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Effect of MDP concentration and addition of iodonium salt on the dentin bond strength of experimental adhesives

Efeito da concentração de MDP e adição de sal de iodônio na resistência de união à dentina de adesivos experimentais

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ABSTRACT

Objective: The aim was to evaluate the bond strength of six experimental adhesives containing binary or ternary photoinitiator systems, associated with three different MDP concentrations (0-12wt%) after 12 months of storage in distilled water. **Material and Methods:** Experimental adhesives were prepared with: bis-GMA, UDMA, bis-EMA, TEGDMA, HEMA, BHT, and ethanol, containing binary (CQ/amine) or ternary (CQ/amine/DPIHFP (diphenyliodonium hexafluorophosphate) – 0.5wt%) photoinitiator systems. It was added to this composition 0wt%, 6wt%, or 12wt% of MDP. The adhesive systems were applied following the etch-and-rinse protocol. Transparent cylindrical molds were placed on the hybridized dentin, filled with composite resin and light-cured. The specimens were stored in distilled water at 37°C for 24h or 12 months and submitted to a microshear bond strength test (n=6). Data were analyzed by three-way ANOVA and Tukey's test (α=5%). **Results:** For the binary system, after 12 months of storage, all bond strength values were similar. Regarding bond strength degradation over time, 6wt% and 12wt% MDP formulations showed more stable bond strength than 0wt% MDP. For the ternary system, all formulations (0wt%, 6wt% and 12wt%) showed stable bond strength over time. **Conclusion:** For the binary photoinitiator system, the 6wt% and 12wt% MDP concentrations were able to keep bond strength stable over time, while for the ternary system, bond strength stability was achieved regardless the MDP concentration.

KEYWORDS

Dental bonding; Dental photoinitiators; Dentin-bonding agents; MDP.

RESUMO

Objetivo: O objetivo foi avaliar a resistência de união de seis adesivos experimentais contendo sistemas fotoiniciadores binários ou ternários, associados a três diferentes concentrações de MDP (0-12% em peso) após 12 meses de armazenamento em água destilada. **Material e Métodos:** Adesivos experimentais foram preparados com: bis-GMA, UDMA, bis-EMA, TEGDMA, HEMA, BHT e etanol, contendo sistemas fotoiniciadores binários (CQ/amina) ou ternários (CQ/amina/DPIHFP – 0,5% em peso). Foi adicionado a esta composição 0%, 6% ou 12% em peso de MDP. Os sistemas adesivos foram aplicados seguindo o protocolo com condicionamento ácido. Moldes cilíndricos transparentes foram colocados sobre a dentina hibridizada, preenchidos com resina composta e fotopolimerizados. Os corpos-de-prova foram armazenados em água destilada a 37°C por 24h ou 12 meses e submetidos ao ensaio de microcisalhamento (n=6). Os dados foram analisados por ANOVA de três fatores e teste de Tukey (α=5%). **Resultados:** Para o sistema binário, após 12 meses de armazenamento, todos os valores de resistência de união foram semelhantes. Em relação à degradação da resistência de adesão ao longo do tempo, as formulações de MDP de 6% e 12% mostraram resistência de união mais estável do que 0% de MDP. Para o sistema ternário, todas as formulações (0%, 6% e 12% em massa de MDP) mostraram resistência de união estável em função do tempo. **Conclusão:** Para o sistema binário, as concentrações de 6 e 12% em massa de MDP foram capazes de manter a resistência de união em função do tempo, enquanto que para o sistema ternário, a estabilidade de união foi obtida independentemente da concentração de MDP. Pode-se concluir que, para a formulação dos adesivos utilizados neste estudo, independente do sistema fotoiniciador, a concentração de 12% MDP apresentou valores de resistência de união mais estáveis após 12 meses de armazenamento em água.

PALAVRAS-CHAVE

Adesão dental; Fotoiniciadores dentais; Sistemas adesivos; MDP.

