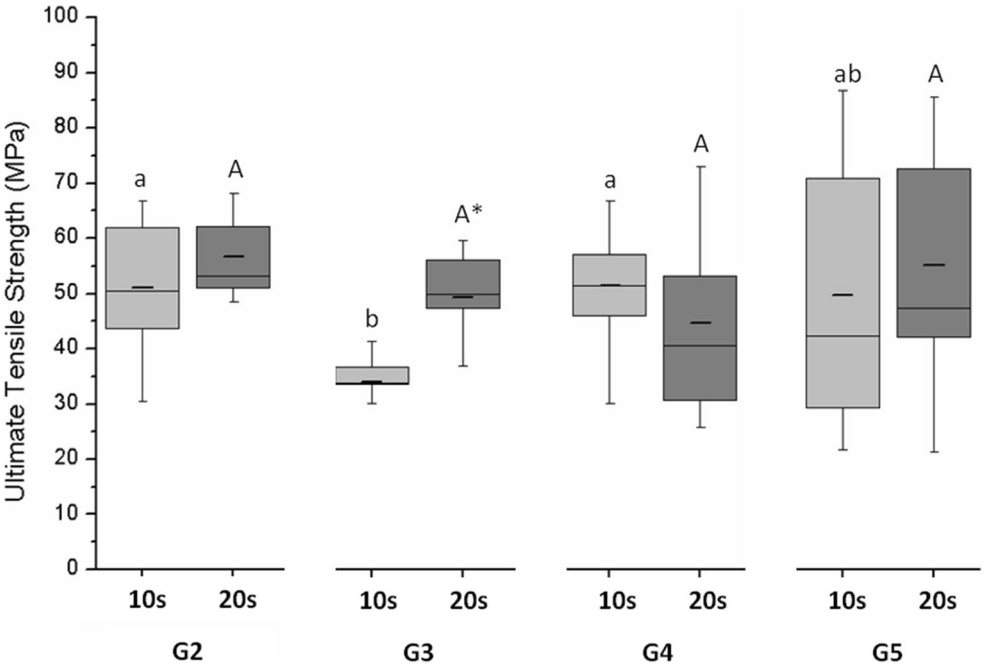

Fig. 2 Ultimate tensile strength (MPa) after different polymerization times (10 and 20 s). Lowercase letters indicate differences between groups after 10 s of photoactivation. Uppercase letters indicate differences between groups after 20 s of photoactivation. Asterisk indicates differences between the photoactivation times. The ultimate tensile strength of G1 could not be measured because it was not possible to obtain specimens. Data are expressed as median ± SD ($n = 10$)

