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Effect of the Bis-Dimethylamino Benzydrol Coinitiator on the Mechanical and Biological Properties of a Composite

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To examine the effect of the alternative coinitiator 4,4'bis dimethylamino benzydrol (BZN) in degree of conversion (DC), mechanical and biological properties of experimental composites. The coinitiator BZN was used in three concentrations (0.2, 0.5 and 1.2%), and the coinitiator DMAEMA was used as control at the same concentrations as above. The molar concentration of camphorquinone (CQ) and coinitiators was kept constant (1:1). The composites were manipulated and submitted to microhardness test (VHN), flexural and compressive strength (in MPa), elastic modulus (GPa), DC (FT-IR) and in vitro cytotoxicity (against 3T3 fibroblastic cells) of the experimental resins. Data were subjected to two-way ANOVA and Tukey post-test (α=0.05). The experimental composite resin with BZN showed higher DC values compared to control DMAEMA groups. For the mechanical properties, microhardness values were higher in BZN groups; flexural strength and elastic modulus were similar between all the groups. Compressive strength for groups BZN0.5 and DMAEMA0.5 were not statistically different, being the lowest values attributed to group BZN0.2. The experimental resins with BZN and DMAEMA were considered nontoxic against 3T3 fibroblasts. The inclusion of the coinitiator BZN in experimental composites was considered nontoxic against 3T3 fibroblast cells, without compromising DC and mechanical properties.

Key Words: composite resins, products with antimicrobial action, Raman spectrum analysis, physical and chemical properties.

Introduction

The light curing systems most commonly employed in the composites are the camphorquinone/amine system. The photoinitiator is camphorquinone, which requires a coinitiator to perform the polymerization process. Tertiary amines (aliphatic or aromatic) do not absorb light, but interact with the activated camphorquinone to produce reactive species (1). However, this photoinitiator system has some disadvantages, such as the tendency to promote yellowing to resin materials (2,3), low compatibility with oral tissues (4) and even mutagenic characteristics (5).

It is well understood that factors such as type of photoinitiator (2), photoinitiator/coinitiator ratio (3,6,7), type and concentration of coinitiator (8) may influence the properties of the resin materials. These molecules must show high reactivity and high degree of monomer conversion, since no reactive monomers may diffuse out of the polymer matrix to the oral cavity (8).

In order to reduce toxicity without compromising the physical and mechanical properties of resin composites, alternative coinitiators already used on the market for other purposes are widely studied in the literature (9,10). Among the investigated coinitiators, a tertiary amine, developed by Vàzquez et al. (4) (N,N`-dimethylaminobenzyl alcohol - DMOH) to be used as an activator in bone cements, was evaluated by Schroeder et al. (8) with the purpose to be an alternative coinitiator for Bis-GMA and TEGDMA-based dental composites. The authors found satisfactory degree of conversion values with DMOH and camphorquinone (CQ) in low concentrations (less than 0.25 wt%). In this study, the radical formation and final monomer conversion of CQ/DMOH was higher than CQ with dimethylaminoethylmetacrylate (DMAEMA) – a widely used coinitiator in dental composites. Also, the coinitiator 4,4`bis-dimethylamino benzydrol (BZN) was studied by de la Torre et al. (11), to be a substitute activator in acrylic bone cements. This activator showed great results regarding the biocompatibility, as low citotoxicity and antibacterial and antisseptic properties. So far, there are no studies in the literature which analyze BZN in photocured dental composites.

Considering the above mentioned information, the objective of this study is to analyze the effect of the alternative coinitiator BZN in degree of conversion, mechanical and biological properties of experimental dental composites. The null hypotheses were: the inclusion of BZN would not influence the (1) degree of conversion, (2) mechanical or (3) the biological properties of the resins.