INTRODUCTION

The in vitro and clinical performance of different adhesive systems in relation to their bond strength, degradation of the hybrid layer and decrease in bond strength over time may vary significantly, depending on the composition and inclusion of functional monomers in their formulation [1, 2]. The inclusion of acidic monomers in self-etching and universal adhesive systems, such as 10-methacryloyloxydecyl dihydrogen phosphate (MDP), allow them to demineralize and simultaneously infiltrate the dentin to form the hybrid layer. The chemical and morphological characteristics at the bonding interface and the quality of the hybrid layer depend on the interaction between these acidic monomers and the dentin substrate [3].

In general, the interaction between adhesive systems and demineralized dentin is predominantly micromechanical. However, the inclusion of acidic monomers such as MDP in the formulations has also permitted occurrence of chemical interaction, through the bonding of these monomers with Ca^{+2} ions deposited under the collagen network or in the hybrid layer, resulting in MDP-Ca salt [4]. Thus, the MDP acidic monomer may form stable calcium-phosphate complexes and be in the form of a regular layer structure on hydroxyapatite surface. These MDP-Ca salts exhibit high hydrolytic stability, which can be attributed to the presence of long and relatively hydrophobic chains of MDP monomer. The unique chemical structure of MDP and the calcium-stable chemical bond present in hydroxyapatite are important factors contributing for bond durability as well as to improve the initial bond strength of self-etching adhesive systems [2, 4, 5]. Universal adhesives also contain acidic monomers in their composition [6, 7] and the function of MDP is not yet completely comprehended when these universal adhesives are applied using the etch-and-rinse mode. It has been reported that the hydrophobic aliphatic portion of the MDP can chemically interact with the collagen present in the demineralized dentin layer [8].

The inclusion of MDP in the composition of adhesive systems has contributed to the increase of bond strength and clinical longevity, also decreasing the degradation of the hybrid layer over time [5, 9, 10]. However, little is known about the ideal concentration of this monomer in the different formulations of adhesive systems to have the aforementioned benefits and advantages [11-14].

The most contemporary adhesive systems and resin composites have camphorquinone (CQ) as photoinitiator and a tertiary amine, such as ethyl 4-dimethylamino benzoate (EDMAB) or dimethylaminoethyl methacrylate (DMAEMA), as a co-initiator. This is known as a binary photoinitiator system (CQ/amine). The CQ, despite being an efficient photoinitiator, has an intense yellowish color, which limits its use in higher concentrations in materials for aesthetic restorations and it can compromise the color stability of restorative materials [15, 16]. In order to improve the optical properties of adhesive systems and resin composites, the use of alternative photoinitiators such as bisacylphosphine oxide (BAPO), phenylpropanedione (PPD) and diphenyliodonium hexafluorophosphate (DPIHFP) have been proposed [17-20]. Thus, the use of ternary photoinitiator systems, in which iodonium salts are added to traditional CQ/amine photoinitiator system, has increased in the last years [17, 18, 19, 21]. The use of DPIHFP and diphenyliodonium chloride as co-initiators in adhesive systems is advantageous, due to their ionic character and miscibility in aqueous medium [22]. Since adhesive systems have hydrophilic character and may be prone to phase separation, iodonium salts may be of interest in improving the polymerization of polar monomers in dental adhesives [23].

Furthermore, there is the possibility that the acidic monomers of the adhesives interact with the amines. The acid-base reaction between the acid monomers and amines can reduce the concentration of the latter components, by decreasing the amount of free radicals formed. In this case, the polymerization efficiency of these adhesives can be compromised [1, 24]. Thus, it is important to study alternative photoinitiators for different adhesive systems that contain acidic monomers to avoid unwanted interference with other components and decrease of polymerization efficiency [25]. The aim of this study was to evaluate the bond strength of six experimental adhesives containing binary (CQ/amine) or ternary (CQ/amine/DPIHFP) photoinitiator systems, associated with three different MDP concentrations (0wt% 6wt% or 12wt%) after 12 months of storage in distilled water at 37°C. The null hypotheses were: i) the different MDP concentrations would not influence the bond strength of the experimental adhesives to dentin; ii) the addition of diphenyliodonium as a photoinitiator would not influence the dentin bond strength of the experimental adhesives, and iii) the storage time would not influence the bond strength of the experimental adhesives tested.