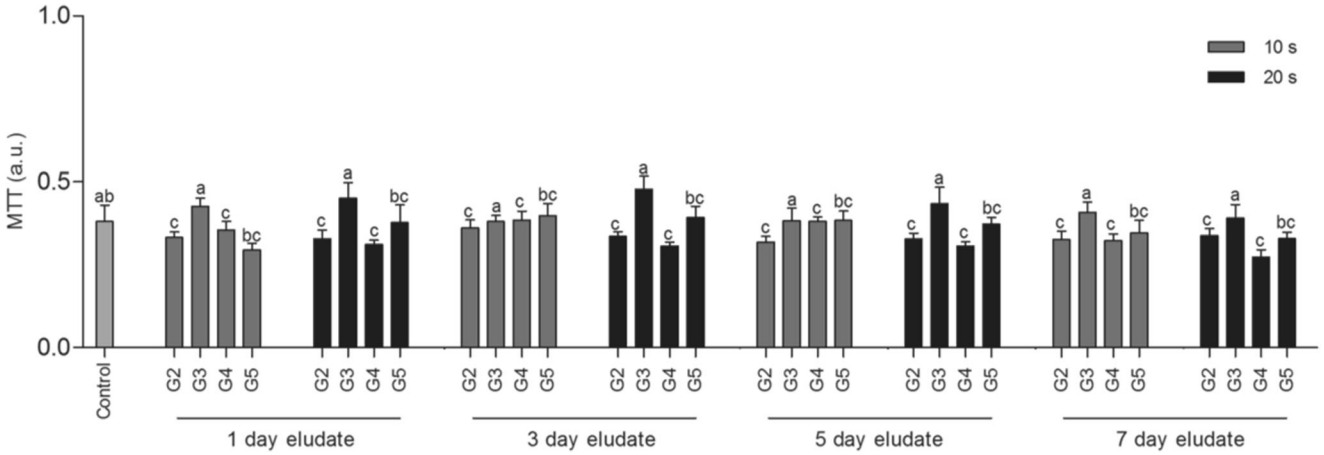

Fig. 3 Cell viability after 24 h of exposure of different initiation systems (G2, G3, G4, and G5), eluate incubation times (1, 3, 5, and 7 days), and polymerization times (10 and 20 s). Lowercase letters indicate significant differences among the four initiation systems groups ($P = 0.000$). There were no significant differences in the eluate incubation and polymerization time ($P = 0.258$ and $P = 0.812$, respectively). Data are expressed as the means ± SEM ($n = 12$)

Genzel apodization, at a range of 1750–1550 cm^{-1}, 12 scan co-addition, resolution of 4 cm^{-1}, and mirror speed of 2.8 mm/s at a controlled room temperature of 23 °C (± 2 °C) and 60% (± 5%) relative humidity. After the monomer reading, the material was immediately photoactivated for 10 s and the reading was repeated. The same occurred after 20 s of photoactivation time. The photoactivation was realized with a halogen lamp curing light (XL 3000, 3M ESPE, St. Paul, MN, USA). In order to confirm the 800 mW/cm^2 irradiation, a potentiometer (Ophir Optronics, Danvers, MA, USA) was used. A support was coupled to the spectrometer to hold the curing unit and standardize a 0.5-mm distance between the fiber tip and material. The DC was calculated considering the intensity of carbon-carbon double bond stretching vibrations (peak height) at 1635 cm^{-1} and using the symmetric ring stretching at 1610 cm^{-1} from the polymerized and non-polymerized samples as an internal standard [5]. The degree of conversion was carried out in five replications.

Ultimate tensile strength

The dental adhesive resins were dropped into a silicon matrix, covered with an acetate strip and a glass slide before light activation. All specimens were light-activated for 10 s on which surface; the same was repeated with 20 s of photoactivation. After polymerization, specimens were measured with a digital caliper and stored at 23 °C for 24 h. The photoactivation was realized with a halogen lamp curing light (XL 3000, 3M ESPE, St. Paul, MN, USA). In order to confirm the 800 mW/cm^2 irradiation, a potentiometer (Ophir Optronics, Danvers, MA, USA) was used. Hourglass-shaped specimens ($n = 10$) with a cross-sectional area of 1 mm^2 at the constriction were fixed in a specific device with cyanoacrylate adhesive and loaded under tension. Tests were performed in a universal test machine Emic DL-500 (Emic; São José dos Pinhais, PR, Brazil) at across head speed of 0.5 mm/min. Ultimate tensile

