Long-term bonding efficacy of adhesives containing benzodioxioles as alternative co-initiators

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Abstract: This study evaluated the three-year lifespan of the bond to dentin of experimental self-etch adhesives containing benzodioxole derivatives – 1,3-benzodioxole (BDO) and piperonyl alcohol (PA) – as co-initiator alternative to amines. Adhesive resins were formulated using Bis-GMA, TEGDMA, HEMA, camphorquinone and different co-initiators: BDO, PA or ethyl 4-dimethylamino benzoate (EDAB – amine). An experimental self-etch primer was used to complete the two-step, self-etch adhesive system. Clearfil SE Bond (CSE) was used as commercial reference. Bond strength to human dentin was assessed by microtensile bond strength (μTBS) test, and failure mode was classified. Morphology of the dentin bonding interface was assessed via scanning electron microscopy (SEM). Irrespective of the dental adhesives evaluated, μTBS was higher after 24 hours compared with that after 1.5 and 3 years ($p \leq 0.001$). However, adhesives with BDO and PA as co-initiators showed significantly higher bond strength than the bonding resin with EDAB ($p \leq 0.002$), independent of the time evaluated. The commercial adhesive CSE showed similar bond strength compared with the other groups ($p \geq 0.05$). Mixed failures were mainly observed after 24 hours, while adhesive failures were more frequently observed after 1.5 and 3 years. No notable differences in homogeneity and continuity along the bonded interfaces were detected among the materials in the SEM analysis. In conclusion, benzodioxole derivatives are feasible alternative co-initiators to tertiary amine in camphorquinone-based self-etching dental adhesive formulations.

Keywords: Adhesives; Dentin; Dental Cements; Microscopy; Dental Materials.

Introduction

The clinical success of resin composite restorations is related to durability of the bonding between the adhesive system and dental substrate.[1,2] The longevity of bonding depends on the mechanical strength and hydrolytic stability of the adhesive polymer, which are modulated by the effectiveness of the polymerization reaction promoted by the photo-initiation system.[3,4] The initiation system must also be biocompatible with the oral tissues.[5] There is evidence that unreacted monomers may cause hydrolytic degradation of the polymer over time[3,6,7] and can be toxic, allergic, carcinogenic, or mutagenic to pulp cells.[4,8,9] This is because an increase in residual monomers may affect the balance between healing and chronic inflammation of damaged dental tissues.[10]

The most commonly used photo-initiator system in dental polymers is based on the visible-light photosensitizer, camphorquinone (CQ). Satisfactory C=C conversion and mechanical properties are obtained through a combination of CQ as a photosensitizer with different types of amines as co-initiators.[4,9,11] However, amines are known for their toxicity and mutagenicity potentials.[12,13] In addition, the conventional CQ/amine photo-initiation system tends to suffer degradation over time[14] and is chemically unstable in acidic media.[6] This has encouraged research efforts to identify alternative co-initiators as substitutes for amines to improve the biocompatibility of photo-initiating systems without impairing the polymerization reaction and the bonding ability of dental adhesives.[15,16] Benzodioxole derivatives found in natural components have been reported to provide initiating reactivity comparable to amines,[17,18] as well as to be nontoxic.[19] Benzodioxoles are present in plant products included in the diets of humans and are widely available in nature.[20] Furthermore, these components have been shown to have biological properties including antifungal, antibacterial, antioxidant, and antitumor activities.[20,21,22]

Previous studies have evaluated the use of benzodioxoles as potential co-initiators for unfilled dental resins.[17,18,23,24] These components were reported as effective co-initiators for CQ-based initiator systems, leading to similar or even improved polymer properties compared with amines.[17,18,23,24,25] However, the literature still lacks evidence of the dentin bonding performance of dental adhesives containing benzodioxole derivatives as co-initiators.

The aim of this study was to evaluate the influence of benzodioxole derivatives on the immediate and long-term dentin bond strength of experimental two-step, self-etch adhesives. The null hypothesis was that adhesives with the benzodioxole derivatives would have a similar bonding performance than the adhesive containing an amine co-initiator.