Material and Methods

Manipulation of the Experimental Composites

Due to the novelty, the patent for the material was

deposited under the number BR 10 2015 015015 6. The experimental composites were formulated as follows: the organic matrix (25 to 35 wt%) was composed by bisphenol-A glycidylmetacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) (Sigma Aldrich). The photoinitiator was camphorquinone (CQ), and 2,6-Di-tert-butyl-4-methyl-phenol (BHT, Sigma Aldrich) was used as inhibitor (0.1 wt%). The coinitiators tested were: DMAEMA for control and BZN. The CQ/coinitiators molar ratio was kept constant (1:1) and only the concentration was varied (0.2, 0.5, or 1.2). The choice for these CQ/coinitiator molar ratios was that the first was the minimum CQ/BZN ratio that was comparable to CQ/DMAEMA, tested by the authors in a pilot study, which showed similar degree of conversion results. In addition, other study have demonstrated the same founds with the 0.2 CQ/DMAEMA molar ratio (7). Silanated barium borosilicate glass fillers were added to the organic phase (mean filler size: 0.7 µm – 65 to 75 wt%, Esstech Inc., Essington, PA, USA) (Table 1). All the compounds were used as received, except BZN, which was purified.

The experimental composite resins were homogeneously mixed for one min at 2,400 rpm (DAC 150.1 FV SpeedMixer; Flacktek, Landrum, SC, USA) in dark environment.

Surface Microhardness Evaluation

The specimens were made in a metallic matrix of 2 mm thick and 5 mm diameter. For standardize the surface smoothness of the specimens, a Mylar strip was placed over the specimens, and gently pressed with a glass slide. The specimens (n=8) were light cured with a LED device (Radii Plus; SDI, Bayswater, Victoria, Australia) for 20 s with a power intensity of 1200 mW/cm^2, confirmed by a radiometer. The specimens were then stored in distilled water in dark environment at 37 °C for 24 h. For the Vickers microhardness measurement (VHN), the surface of the specimens was divided in 4 quadrants, and 5 indentations were performed with a load of 50 g for 15 s each (Shimadzu, Kyoto, Japan).

Compressive Strength Test

Cylindrical specimens (6 mm in height and 4 mm in diameter) were prepared for testing the compressive strength, according to ISO 4049 and ANSI/ADA #27 (11). These specimens (n=6) were light cured and stored as per the procedure above described. The compressive strength was determined by loading in using a mechanical testing machine (Autograph AG-I; Shimadzu, Tokyo, Japan) at a crosshead speed of 1 mm/min and 10 KN, until specimen failure. Data were expressed in MPa values.

Flexural Strength and Elastic Modulus Test

Flexure strength and elastic modulus were conducted in a three-point bending apparatus with a 20-mm span at a crosshead speed of 0.75 mm/min according to ISO 4049 and ANSI/ADA #27 (11). The samples were tested until fracture in testing machine (Autograph AG-I" Shimadzu). Rectangular specimens of 25x2x2 mm were made (n=6) in a metallic matrix. Light curing and storage procedures were the same as described above. The flexural strength was calculated according to Eq. (1) and elastic was calculated according to Eq. (2), where F is the load (N), l is the distance between supports (mm), w and t are the width and thickness of the sample (mm) and d is the deflection due to the load F (mm).

(1) $RF=3Fl/2wt^2$

(2) $EM = FL^3/4wt^3d$

Degree of Conversion

Six specimens per group were made for this test under the same conditions as those previously mentioned. After 24 h, the middle of the composite resin specimen was pulverized and maintained in a dark room until the moment of the FT-IR (IR Prestige-21) analyses. One milligram of the ground powder was thoroughly mixed with 100 mg of KBr powdered salt. This mixture was placed into a pelleting device and then pressed in a hydraulic press (SSP- 10A model; Shimadzu) with a load of 80 KN for 3 min to obtain

Table 1. Composition of the experimental composites used in the study (in wt%)*

Experimental composites	Organic matrix (35 wt%)		Inorganic matrix (65 wt%)
	Monomers	Photoinitiator system	
DMAEMA0.2	Bis-GMA (22.75%), TEGDMA (11.76%), BHT (0.1%)	CQ/DMAEMA0.2/0.19%	Silanated barium borosilicate glass
dmaema0.5	Bis-GMA (22.75%), TEGDMA (11.17%), BHT (0.1%)	CQ/DMAEMA 0.5/0.48%	
DMAEMA1.2	Bis-GMA (22.75%), TEGDMA (9.81%), BHT (0.1%)	CQ/DMAEMA 1.2/1.14%	
bzn0.2	Bis-GMA (22.75%), TEGDMA (11.62%), BHT (0.1%)	CQ/BZN 0.2/0.33%	
bzn0.5	Bis-GMA (22.75%), TEGDMA (10.83%), BHT (0.1%)	CQ/BZN 0.5/0.82%	
bzn1.2	Bis-GMA (22.75%), TEGDMA (8.99%), BHT (0.1%)	CQ/BZN 1.2/1.96%	