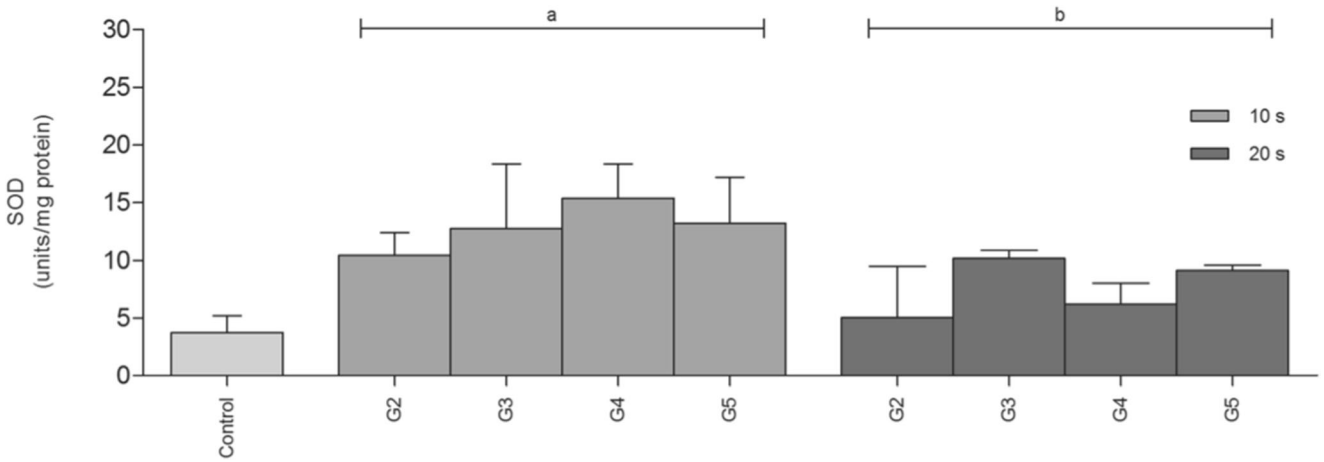

Fig. 4 SOD levels in NIH/3T3, after 24 h of exposure of different initiation systems (G2, G3, G4, and G5) and polymerization times (10 and 20 s). Lowercase letters indicate differences between polymerization times ($P = 0.046$). Data are expressed as the means ± SEM ($n = 6$)

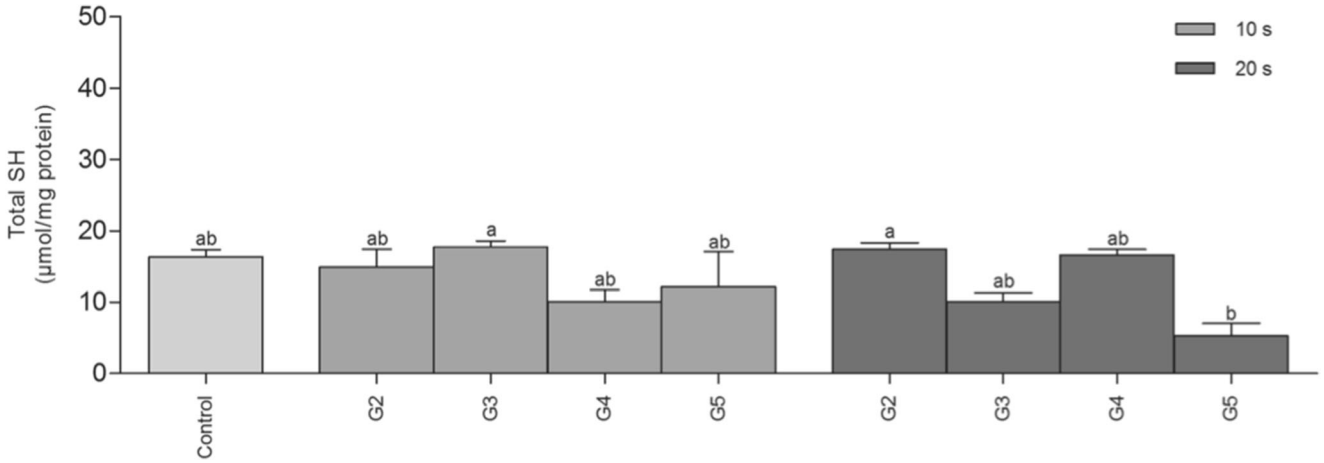

Fig. 5 Total SH levels in NIH/3T3 after 24 h of exposure of different initiation systems (G2, G3, G4, and G5) and polymerization times (10 and 20 s). Lowercase letters indicate differences between polymerization times and initiation systems groups ($P = 0.026$). Data are expressed as the means ± SEM ($n = 6$)

strengths were calculated in MPa. Camphorquinone (G1) was not able to provide specimens [31]. The ultimate tensile strength test was conducted once with $n = 10$ per group.