Methodology

Materials

Bisphenol-A glycidyl dimethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA) and camphorquinone (CQ) were supplied by Esstech Inc. (Essington, USA) and used without further purification. The acidic phosphate monomer HEMA-P, which is an equimolar mixture of methacryloyloxyethyl dihydrogen phosphate/bis(methacryloyloxyethyl) hydrogen phosphate, was synthesized as previously described.[7] Ethanol was obtained from Synth (Diadema, SP, Brazil). The co-initiators ethyl 4-dimethylamino benzoate (EDAB), 1,3-benzodioxole (BDO) and piperonyl alcohol (PA) were purchased from Sigma-Aldrich (Milwaukee, USA) and used as received. Figure 1 shows the structural formulas of the co-initiators evaluated.

Formulation of the experimental adhesives

An experimental self-etch primer was formulated using a mixture of 30 wt% HEMA-P, 30 wt% HEMA, 20 wt% distilled water, and 20 wt% ethanol. The

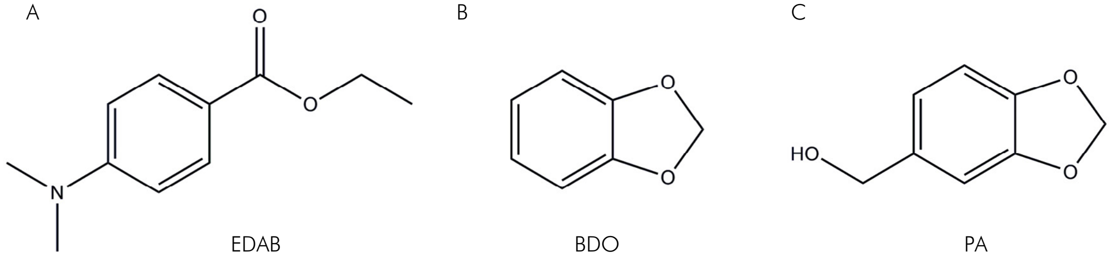

Figure 1. Molecular structure of the co-initiators used in the present study.

pH of the solution was measured with a digital pH meter (An2000; Analion, Ribeirão Preto, Brazil). An experimental adhesive resin was prepared using a mixture of 50 wt% Bis-GMA, 25 wt% TEGDMA, and 25 wt% HEMA. An optimal photo-initiator concentration of 1 mol% CQ combined with 1 mol% EDAB, 4 mol% BDO or 4 mol% PA was established in previous studies testing the degree of C=C conversion of the adhesives,[33-35] and added to the adhesive resin. The adhesive resin of Clearfil SE Bond – CSE (Kuraray, Osaka, Japan) was tested as commercial reference. Photo-activation procedures were carried out using a halogen light curing unit (XL3000; 3M ESPE, St. Paul, USA) with 650 mW/cm^2 irradiance confirmed using a digital power meter (Ophir Optronics, Danvers, USA).

Dentin bond strength and failure analysis

Forty sound human third molars, removed for orthodontic reasons, were used under approval of the Research Ethics Committee (process 090/09). The teeth were cleaned and stored in a 0.5% chloramine-T solution for seven days. Ten teeth were randomly allocated to each group according to the storage time (24 hours, 1.5 years, or 3 years) or bonding resin tested (CSE, EDAB, BDO, or PA). The buccal faces were wet-ground to create a flat surface in medium dentin. To standardize the smear layer, the dentin surfaces were wet-polished with 600-grit SiC paper for 60 s. Dentin moisture was controlled with absorbent paper until no surface water was observed. The dentin surfaces were vigorously etched with experimental primers for 30 s and air-dried for 10 s. One coat of adhesive resin was applied and light-activated for 20 s. Resin composite restorations (Charisma; Heraeus Kulzer, Hanau, Germany) were incrementally built-up with each increment light-activated for 20 s.