*DMAEMA: dimethylaminoethylmetacrylate; BZN: 4,4`- bisdimethylamino benzydrol; Bis-GMA: bisphenol-A glycidylmetacrylate; TEGDMA: triethylene glycol dimethacrylate; BHT: 2,6-Di-tert-butyl-4-methyl-phenol; CQ: camphorquinone.

a pellet. The pellet was placed into a holder attachment into the spectrometer. Uncured composite specimens were also made. The absorbance spectra of both uncured and cured samples were analyzed in the range of 400 to 4,000 cm^{-1} operating under the following conditions: 64 scans and 4 cm^{-1} resolution. DC was calculated based on the ratio between 1,637 cm^{-1} peak (aliphatic C=C double bonds of the resin) and 1,608 cm^{-1} peak (internal standard), as the following equation (3), where: $R_{polymer}$=Area 1,637 cm^{-1}/Area 1,608 cm^{-1} in polymerized composite, and $R_{monomer}$=Area 1,637 cm^{-1}/Area 1,608 cm^{-1} in composite before light polymerization, arranged on a glass slide:

$$DC = 1 - R_{polymer}/R_{monomer} \times 100$$

Cytotoxicity

The cytotoxicity evaluation followed ISO 10993-5 protocol (Biological Evaluation of Medical Devices Part 1: Evaluation and Testing) (12). Specimens were made in the same way as described for surface microhardness test (n=4). The test was triplicated. The specimens were stored in distilled water and atmosphere containing CO2 at 37 °C. After this period, the specimens were ultrassonically cleaned for 30 min, sterilized by ultraviolet light for 15 min each side and placed in wells of a 24-well culture plate containing 1 mL of DMEN supplemented with 10% fetal bovine serum (FBS), with 100 IU/mL of penicilin, 100 µg/mL of streptomicin and 2 mmol/L of glutamin. After 24 h, the samples were removed and 200 µL of each formed extract was transferred in quadruplicate in wells of a 96-well culture plate, which were previously adhered by 3T3 fibroblastic cells for 24 h (1 x 10^5 cells/plate). The positive control was simulated with 10% FBS (13).

The viability of the cells was quantitatively analyzed by MTT (methylthiazolydiphenyl-tetrazolium bromide) assay. In this technique, MTT reagent produces a dark-blue formazan product with viable cells; the level of cell viability was determined by an ELISA reader (Biotek EL 800, Biotek, Winooski, VT, USA, 570 nm).

Results

Data were analyzed by two-way ANOVA and Tukey post-test (α=0.05 and power of the test of 90% - factors: concentration and coinitiator). The tests with BZN1.2 experimental composites were not performed, as this blend did not sufficiently polymerize.

Surface Microhardness Evaluation

For microhardness values, only the factor coinitiator type was significant (p<0.0001). The interaction between the factors and the factor concentration were not significant (p>0.05 and p=0.75, respectively). Both 0.2 BZN and 0.5 BZN groups were statistically higher than DMAEMA controls, for all concentrations (Table 2).

Compressive Strength Test

For compressive strength, the interaction and the isolated factor coinitiator type were not significant (p=0.5 and p=0.65, respectively). The factor concentration was significant (p<0.0001). BZN0.5 was similar to its control DMAEMA0.5, while BZN0.2 differs significantly from the control DMAEMA groups, showed in Table 2.

Flexural Strength and Elastic Modulus Test

For flexural strength, the interaction between the factors were not significant, neither the isolated factors (concentration: p=0.07 and coinitiator type: 0.76). So, there were no significant differences between all the experimental groups for flexural strength. For elastic modulus, the same observations may be visualized (Table 3). The experimental groups were statistically similar (p>0.05).

Degree of Conversion

For degree of conversion, only the factor coinitiator type was significant (p=0.001). The composite BZN0.5 showed the highest values between the groups. All DMAEMA resin blends showed similar results, regardless of the concentration (Table 2).