Preparation of specimens for the cytotoxicity and oxidative stress assay

All specimens were manufacture in triplicate, in a total of 96 specimens, 24 specimens per group (G2, G3, G4, and G5). Each specimen was manufacture in a silicone matrix with 5-mm diameter and 2-mm thick. Dental adhesive resins were dispensed in the matrix and photoactivated for 10 and 20 s with a halogen lamp curing light (XL 3000, 3M ESPE, St. Paul, MN USA), with a 5-mm distance from the matrix. In order to confirm the 800 mW/cm^2 irradiation, a potentiometer (Ophir Optronics, Danvers, MA, USA) was used. Each specimen was then incubated with 1 mL of DMEM, according to ISO 10993-5, in an atmosphere of 95% humidified air and 5% CO_2, for 1, 3, 5, and 7 days. After these periods specimens were removed and the eluate formed by the triplicates was combined and filtered (0.22 µm, Millex, Millipore, São Paulo, Brazil). The eluates obtained were then used in experiments in order to determine cell viability and biochemical assays.

Cell viability assay

The viability of the NIH/3T3 cells was determined by measuring the reduction of soluble MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to water insoluble formazan [25]. Briefly, cells were seeded at a density of 2×10^4 cell per well in a volume of 100 µL in 96-well plates and grown at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 24 h before being used in the MTT assay. Cells were incubated with eluates for 24 h. After incubation, 20 µL MTT (5 mg MTT/mL solution) was added to each well. The plates were incubated for an additional 4 h and the medium was discarded. Two hundred microliters of dimethyl sulfoxide

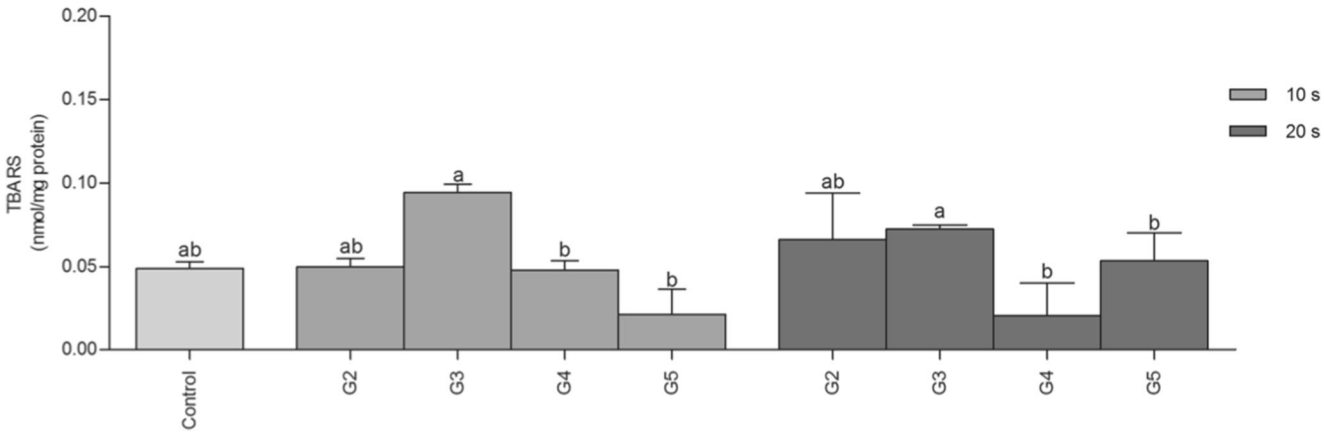

Fig. 6 TBARS levels, in NIH/3T3, after 24 h of exposure of different initiation systems (G2, G3, G4, and G5) and polymerization times (10 and 20 s). Lowercase letters indicate differences between initiation systems groups ($P = 0.027$). Data are expressed as the means ± SEM ($n = 6$)

(DMSO) was added to each well, and the formazan was solubilized on a shaker for 5 min at 150 rpm. The absorbance of each well was evaluated in a microplate reader (MR-96A, Mindray Shenzhen, China) at a wavelength of 450 nm. All observations were validated by at least three independent experiments, and for each experiment, the analyses were performed in quadruplicate.