After storage in distilled water at 37°C for 24 hours, the restored teeth were sectioned perpendicularly to the bonded interfaces with a refrigerated low-speed diamond saw (ISOMET 1000 Buehler – Lake Bluff, USA) in both the mesio-distal and occluso-cervical directions. This yielded beam-shaped microtensile specimens with each cross-sectional surface area of approximately 0.7 mm^2. For each tooth, 6 beams were randomly allocated into three storage times (24 h, 1.5 years, or 3 years). The storage media was renewed weekly during aging.[26]

After the predetermined storage period, the specimens were fixed into the grips of a microtensile device and tested in a mechanical testing machine (DL500; EMIC, São José dos Pinhais, Brazil) at a crosshead speed of 0.5 mm/min until failure. Microtensile bond strength values were recorded in MPa. The data was analyzed using a two-way analysis of variance (co-initiator type vs. storage period). All pairwise multiple comparison procedures were performed using the Tukey's method ($p < 0.05$). Failure analyses for all micro-specimens tested were performed using light microscopy at 100× and 500× magnifications. The failure mode was classified as adhesive failure (interfacial), cohesive failure within resin, cohesive failure within dentin, and mixed failure, *i.e.*, when there is more than one type of failure. Premature failures (pre-testing) were also recorded.

Morphology of the dentin-bonded interfaces

The protocol used for preparing the specimens was based on a previous study.[27] Dentin discs (thickness 2 mm) were obtained from the mid-coronal portion of human molars. The dentin surfaces were polished and the primer/adhesive resins were applied as previously described. Two dentin discs were bonded to each other using a thin layer of light-activated composite, generating a dentin–composite–dentin sandwich specimen. The specimens were embedded cross-sectionally in epoxy resin in order for the dentin–cement interfaces to be visible. After 24 h, the specimens were wet-polished with 600, 1200, 1500, 2000, and 2500-grit SiC papers, followed by polishing with 3, 1, 0.25 and 0.1-µm diamond suspensions. The surfaces were etched with 50% phosphoric acid solution for 5 s and deproteinized by immersion in 2.5% NaOCl solution for 10 min. The specimens were ultrasonically cleaned in distilled water, dry-stored at 37°C for 2 h, and coated with gold. The bonded interfaces were examined using scanning electron microscopy – SEM (SSX-550; Shimadzu, Tokyo, Japan), at 15 kV.

Results

Table shows the results for the bond strength of the adhesives evaluated. The factors 'co-initiator type' and 'storage period' were both significant ($p \leq 0.001$). The interaction between the two factors was not significant. The power of the performed statistical test was above 0.8 for all conditions. Irrespective of the adhesive evaluated, bond strengths at 24 h were significantly higher than those at 1.5 and 3 years ($p \leq 0.001$). The adhesives with BDO and PA as co-initiators presented significantly higher bond strengths ($p \leq 0.002$) than the adhesive with EDAB as co-initiator, irrespective of the storage time. The commercial adhesive CSE showed similar bond strength compared with the BDO and PA adhesives ($p \geq 0.05$).

Figure 2 shows the distribution of failure modes. For all groups, mixed failures were predominant at 24 h while adhesive failures were predominantly observed after 1.5 and 3 years. Pre-testing failures were also observed only after storing in water for 1.5 or 3 years. Figure 3 shows representative SEM pictures of the bonded interfaces provided by the experimental adhesives evaluated. The hybrid layer

Table. Bond strength (means (standard deviations)), in MPa, of adhesives evaluated.

Co-initiator	24 hours	1.5 years	3 years	Pooled average
CSE	49.7(13.7)^{aAB}	26.6(5.0)^{bAB}	24.6(8.0)^{bBC}	33.5(15.3)^{AB}
EDAB	45.7(14.0)^{aB}	18.23 (7.46)^{bB}	18.9(9.4)^{bC}	28.7(16.7)^{B}
BDO	54.5(16.64)^{aAB}	29.4(13.7)^{bAB}	32.3(10.4)^{bAB}	39.1(17.9)^{A}
PA	61.9(13.4)^{aA}	33.4(17.9)^{bA}	35.1(12.8)^{bA}	42.5(19.7)^{A}
Pooled average	53.2(15.6)^{a}	27.2(14.1)^{b}	27.8(12.0)^{b}	-

Distinct uppercase (column) or lowercase (line) letters indicate significant statistical difference ($p < 0.05$).