Cytotoxicity

For cytotoxicity, the interaction neither the isolated factors were significant (p>0.05). The cytotoxicity test using 3T3 fibroblast cells demonstrated no differences between the experimental groups, once all of them were significant similar to the positive control, irrespective of

Table 2. Mean values (standard deviation) of degree of conversion (DC - in %), surface microhardness (VHN) and compressive strength (in MPa) according to each experimental group*

	DMAEMA 0.2	DMAEMA 0.5	DMAEMA 1.2	BZN 0.2	BZN 0.5	BZN 1.2
DC	47.3 (1.5) a	43.8 (2.4) a	47.9 (2.1) a	64.3 (8.9) a,b	74.3 (8.7) b	--
Microhardness	20.7 (3.9) a	31.6 (5.1) a	28.4 (3.8) a	49.3 (4.1) b	51.9 (8.4) b	--
Compressive strength	543.1 (37.3) b	465.8 (45.7) a,b	381.2 (33.1) a	260.7 (47.7) c	399.2 (44.5) a	--

*Distinct letters are statistically different only in the same lines

the coinitiator or the concentration used (Table 4).

Discussion

Several studies regarding alternative coinitiators and photoinitiator systems has been conducted in an attempt to ensure appropriate physical and mechanical properties of composite resins, concomitantly to a significant improvement in the biocompatibility of these materials (14-16). Biocompatibility is an essential attribute to a dental material. However, it should be resistant against the dynamic oral environment, in order to ensure favorable mechanical properties as high microhardness, compressive and flexural strength (17).

The first null hypothesis tested in this study was rejected, as BZN influence degree of conversion values. These values obtained from the experimental composites with the alternative coinitiator BZN were higher than that with the control DMAEMA. DMAEMA was used as control because it is a radical-containing methacrylate, which acts as a photopolymerizable amine and may increases DC values and consequently the physical-mechanical properties of the polymer, even when used in small amounts (18). Which is expected from a coinitiator is that it forms a great amount of free radicals coupled with camphorquinone, after reaching its "excitatory" state (19) and should be sufficient to promote the breaking of aliphatic carbon double bonds of the monomers (20). DMAEMA and BZN have the same efficiency (the capacity to form free radicals per mol). It may be speculated that high molecular weight coinitiators (BZN=270.37 and DMAEMA=157.21) could enhance the polymerization kinetics and biocompatibility of the experimental composites (21), which may explain the higher degree of conversion values from BZN groups.

Mechanical properties, such as microhardness, compressive and flexural strength, elastic modulus, predict the materials performance in the oral environment. The experimental composites were analyzed in all this properties, once coinitiator type and concentration may influence all the above mentioned characteristics (7). The second null hypothesis was partially rejected. Microhardness values from BZN experimental groups were higher than DMAEMA, regardless of the concentration. According to ADA (12), the results from BZN groups are satisfactory, in terms of compressive and flexural strength. For compressive strength, between the three analyzed concentrations, BZN0.5 showed the highest values, which was compared to the control DMAEMA0.5. For flexural strength, there was no significant differences between the groups, being all them acceptable to ADA specifications (not lower than 50 MPa). The elastic modulus found are similar to those found in the literature for low viscosity composites, probably by the filler content presented in the experimental composites (65 wt%) (20,21). One could expected that higher elastic modulus values would be found if the experimental composites showed 75 wt% filler content, which sets them as a microhybrid composite.

The cytotoxicity of experimental composites against 3T3 fibroblast cells was also evaluated. This property estimates the degree of pulp and gingival cell impairment in contact with the composites. In the methodology used, it was possible to quantitatively estimate the cellular mitochondrial activity. The composites were considered non-toxic against 3T3 fibroblasts. Thus, the third null hypothesis was accepted. Similar results were obtained by authors who have investigated BZN in bone cements (11), where low toxicity and high *in vivo* biocompatibility results were found. Also, the formation of a connective tissue was observed, attributed to the non-cytotoxic effect of BZN in adjacent bone tissues.