Biochemical assays

Briefly, cells were seeded at a density of 3×10^6 cell per well in a volume of 2000 μL in 6-well plates and grown at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 24 h before the oxidative stress assay. The culture medium was removed and cells were incubated with 2000 μL of eluates for 24 h. After incubation, the medium was removed and 500 μL of PBS was added; the cell monolayer was then gently detached and stored at − 20 °C. Protein content was measured using bicinchoninic acid (BCA) assay using bovine serum albumin as standard. Biochemical assays were performed by two independent experiments.

Superoxide dismutase assay

Total superoxide dismutase (SOD) activity was measured by the method described by Misra and Fridovich (1972) [26]. This method is based on the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer adjusted at 480 nm. The specific activity of SOD was expressed as UI SOD/mg protein.

Total sulfhydryl content assay

This assay measures protein and non-protein thiols and was performed as described by Aksenov and Markesbery (2001) [27]. This method is based on the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by thiols, which becomes oxidized (disulfide) generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm. Briefly, homogenates were added to PBS buffer pH 7.4 containing EDTA. The reaction was started by the addition of DTNB. Results were reported as micromole TNB/mg protein.

Thiobarbituric acid reactive species formation assay

Lipid peroxidation was determined by thiobarbituric acid reactive species (TBARS) production, performed by malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), according to the protocol described by Esterbauer and Cheeseman (1990) [28]. Briefly, homogenates were mixed with 10% trichloroacetic acid and 0.67% thiobarbituric acid and incubated for 25 min at 95 °C. TBARS was determined in a spectrophotometer at 535 nm. Results were reported as nanomole TBARS/mg protein.

Gas chromatograph

The samples were analyzed on a Shimadzu 2010 Gas Chromatograph equipped with a Rtx-Wax polyethylene glycol capillary column (0.32 mm 30 m). The mass spectra were obtained on a Hewlett Packard 6890/MSD5973 GC-MS with a split-splitless injector and equipped with a HP-Innowax capillary column (30 m 250 lm); helium was used as the carrier gas (56 Kpa).

Data analysis

DC and UTS were analyzed by Kruskal-Wallis and Holm-Sidak test. Cell viability, SOD, SH, and TBARS data were analyzed by two-way or three-way analysis of variance (ANOVA) flowed by Tukey post hoc test. The value of $P < 0.05$ was considered significant. Graphic for DC and UTS was made using Microsoft Excel, and cell viability, SOD, SH, and TBARS using GraphPad Prism Program 4.00 version (GraphPad Software, San Diego, USA).

Results

Degree of C=C conversion

The results show that photoactivation time statistically affected the DC, with higher values in all groups evaluated after 20 s ($P = 0.002$). After 10 s, the presence of DPI increased the DC, G1 < G3 < G2 < G4 = G5 (G1 4.76 ± 0.91; G2 64.39 ± 1.18; G3 61.43 ± 1.32; G4 77.56 ± 1.59; G5 76.98 ± 0.52; $P = 0.02$). After 20 s, the ternary systems (CQ + EDAB + DPI) showed higher degree of conversion (G4 79.96 ± 1.06; G5 79.46 ± 0.44) when compared with the groups G1, G2, and G3 (G1 10.93 ± 1.46; G2 69.87 ± 0.77; G3 68.88 ± 3.72; $P = 0.017$) (Fig. 1).

Ultimate tensile strength

The experimental group G3 was the only one that showed statistically significant difference between 10 and 20 s of polymerization (10 s 51.05; 20 s 56.7; $P = 0.02$), with better performance for 20 s. All of the other experimental groups showed no statistically significant differences between themselves ($P = 0.125$) and with the polymerization time ($P = 0.132$) (Fig. 2).

Cell viability assay

In order to evaluate NIH/3T3 cell viability towards four different initiation systems exposed to different times of polymerizations and eluate exposures, a MTT assay was carried out. Results show no statistically significant differences between the time of eluate exposure (1, 3, 5, and 7 days) ($P = 0.258$) and polymerization time (10 and 20 s) ($P = 0.812$). When the initiation system groups (G2, G3, G4, and G5) were compared to controls (0.380 ± 0.05), G2 (0.33 ± 0.02) and G4 (0.32 ± 0.02) showed the lower cell viability ($P = 0.000$) (Fig. 3).