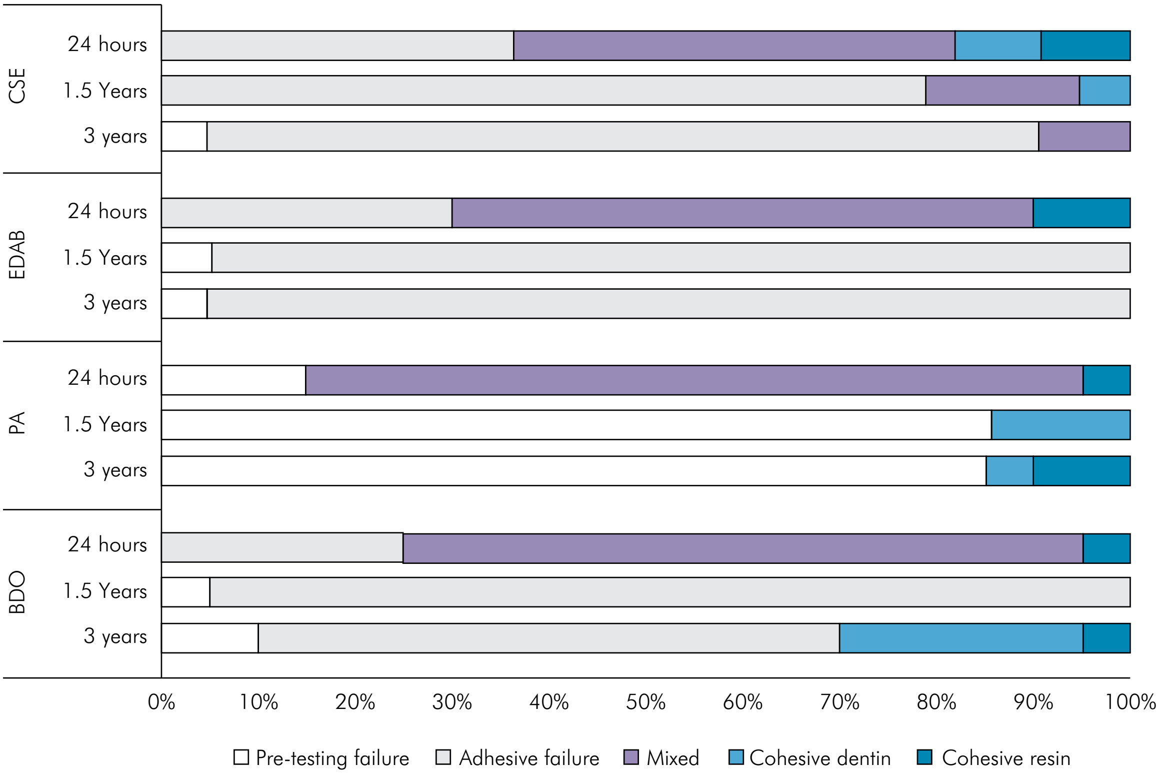

Figure 2. Distribution of failure modes for all groups (mixed failure, adhesive failure, cohesive resin, cohesive dentin).