MATERIALS AND METHODS

Three factors were evaluated: photoinitiator system (in two levels), MDP concentration (in three levels), and storage time for microshear bond strength evaluation (in two levels). Six experimental adhesives were prepared with and without DPIHFP as shown on Table 1. To these compositions 0wt%, 6wt% or 12wt% of MDP were added.

Table 1 - Composition by weight of the experimental adhesives with binary and ternary photoinitiator systems

	Binary (CQ/amine)	Ternary (CQ/amine/DPIHFP)
CQ	0.5	0.5
DABE	1.0	1.0
BHT	0.2	0.2
DPIHFP	0.0	0.5
HEMA	10.1	10.0
TEGDMA	15.1	15.0
Bis-EMA	25.1	25.0
Ethanol	10.0	10.0
UDMA	25.1	25.0
Bis-GMA	12.9	12.8

HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: glycerolate dimethacrylate; DABE: 1,2 diaminobenzene; CQ: camphorquinone; BHT: butylated hydroxytoluene; DPIHFP: diphenyliodonium hexafluorophosphate; TEGDMA: triethylene glycol dimethacrylate; Bis-EMA: ethoxylated bisphenol A dimethacrylate; UDMA: urethane dimethacrylate.

Seventy-two freshly extracted human molars were used after approval of the Institutional Review Board (CAAE 65473117.0.0000.0093, approval protocol 2.009.823). The crowns were separated from the roots, cut in half in the mesiodistal direction and each half was included in PVC cylinders (1.2x2.5 cm) with self-curing acrylic resin (Jet, São Paulo, Brazil). For smear layer standardization, dentin surfaces were wet-sanded with 600-grit silicon carbide paper (Buehler MetaServ 250, Lake Bluff, IL, USA) for 1 minute under constant water irrigation.

The dentin surfaces were randomly distributed in the experimental groups, according to the adhesive system used (photoiniciatior system and MDP concentration). Each adhesive system was applied with the following protocol: i) etch with 37% phosphoric acid (Condac 37, FGM, Joinville, SC, Brazil) for 15 s; ii) apply air/water spray for 10 s; iii) remove rinsing water with absorbent paper; iv) apply two adhesive layers, remove solvent with dry air from an air-water syringe for 10 s; and v) light activation for 15 s. Light activation was performed with a LED curing unit (Radii-cal, SDI, Victoria, Australia) with output irradiance of 1200 mW/cm^2.

Cylindrical transparent molds with 1.4 mm in diameter and 1 mm in height were placed on hybridized dentin surfaces and the internal volume was filled with a resin composite (Charisma Diamond, Kulzer, Hanau, Germany – shade A1). The resin composite cylinders were light activated for 25 s. Two or three composite resin cylinders were bonded in each dentin surface. After 10 min, the molds were removed to expose resin composite cylinders with a bonding area of 1.54 mm^2. Specimens were stored in distilled water at 37°C for 24 h or 12 months.

Microshear bond strength (μSBS) (n=6) was evaluated with a wire-loop method on a universal testing machine (DL2000, EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min. Teeth were aligned to allow the loading steel wire-loop (0.2 mm diameter) to be placed as close as possible to the bonded interface at the base of the resin composite cylinders. The μSBS (in MPa) was calculated by dividing the maximum force (in N) by the bonded area (in mm^2). Debonded surfaces were examined under a stereomicroscope with 57x magnification (SZX9, Olympus, Tokyo, Japan) to determine the failure mode, classifying them as adhesive (at the bonding interface), cohesive (in dentin or in resin composite) or mixed failures.