Biochemical assays

Superoxide dismutase assay

Since oxidative stress can generate alteration in cell viability, we evaluated the levels of SOD, SH, and TBARS. Based on the viability results that showed no statistical differences between the times of eluate incubation (1, 3, 5, and 7 days), we evaluated SOD, total SH, and TBARS in 1 day of eluate exposure. The results show higher levels of SOD in the group polymerized for 10 s ($P = 0.046$) in comparison with 20 s of polymerization. No statistically significant differences were showed between the different initiation systems tested (Fig. 4).

Total sulfhydryl content

Results show higher levels of total SH in G3 (17.73 ± 0.88) with 10 s of polymerization, which reduces when polymerization is increased to 20 s (10.07 ± 1.25; $P = 0.026$). The G2 group showed higher levels of total SH, with 20 s of polymerization (17.38 ± 0.95; $P = 0.026$). The G5 group polymerized for 20 s showed the lowest levels of total SH (5.32 ± 1.72; $P = 0.026$) (Fig. 5).

Thiobarbituric acid reactive species formation

Results show statistical differences between the initiation systems tested, where the higher levels of TBARS were observed in the G3 group (0.094 ± 0.005) and the lower levels in the G4 (and G5 group ($P = 0.027$)). No statistical differences were found between times of polymerization ($P = 0.99$) (Fig. 6).

Gas chromatography

The samples were analyzed in mass spectrometer in the described conditions. The residues of volatile organic compounds were not detected in this technique.

Discussion

Several researches in dentistry have been looking deeper in new photoinitiators in an attempt to obtain a more efficient adhesive systems with suitable mechanical [4] and biological properties [29]. According to the literature, an adhesive with ternary photoinitiation system, comprising of CQ, EDAB, and iodonium salt, can improve restoration clinical performance, due to its capacity to increase bond strength to dentin, immediately and longitudinally [30]. In this sense, our study emphasizes the relevance of incorporating the third element—iodonium salt—in a binary adhesive systems composed exclusively of CQ and EDAB.

The incorporation of iodonium salts in dental adhesive systems has been considered very interesting, since they may act as catalysts, reducing the energy of activation [1]. Furthermore, they can act as co-initiators in dental adhesives due to their ionic character, thus contributing to the polymerization of hydrophilic monomers preventing phase separation [6], increasing, therefore, the polymerization reaction rate and the percentage of monomer conversion. The literature has shown that DPI can improve the mechanical properties of the hybrid and adhesive layers formed by etch-and-rinse adhesives, reducing nano-leakage at the adhesive interface [31].

According to our viability assay result, although higher degree of conversion was shown with 20 s of polymerization in comparison to 10 s, this did not seem to affect NIH/3T3 cell viability, since there were no statistically significant differences in cell viability between 10 and 20 s of polymerization. This could be explained by the higher degree of conversion showed by the different initiation systems in comparison to the control group (10 s—G1 < G3 < G2 < G4 = G5; 20 s—G1 < G2 = G3 < G4 = G5). The UTS showed no statistical significance in the DPI group between 10 and 20 s of polymerization. This could be a favorable aspect when considering methods to reduce clinical time spent to perform a dental restoration. According to other studies, we found that the ternary photoinitiation system is able to perform improvements in polymerization rate, raising the conversion of monomers in a short period of curing, and the strength bond of dental adhesive even at low concentrations of iodonium salt, in relation to the binary systems [4, 32, 33].