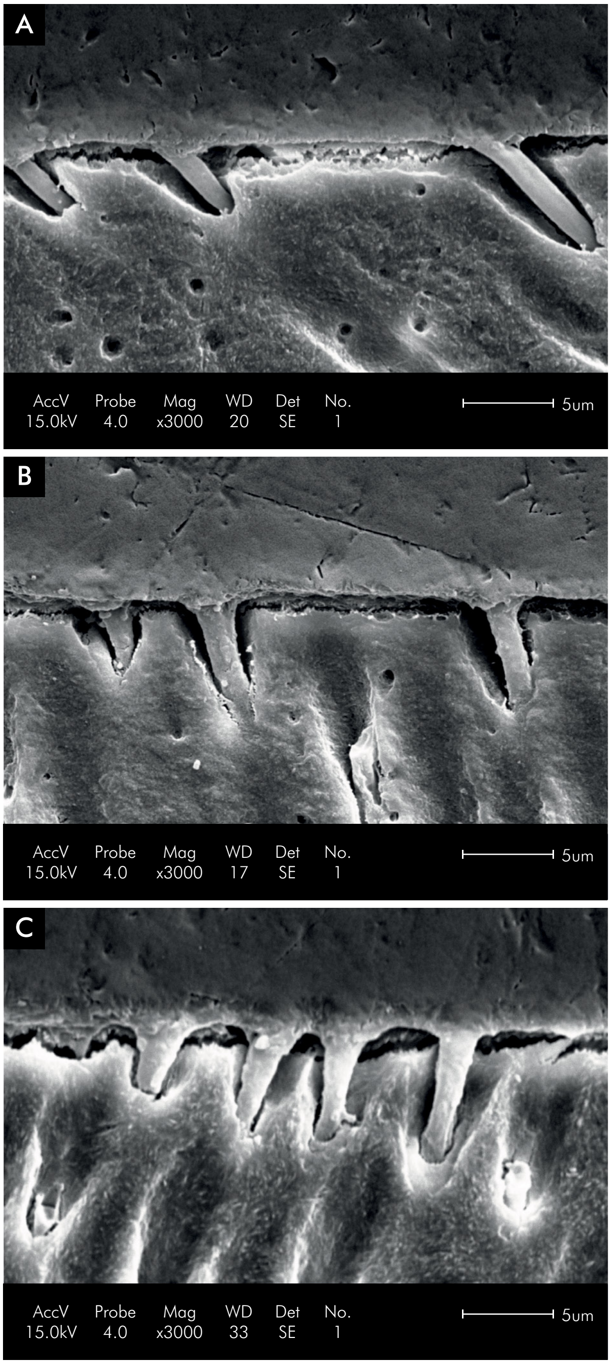

Figure 3. SEM pictures of bonded interfaces. The resin tags observed for the groups BDO and PA had a trunk-conical-shape with a rough surface similar to EDAB.

for all groups was observed to be relatively shallow (thickness < 2 µm). No appreciable differences in homogeneity and continuity of the hybrid layer along the interfaces were detected among the materials. The resin tags observed for the BDO and PA groups had trunk-conical shape similar to EDAB. For the group PA, the surface of the resin tags was slightly rougher compared with BDO, with the presence of accessory hybridized tags.

Discussion

The results of this study provide evidence that benzodioxole derivatives are promising alternative co-initiators to tertiary amines in CQ-based, self-etch dental adhesive formulations. The addition of BDO or PA as co-initiators improved the dentin bond strength compared with adhesive resin with tertiary amine EDAB as co-initiator, irrespective of the storage time. Therefore, the null hypothesis was rejected.

A co-initiator is necessary for effective photopolymerization of CQ-based dental materials,[28,29,30,31] because when exposed to light, CQ quickly reacts and starts the process of radical polymerization.[32] The photo-initiation reaction of binary systems involves hydrogen abstraction from the excited sensitizer to the initiator.[30,31] Tertiary amines are very effective in reacting with the excited CQ* species as an H-donor and by oxygen scavenging reaction to generate free radicals that can start the polymerization process.[30] Polymers formed using CQ/EDAB have a high degree of C=C conversion,[4,6,25,28] which could explain the immediate bond strength being similar to that of the commercial adhesive CSE. This is because the degree of conversion is strongly related to the mechanical properties of the adhesive layer. Amines are organic nitrogen compounds derived from ammonia. They are considered basic, because they have a pair of free electrons that can bind to a cation through a dative covalent bond and are excellent proton donors. However, the amine in contact with the acidic monomer present in the self-etch adhesive system may generate an acid-base reaction, impairing the polymerization reaction and therefore the bond strength of this material over time.[4] This might explain the dentin bonding performance observed for the EDAB-based adhesive during storage.