The experimental composites of group BZN1.2 did not

Table 3. Mean values (standard deviation) of flexural strength (in MPa) and elastic modulus (in GPa) according to each experimental group*

	DMAEMA 0.2	DMAEMA 0.5	DMAEMA 1.2	BZN 0.2	BZN 0.5	BZN 1.2
Flexural strength	86.4 (16.7) a	73.9 (18.2) a	67.7 (33.3) a	55.1 (18.1) a	59.9 (9.1) a	--
Elastic modulus	2.9 (0.7) a	3.1 (0.8) a	3.1 (2.0) a	1.8 (0.6) a	2.5 (0.5) a	--

*Distinct letters are statistically different only in the same lines.

Table 4. Mean values (standard deviation) of cell viability (3T3 fibroblasts) according to each experimental group*

	DMAEMA 0.2	DMAEMA 0.5	DMAEMA 1.2	BZN 0.2	BZN 0.5	BZN 1.2
In vitro cytotoxicity	1.14 (0.08) a	1.21 (0.15) a	1.21 (0.09) a	1.26 (0.03) a	1.37 (0.10) a	--
Control	1.18 (0.09) a					

polymerize, and the tests could not be conducted. Probably, the composites suffered alterations in polymerization kinetics and degree of conversion, resulting from the lower TEGDMA monomer content (22), specifically in propagation phase, when the monomers have low mobility inside the polymeric chain, reaching termination phase faster (22). Furthermore, the color of the experimental resin was yellower than the other two experimental composites, which was not clinically acceptable.

This is a preliminary *in vitro* study of the experimental composite with the BZN coinitiator. The results are promising, especially those found in microbial adherence tests. However, they should be carefully interpreted, since in a laboratory study there is not the acquired pellicle formation and saliva, which may influence the adhesion of microorganisms to the composite resin (23,24). Clinical extrapolations are not possible until this moment, and further *in vitro* studies, especially concerning the ideal BZN concentrations are required to support our findings.

The experimental composites formulated with the alternative coinitiator BZN demonstrated satisfactory mechanical properties, degree of conversion values, and was considered non-toxic against 3T3 fibroblasts, compared to DMAEMA control.

Resumo

Analisar o efeito do co-iniciador alternativo 4,4'bisdimetilaminobenzidrol (BZN) no grau de conversão (GC) e nas propriedades mecânicas e biológicas de resinas compostas experimentais. O co-iniciador BZN foi utilizado em três concentrações (0,2, 0,5 e 1,2), e o co-iniciador DMAEMA como controle, nas mesmas concentrações acima. A concentração molar entre canforoquinona (CQ) e os co-iniciadores foi mantida constante (1:1). As resinas compostas foram manipuladas e submetidas aos testes de microdureza (VHN), resistência à compressão e flexural (em MPa), módulo de elasticidade (em GPa), GC (em %, por meio de espectroscopia micro-Raman e FTIR com KBr), citotoxicidade in vitro (frente às células fibroblásticas 3T3) das resinas experimentais. Os resultados foram submetidos ao teste ANOVA 1 fator e pós-teste de Tukey (α=0,05). As resinas compostas experimentais com o BZN apresentaram GC e propriedades mecânicas satisfatórias, além de serem consideradas atóxicas a fibroblastos 3T3. A inclusão do co-iniciador BZN à resina composta foi considerada não tóxica frente a células fibroblásticas 3T3 e sem comprometer o grau de conversão e as propriedades mecânicas da mesma.