Studies have reported that the addition of iodonium salt, in an experimental resin, increases degree of conversion [4, 34]. Thus, our findings corroborate with the literature since groups in which DPI was added showed higher levels of degree of conversion. In this context, considering that the degree of conversion is closely related to the cross-linking of polymers [35], it can be implied that a higher degree of conversion is required for a more resistant polymer. The increase in the degree of conversion values also can significantly affect the biocompatibility of the adhesive system and may inhibit adverse effects on pulp tissue [36]. The viability assay showed

slightly lower values for G2 (CQ + DPI) and G4 (CQ + higher concentrations of EDAB + DPI) groups, both groups with DPI. However, the G5 group (CQ + lower concentration of EDAB + DPI) showed no reduction in cell viability when compared with controls, and G3 group (CQ + EDAB) induced NIH/3T3 proliferation. Together, these results can indicate that EDAB in low concentration (1 mol%) can trigger cell proliferation; however, when the concentration is increased (2 mol%), it becomes cytotoxic for NIH/3T3 cells. When EDAB is maintained at low concentration (1 mol%), and therefore a concentration that induces proliferation, the slight cytotoxicity observed by the DPI (G2 group) seems to be attenuated; we hypothesize that a balance between proliferation and cytotoxicity of both components occurs.

There are a few studies that are dedicated to investigate the disequilibrium between the production and removal of ROS and RNS generated by dental materials. Many materials have in their composition cytotoxic elements that are capable of generating apoptotic effects and, therefore, could be linked to the induction of oxidative stress [21, 37]. In this context, it is important to investigate the levels of antioxidant activity, such as SOD and total SH, as well as the oxidative damage to lipid structure, which indicates damage to cell structures throughout oxidative stress.

The behavior of DPI seems paradoxical in relation to oxidative stress [37]. Two pathways of DPI action has been described: DPI can play a role as an antioxidant by inhibiting enzymes that produces ROS, and on the contrary, DPI can act in a deleterious way, by inducing mitochondrial superoxide production, which can decrease mitochondrial membrane potential and lead to cytochrome c release, promoting cell apoptosis [38]. In the present study in relation to SOD levels, there were no statistical differences between the different initiation systems. However, the time of polymerization showed higher levels of SOD for 10 s of polymerization in comparison to 20 s. The only parameter that showed significant differences between polymerization times was SOD levels. Since SOD is an antioxidant enzyme that inactivates superoxide, its higher levels in 10 s of polymerization indicate a slight cell reaction that could be related to leached monomers from the initiation systems tested. This cell insult is possible of low proportions since the other parameter did not indicate cell alteration.

It is well described that oxidative damage (that can be caused by ROS) is the oxidation of polyunsaturated fatty acid chains (lipid peroxidation), which are present in membrane structures [39]. Within the aim to verify membrane damage by lipid peroxidation, we evaluated the ability of different initiation systems to form TBARS. In this context, the inhibitory systems containing DPI and EDAB (G4 and G5) presented the lower values of lipid peroxidation in NIH/3T3 cells. These results demonstrate that the ternary systems did not induce membrane damage and in some degree protected NIH/3T3 cells from lipid peroxidation, since TBARS values were significantly lower than those presented by the control group. Thiols are efficient antioxidant, which protect cells against damage induced by free radical [40, 41]. The total SH assay, therefore, measures protein and non-protein thiols. In our study, a significantly lower level of total SH in the G5 group was shown, which is in association to the TBARS results. This corroborates with the possible benefit effects of ternary systems with DPI and low concentration of EDAB. In relation to gas chromatography, our results did not present any leached compound, endorsing the positive behavior of the proposed formulation with iodonium salt.

Conclusion

This study showed that although a slight decrease in cell viability was observed when DPI was associated with CQ and high concentration of EDAB, this reduction was attenuated when lower concentration of EDAB were used. Also, EDAB (in lower concentration) associated with DPI did not affect SOD levels and was unable to induce oxidative damage (TBARS), presenting the lowest levels of SH. In addition, this group presented the highest degree of conversion, which did not affect the ultimate tensile strength. Therefore, these results indicate that EDAB (in lower concentration) associated with DPI can increase the degree of conversion, without causing oxidative stress damage and favoring cell viability. Also, our studies showed that the polymerization time (10 and 20 s) did not alter cell viability, total SH, and TBARS; however, a slight increase was shown in SOD levels, which can indicate a discreet cell defense towards possible monomer release.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

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