The mean bond strength of all resin cylinders from the same hemi-tooth was averaged for statistical purposes; and each hemi-tooth was considered an independent experimental unit for the statistical analysis. The μSBS data were analyzed by three-way ANOVA and Tukey's test with a significance level of 5%. Post-hoc power analysis was also performed and the observed power was 0.816.

RESULTS

For both photoinitiator systems, the results of dentin bond strength as a function of MDP concentration and storage time are described in Table 2. The results showed statistically significant differences for the storage time (p=0.009). MDP concentration and photoinitiator system were not statistically significant (p=0.890 and p=0.525, respectively). Only the double interaction MDP concentration*photoinitiator systems and the triple interaction were statistically significant (p=0.032 and p=0.008, respectively).

Table 2 - Mean values and standard deviations for the bond strength values according to photoiniciator system, MDP concentration, and storage time

Photoinitiator system	% MDP	Bond strength (MPa)	
		24 h	12 months
Binary (CQ/amine)	0	10.04±6.34 ^{a}	3.86±1.80 ^{b}
	6	4.98±2.64 ^{ab}	5.19±0.80 ^{ab}
	12	5.06±1.98 ^{ab}	5.95±2.26 ^{ab}
Ternary (CQ/amine/ DPIHFP)	0	4.07±1.27 ^{ab}	3.52±1.61 ^{b}
	6	9.13±4.93 ^{ab}	3.72±1.17 ^{b}
	12	6.29±2.34 ^{ab}	5.59±1.49 ^{ab}

MDP: 10-methacryloyloxydecyl dihydrogen phosphate; Values followed by the same superscript letters are statistically similar (p>0.05).

For the binary system (CQ/amine), all bond strength values were similar considering the storage times of 24 h and 12 months. Regarding bond strength degradation over time, 6% and 12% MDP formulations showed more stable bond strength than 0% MDP (bond strength reduction of 62%). For the ternary system (CQ/amine/DPIHFP), after 24 h and 12 months of storage, all bond strength values were similar. Formulations with 0% and 12% MDP showed more stable bond strength (bond strength reductions of 14 and 11%, respectively) than the adhesive with 6% MDP (bond strength reduction of 62%).

The results of the failure mode analysis are described in Figure 1. For all groups evaluated, the failures were predominately adhesive (between 60 and 100%), followed by mixed failures (between 0% and 40%). Cohesive resin failures were found in a few groups and ranged from 0 to 7%.

DISCUSSION

Previous studies have shown that the inclusion of MDP provides an additional chemical interaction by bonding with calcium ions and forming the acid-resistant zone, improving bond strength and positively interfering with adhesive process [13]. The MDP was classified as the most promising monomer for chemical bonding to hydroxyapatite of enamel or dentin. In fact, adhesives containing MDP showed favorable bond strength, particularly in relation to long-term adhesion durability [5, 14].