This study shows that an alternative to enhance the polymerization[25] of CQ-containing self-etch adhesives and their dentin bond strength is the use of benzodioxole derivatives. The use of these natural components may also decrease the cytotoxicity and improve the biocompatibility of dental biomaterials,[19] especially those placed in deep cavities. The free-radical formation mechanism of PA and BDO in CQ-based materials involves hydrogen abstraction through cyclic acetyl radicals generated by the photo-irradiation onto cyclic acetyl compounds. This might be rearranged rapidly by ß-scission to the corresponding ester radicals.[25] For PA, the hydrogen abstraction from the methylene-bridge carbon could also occur to form a methylene dioxybenzene radical and initiate polymerization with the methacrylate monomers.[18,25] PA showed improved immediate bond strength compared with CQ/EDAB and similar bond strength to the other groups tested.

The similar molecular structure of the two benzodioxole derivatives tested herein could explain the similar behavior of adhesive systems containing BDO or PA. The dentin hybridization observed for these two materials suggests good interaction with the dentin substrate. However, the SEM analysis suggested a better interaction of PA with dentin. This finding might be related to the higher hydrophilicity of PA due to the presence of the methanol group. The hydrophilicity increases the solubility of the adhesive resin with co-initiator and facilitates hybridization to dentin, which is a naturally moist substrate.[33] To achieve optimal dentin bond strength, the adhesive needs to hybridize with the intertubular and peritubular dentin, since the actual contribution of hybridized resins tags to bond strengths is not sufficiently explained.[34] EDAB showed more adhesive failures than the alternatives co-initiators. It is hypothesized that the acid-base reaction between the amine and self-etch monomers led to lower C=C conversion.[4]

Irrespective of the co-initiator tested, all adhesives showed a reduction in bond strength values after 1.5 and 3 years of storage in water. This reduction may be associated with the degradation of the hybrid layer due to hydrolytic phenomena. The polymer absorbs water which triggers a relaxation of polymer chains, causing a reduction in bond strength.[35] In addition, the ester group of the methacrylate monomers undergoes hydrolysis when the medium is aqueous and acidic, as for example in self-etching adhesive systems.[36] Another factor accounting for the decrease of the bond strength is the extracellular matrix metalloproteinases (MMPs). These enzymes have been associated with the degradation of the collagen, which forms the hybrid layer. They are endogenous enzymes that are released from the matrix and activated. The inhibition of these enzymes by the host is of great importance to the longevity of adhesive restorations.[37,38]

Bond strength tests are limited in terms of extrapolation of data for clinical performance. These tests help to select between different compositions of material. Among the bond strength tests, the microtensile test has a uniform distribution of stresses and has the ability to correlate with clinical retention data. Storage of specimens in water should be performed to evaluate possible degradation of the material. The microtensile test can be used to evaluate the bond strength in clinically critical areas, such as the cervical Class II restorations. This critical area can be adopted as a reference to improve the composition and hence the effectiveness of the adhesive systems.[39]

The literature points out the need for further studies on water-compatible photo-initiator systems.[40] The use of BDO and PA promoted better adhesion to the substrate even over time, making them viable alternatives. These co-initiators exhibit water-compatible characteristics, which are desirable to overcome the challenges associated with moist environments. Furthermore, they may present the advantage of reduced toxicity during manufacture, as well as reduced production of toxic waste. These can be considered potential clinical and environmental implications related to the use of the alternative co-initiators tested here. However, one should keep in mind that these findings are still restricted to the *in vitro* performance of the molecules tested and further evaluation is required for a better assessment of the feasibility of using these components in actual dental adhesive systems.

Conclusion

This study shows that benzodioxole derivatives can be used as an alternative to the conventional tertiary amine co-initiators in CQ-based, self-etch dental adhesive formulations. After prolonged storage, adhesives with 1,3-benzodioxole or piperonyl alcohol showed higher dentin bond strengths than ethyl 4-dimethylamino benzoate-containing adhesives.

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