References

1. Jakubiak J, Wrzyszczynski A, Linden LA, Rabek JF. The role of amines in the Camphorquinone photoinitiated polymerization of multifunctional monomer. J Macromol Sci 2007;44:239-242.
2. Schneider LFJ, Pfeifer CSC, Consani S, Prahl SA, Ferracane JL. Influence of photoinitiator type on the rate of polymerization, degree of conversion, hardness and yellowing of dental resin composites. Dent Mater 2008;24:1169-1177.
3. Guimarães T, Schneider LF, Braga RR, Pfeifer CS. Mapping camphorquinone consumption, conversion and mechanical properties in methacrylates with systematically varied CQ/amine compositions. Dent Mater 2014;30:1274-1279.
4. Vàzquez B, Elvira C, Levenfeld B, Pascual B, Goni I, Gurruchaga M, et al.. Application of tertiary amines with reduced toxicity to the curing process of acrylic bone cements. J Biomed Mater Res 1997; 34:129–136.
5. Kucybala Z, Pietrzak M, Paczkowski J. Kinetic studies of a new photoinitiator hybrid system based on Camphorquinone-N-phenylglicyne derivatives for laser polymerization of dental restorative and stereolithographic (3D) formulations. Polymer 1996;37:4585-4591.
6. Musanje L, Ferracane JL, Sakaguchi RL. Determination of the optimal photoinitiator concentration in dental composites based on essential material properties. Dent Mater 2009;25:994-1000.
7. Camargo FM, Della Bona Á, Moraes RR, Coutinho de Souza CR, Schneider LF. Influence of viscosity and amine content on C=C conversion and color stability of experimental composites. Dent Mater 2015;31:e109-e115.
8. Schroeder WF, Cook WD, Vallo CI. Photopolymerization of N,N-dimethylaminobenzyl alcohol as amine co-initiator for light-cured dental resins. Dent Mater 2008;24:686-693.
9. Shi S, Nie J. A natural component as coinitiator for unfilled dental resin composites. J Biomed Mater Res B Appl Biomater 2007;82:44-50.
10. Manojlovic D, Dramićanin MD, Miletic V, Mitić-Ćulafić D, Jovanović B, Nikolić B. Cytotoxicity and genotoxicity of a low-shrinkage monomer and monoacylphosphine oxide photoinitiator: Comparative analyses of individual toxicity and combination effects in mixtures. Dent Mater 2017;33:454-466.
11. de la Torre B, Salvado M, González Corchón MA, Vázquez B, Collía F, De Pedro JA, et al.. Biological response of new activated acrylic bone cements with antiseptic properties. Histomorphometric analysis. J Mater Sci: Mater Med 2007;18:933–941.
12. American National Standard. American Dental Association Specification n. 27 for Resin-based filling materials; 1993.
13. International Standard ISO 10993-5. "Biological Evaluation of Medical Devices Part 1: Evaluation and Testing"; 1995.
14. Campanha NH, Pavarina AC, Giampaolo ET, Machado AL, Carlos IZ, Vergani CE. Cytotoxicity of hard chair-side reline resins: effect of microwave irradiation and water-bath post-polymerization treatments. Int J Prosthodont 2006;19:195-201.
15. Bakopoulou A, Papadopoulos T, Garefis P. Molecular toxicology of substances released from resin–based dental restorative materials. Int J Mol Sci 2009;10:3861-3899.
16. Schmalz G, Galler KM. Biocompatibility of biomaterials - Lessons learned and considerations for the design of novel materials. Dent Mater 2017;33:382-393.
17. Thomaidis S, Kakaboura A, Mueller WD, Zinelis S. Mechanical properties of contemporary composite resins and their interrelations. Dent Mater 2013;29:e132-e141.
18. Yoshida K, Greener EH. Effects of two amine reducing agents on the degree of conversion and physical properties of an unfilled light-cured resin. Dent Mater 1993;9:246-251.
19. Bowen RL, Argentar H. Tertiary aromatic amine accelerators with molecular weights above 400. J Dent Res 1972;51:473-482.
20. Stansbury JW. Curing dental resins and composites by photopolymerization. J Esthet Dent 2000;12:300-308.
21. Braga RR, Hilton TJ, Ferracane JL. Contraction stress of flowable composite materials and their efficacy as stress-relieving layers. J Amer Dent Assoc 2003;134:721-728.
22. Amirouche-Korichi A, Mouzali M, Watts DC. Effects of monomer ratios and highly radiopaque fillers on degree of conversion and shrinkage-strain of dental resin composites. Dent Mater 2009;25:1411-148.
23. Wang S, Guo L, Seneviratne CJ, Huang B, Han J, Peng L, et al.. Biofilm formation of salivary microbiota on dental restorative materials analyzed by denaturing gradient gel electrophoresis and sequencing. Dent Mater J 2014; 33:325-331.
24. Caroline de Abreu Brandi T, Portela MB, Lima PM, Castro GF, Maia LC, Fonseca-Gonçalves A. Demineralizing potential of dental biofilm added with Candida albicans and Candida parapsilosis isolated from preschool children with and without caries. Microb Pathog. 2016;100:51-55.

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