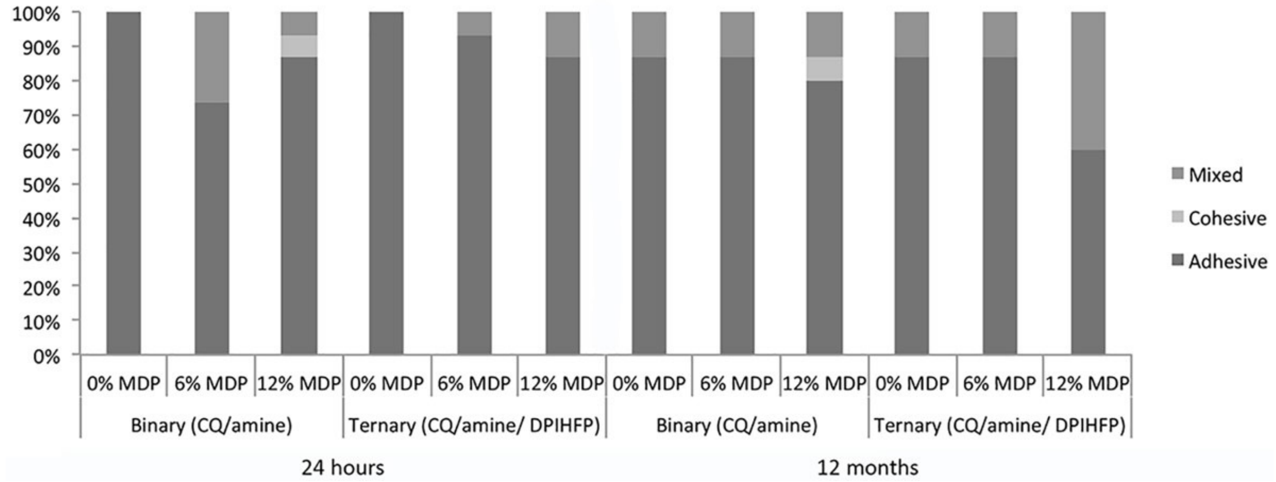

Figure 1 - Distribution of failure modes found after microshear bond strength test for all groups.

These positive results suggest the formation of nano-layers of MDP-Ca salts, which can protect the adhesive interface against hydrolytic degradation. The benefits of this layer include the protection of collagen fibrils from water-induced degradation due to its hydrophobicity, increasing the resistance of residual apatite crystals to acid dissolution and creating a more gradual transition between substrate and adhesive [26]. On the contrary, MDP is a viscous acidic monomer and its increased concentration in the adhesive formulation can result in a material with decreased fluidity and flow, causing partial penetration into the demineralized dentin layer [14].

Furthermore, as MDP is a functional monomer with hydrophilic and hydrophobic portions, its incorporation may bring more hydrophilic characteristics in an adhesive system. Acidic functional monomers may contribute to the increase of initial bond strength to dentin, but would also increase the water sorption of the polymerized adhesive in the long term [12, 25, 27].

The ideal concentration of MDP for different adhesives is not known yet. The monomeric composition, amount and nature of solvents, pH and mode of application should influence the ideal proportion of MDP, interfering in the quality of the hybrid layer and in the acid-resistant zone formed [28, 29]. Few studies have evaluated the effect of different concentrations of MDP on dentin adhesives [11-14], however the results are not clear and it was not possible to establish the ideal concentration of MDP for different adhesive formulations. In general, the studies reported the use of experimental adhesives with MDP concentration ranging from 0% and 15% [11, 13, 14, 30]. Therefore, the design of the present study included experimental adhesives formulated with MDP percentages of 0, 6 and 12%. Concentrations between 10% and 15% of MDP have shown better results in relation to bond strength [14]. This previous study also investigated an experimental adhesive with MDP concentration greater than 15% (20%). In this case, when the MPD percentage was increased to 20%, there was not an improvement in the bond strength values to dentin [14]. Thus, the first null hypothesis, which stated that the different MDP concentrations would not influence the bond strength of the experimental adhesives to dentin, was accepted. In the present study, there was no difference in bond strength when different concentrations of MDP were used.

In self-etching and universal adhesive systems, different functional monomers are added to the formulations. Universal adhesives are similar to single bottle one-step self-etch adhesives systems that can be used with the etch-and-rinse, self-etching, and selective enamel-etching bonding approaches [6, 7]. The experimental adhesive used in the present study can be considered as a universal adhesive system, because it contains a functional monomer in its composition, in addition to being used in the etch-and-rinse strategy [31]. A recent study evaluated the influence of different MDP concentrations (0 wt% to 15 wt%) on the bond strength to dentin of the experimental adhesives with similar compositions to the ones used in the present study, when used in etch-and-rinse and self-etching approaches. Higher bond strength values were observed for the etch-and-rinse mode, regardless of MDP concentration [31]. Thus, despite including an acidic monomer in its composition, and based on these previous results, the experimental adhesives used here were applied using the etch-and-rinse approach. Also, one of the experimental adhesives evaluated in the present study was formulated without MDP in its composition (0%); in this way, this group could not be applied using the self-etching approach.

Although the self-etching mode may seem more favorable for dentin bonding, some studies investigated the bond strength of universal adhesives to dentin using the etch-and-rinse approach. Takamizawa *et al.* demonstrated that the shear bond strength and shear fatigue strength of universal adhesives showed equivalent bonding quality to dentin regardless of etching mode. They also concluded that, for the universal adhesives, total-etch mode did not have a negative impact on dentin bond quality [32]. The dentin bond durability of two universal adhesives with and without MDP in the etch-and-rinse mode after thermocycling and water storage has also been investigated [33]. The results showed that the adhesive containing MDP (Clearfil Universal Bond Quick) had significantly higher shear bond strength than the experimental adhesive with the same composition, but without MDP, regardless of the application mode or degradation method. These results confirm the authors' hypothesis that MDP can affect the quality of bond when used with the etch-and-rinse approach after in vitro aging [33]. The role of MDP is not yet completely understood when universal adhesives are used with the etch-and-rinse approach. However, it has been reported that MDP has a stable chemical interaction with the collagen present in the demineralized dentin layer. This interaction seems to be related to a hydrophobic effect between the hydrophobic aliphatic portion of the MDP monomer and the collagen surface [8]. Hidari *et al.* also demonstrated an effective penetration of the MDP-containing universal adhesive in deeper and etched regions of dentin, when applied in etch-and-rinse mode [33]. In a review that discussed current concepts related to adhesive systems and adhesive materials related especially to their clinical use, it has been concluded that, for universal adhesive systems, etch-and-rinse and selective enamel etching strategies have resulted in excellent clinical behavior [6].

It is important to have in mind that adhesive systems are complex mixtures, containing numerous different monomers, as well as other components. HEMA is a water-soluble monomer that is frequently added to adhesives to improve dentin wetting and increase bond strength [1]. However, it has been demonstrated that HEMA significantly affected the chemical interaction of MDP with hydroxyapatite, reducing nano-layering and the hydroxyapatite-demineralization rate, hindering the formation of MDP-Ca salts. Although HEMA does not completely inhibit the chemical interaction of MDP with hydroxyapatite, the addition of low concentrations of this hydrophilic monomer (8%) was sufficient to interfere with nano-layering [34].

Different photoinitiators can be used in resin-based materials. The photoinitiators can be classified as type I, which are represented by cationic molecules undergoing unimolecular dissociation without the necessity of synergistic amine; and type II, which exhibit the formation of radicals by means of electronic exchange with a co-initiator, generally represented by an amine. In the latter situation, which characterizes a binary system, the CQ requires a tertiary amine reducing agent for efficient polymerization. The CQ should be considered as an initiator of hydrophobic characteristics. Therefore, it is preferentially distributed in the hydrophobic domains of the adhesive systems [23]. The diphenyliodonium salt is characterized by an ionic nature and, as a result, may enhance the compatibility between amphiphilic monomers and initiators, especially in the presence of water[23]. In general, this salt has been associated with the CQ/amine forming a ternary photoinitiator system, acting as an electron scavenger from the dye radical [35].

Previous studies have shown that the addition of the diphenyliodonium salt in the CQ/amine system accelerates the rate of polymerization and improves the degree of conversion, glass transition temperature and crosslink density, as well as some other mechanical properties of the adhesive, especially when the polymerization reaction occurs in a humid environment [23, 36].

The physical properties and the bond strength of the adhesive systems can be affected by their composition, photoinitiator system and interaction between the components. Tertiary amines, for example, can be neutralized by the functional acidic monomers, such as MDP. Thus, acidic monomers can inhibit polymerization by decreasing the conversion of adhesives containing the CQ/amine binary system [30]. Therefore, when the adhesive formulation includes acidic monomers, the addition of a third component in the CQ/amine system, such as iodonium salts, could improve the polymerization.

Few studies have evaluated experimental adhesives containing both MDP and diphenyliodonium salt. The addition of diphenyliodonium salt in an experimental adhesive based on UDMA and MDP showed an increase in the reaction rate and monomer conversion. A decrease in nanoinfiltration was also observed in the interfaces produced with the iodonium salt-containing adhesive [19]. However, the benefits of DPIHFP were not observed for bond strength [19]. Even though there was no immediate difference in bond strength values, it can be hypothesized that the ionic nature of diphenyliodonium salt could increase the compatibility between monomers and initiators, and the higher degree of conversion could improve the mechanical properties of the adhesive layer.

The ternary CQ/amine/DPIHFP system has emerged in the last decade as a possibility of improvement over the binary CQ/amine photoinitiator system. The additional iodonium salt is a hydrogen-accepting compound that can revert the chain-terminating CQ ketyl radical (CQH·) to the original initiator CQ, while generating a highly active and initiation-capable phenyl radical (Ph·) [37]. The triad of terminal radical consumption, regeneration of the photosensitizer and production of additional reactive radicals would contribute to increase the polymerization efficiency of the ternary system [37]. In the present study, although the addition of DPIHFP showed no increase in bond strength, the incorporation of iodonium salt did not affect the polymerization of the experimental adhesive. Thus, the second hypothesis, that the addition of diphenyliodonium as a photoinitiator would not influence the dentin bond strength of the experimental adhesives, was also accepted.

The third null hypothesis was rejected, since the storage time influenced the bond strength, depending on the MDP concentration and photoinitiator present in the experimental adhesive. The literature reports many methods for aging the adhesive interfaces. Among these methods, the most used are storage in water or artificial saliva for long periods, thermocycling or mechanical cycling. For adhesive interfaces, it has been suggested that one of the most efficient ways of aging is through storage in water for at least three months [38]. It is well known that the main deleterious factor for polymeric materials, which can compromise their integrity and clinical longevity, is the hydrolysis and degradation of the components [38]. Water and solvents trapped in the hybrid layer prevent the formation of a highly cross-linked polymer within the collagen fibrils network [39], probably due to separation of hydrophobic and hydrophilic phases, making the polymer weaker that those formed in a water-free environment [40]. This subpolymerized polymer is more prone to the plasticizing effects of water over time and renders the adhesive layer more permeable to water in the oral environment and the underlying dentin [41].

Based on the results shown in Table 2, the groups with 6% and 12% of MDP (binary photoinitiator system) maintained the bond strength after 12 months of storage in water. In the groups with the ternary photoinitiator system, in the presence of DPIHFP, all groups with and without MDP preserved their bond strength after 12 months of aging in water.

One of the limitations of the present study is the use of an experimental adhesive. The use of an experimental formulation can make it more difficult to extrapolate the results for commercial adhesive systems and immediate clinical application, but it also has important advantages, such as a more objective response in relation to the factors evaluated, since the material has a known and controlled composition.

It can be concluded that, for the binary photoinitiator system, the 6wt% and 12wt% MDP concentrations should be applied as both maintained bond strength stable over time. On the other hand, the ternary photoinitiator system was more stable over time, as the concentration of MDP did not influence the long-term bond strength.

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Author Contributions

GRK: Performed the study in partial fulfillment of requirements for MSc degree, wrote draft of manuscript and commented on manuscript at all stages. AYF: Study design, analyzed the data, wrote the manuscript, discussed the results and commented on manuscript at all stages. RMC: Prepared the experimental adhesives, discussed the results, contributed to discussion section and reviewed the manuscript. FSL: Analyzed the data, discussed the results, and commented on manuscript at all stages. GMC: Analyzed the data, discussed the results and commented on manuscript at all stages. CCG: Study design, analyzed the data, wrote the manuscript, discussed the results and commented on manuscript at all stages.

Conflicts of Interest

No conflicts of interest declared concerning the publication of this article.

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Regulatory Statement

The approval code for this study is CAAE 65473117.0.0000.0093, approval protocol 2.009.823.

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