UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

MAYARA HANA NARIMATSU

Influence of the concentration of 10-metacryloxydecyl dihydrogen phosphate (10-MDP) on the degree of conversion of experimental adhesives applied with different manipulation protocols

Influência da concentração de 10-metacriloiloxidecil dihidrogeniofosfato (10-MDP) no grau de conversão de adesivos experimentais aplicados com diferentes protocolos de manipulação

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Orientador: Prof. Dr. Adilson Yoshio Furuse

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DEDICATÓRIA

À Deus, que nos criou e foi criativo nesta tarefa. Seu fôlego de vida em mim me foi sustento e me deu coragem para questionar realidades e propor sempre um novo mundo de possibilidades.

À minha família, vocês são e sempre serão um motivo de orgulho e inspiração para perseverar na batalha pelo amor. Com toda imperfeição vocês são perfeitos para mim.

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Não sou obrigado a vencer mas tenho o dever de ser verdadeiro. Não sou obrigado a ter sucesso mas tenho o dever de corresponder à luz que tenho.

Abraham Lincoln

ABSTRACT

Influence of the concentration of 10-metacryloxydecyl dihydrogen phosphate (10-MDP) on the degree of conversion of experimental adhesives applied with different manipulation protocols

Purpose: To evaluate the influence of different concentrations of 10-MDP in degree of conversion (%DC) of experimental adhesives (EA) applied with different manipulation protocols (MP).

Materials and Methods: EA containing a three-component-photoinitiator system (CQ/DABE/iodonium salt) were prepared and different concentrations of 10-MDP were added A1 = 0%, A2 = 3%, A3 = 6%, A4 = 9%, A5 = 12%, or A6 = 15% by weight. A commercially available adhesive was used as comparison (Ambar, FGM). The adhesives were divided into 4 MP: I) Control (without any manipulation), II) 5 s of evaporation, III) 20 s of evaporation and IV) addition of water to the adhesive. DC was evaluated by FTIR-ATR. The data were analyzed with two-way Anova and Tukey´s HSD ($\alpha = 0.05$).

Results: Significant differences were found between the adhesives ($p < 0.0001$) and MP ($p = 0.000001$). An interaction effect ($p < 0.0001$) was also observed. For adhesives, it was observed that the A5 showed the highest value (89.70% ± 4.21) and the lowest values to EA without MDP (76.35% ± 8.45). Ambar showed intermediate values (83.68% ± 9.46). For MP, the highest DC was observed to Control (88.50% ± 2.72) > 20-s-evaporation (85.13% ± 6.95) ≈ 5-s-evaporation (85.18% ± 6.08) ≈ addition of water (85.80% ± 10.33).

Conclusion: The results suggest that 10- MDP can enhance the %DC of the three-component-photoinitiator system EAs evaluated.

Keywords: Fourier transform infrared spectrophotometer (FTIR), degree of conversion, adhesive, 10-MDP, iodonium salt, solvent evaporation, water.

RESUMO

Influência da concentração de 10-metacriloiloxidecil dihidrogeniofosfato (MDP) no grau de conversão de adesivos experimentais aplicados com diferentes protocolos de manipulação

Propósito: Avaliar a influência de diferentes concentrações do 10-MDP no grau de conversão (%GC) de adesivos experimentais (AE) em diferentes protocolos de manipulação (PM).

Material e Métodos: Os AE contêm um sistema iniciador triplo (CQ/DABE/sal diodônio) e diferentes concentrações de 10-MDP A1 = 0%, A2 = 3%, A3 = 6%, A4 = 9%, A5 = 12% ou A6 = 15% em peso foram adicionadas. Um adesivo comercialmente disponível foi utilizado para comparação (Ambar, FGM). Os adesivos foram divididos em 4 PM: I) Controle (sem manipulação), II) 5 s de evaporação, III) 20 s de evaporação e IV) adição de água. O GC foi avliado com o FTIR-ATR. Os dados foram analisados com Anova a dois critérios e Tukey (α = 0,05).

Resultados: Diferenças significativas foram encontradas entre os adesivos ($p < 0.0001$) e PM ($p = 0.000001$). Também foi encontrado um efeito de interação ($p < 0.0001$). Para os adesivos, foi observado que o A5 obteve o maior resultado (89.70% ± 4.21) e o com menor valor foi o AE sem MDP (76.35% ± 8.45). O Ambar obteve valores intermediários (83.68% ± 9.46). Para o PM, o maior valor foi para: Controle (88.50% ± 2.72) > 20-s-evaporação (85.13% ± 6.95) ≈ 5-s-evaporação (85.18% ± 6.08) ≈ adição de água (85.80% ± 10.33).

Conclusão: Os resultados obtidos sugerem que o 10- MDP pode aumentar a %GC dos AE com o sistema iniciador triplo.

Palavras chave: Espectroscopia Transformada de Fourier Infravermelho (FTIR), grau de conversão, adesivo, 10-metacriloiloxidecil dihidrogêniofosfato (MDP), evaporação de solventes, água.

TABLE OF CONTENTS

1 Introduction

1 INTRODUCTION

The adhesive dentistry has been increasingly used in restorative treatments, whether for direct or indirect procedures, because it allows the preservation of tooth structure since classical retention forms based on cavity configurations and prosthetic preparations are no longer essential for restoration to be maintained in position. Thus, understanding the use of adhesive systems has become critical to a good practice of Restorative Dentistry. However, the characteristics of the materials available are essential to provide a good performance and longevity to the restorations.

There are many variables related to the adhesion of the resin-based materials to dental structures such as dental substrate, adhesive system and adhesive technique. It is known that the adhesion to enamel is durable because it is a highly mineralized homogeneous substrate composed in essence by hydroxyapatite. The adhesion in dentin, however, has not shown the same results. This is mainly due to the heterogeneous nature of this type of substrate and the constant presence of water.(5, 24)

Various adhesive systems with different characteristics are available in the market. Depending on the prior application or not of phosphoric acid on the dental substrate, adhesives can be classified into conventional or self-etching. Conventional adhesives require the prior conditioning with phosphoric acid. These adhesives can be further classified according to the number of operative steps in three steps (phosphoric acid, primer and adhesive) and two steps (phosphoric acid and adhesive). The self-etching adhesives have acidic monomers in their composition and dispense acid etching and can be classified into two steps (acidic primer and adhesive) and one step self-etching adhesives (all components into a single solution).(5) It has been observed that some etch systems with moderate pH (pH ≈ 2) are able to dissolve only a part of the dentin surface, causing a substantial amount of hydroxyapatite crystals remain within the hybrid layer. Thus, the carboxylic groups and phosphate acid monomers of these adhesives can chemically interact with this residual hydroxyapatite (HAp).(27) In terms of durability of the adhesive interface, the micromechanical and chemical adhesion mechanism could be advantageous. The 10-metacryloxydecyl dihydrogen phosphate (10-MDP) is a typical functional phosphate-based acid monomer, that has the potential to interact with HAp and can contribute significantly to the bond durability of dentin-resin interface (6, 28). Thus, considering the differences between the adhesive system available in the market, different behaviors are expected.(3, 7, 17, 21-23)

It is well known that light-cured resin-based materials commonly contain camphorquinone (CQ) and tertiary amines as a two-component-photoinitiator system for allowing visible-light-initiated free-radical polymerization. On the other hand, it has been suggested that the inclusion of a third component to the CQ/amine photoinitiating system such as iodonium salts could improve the photo-activation induced by visible light sources.(4) However, it has been suggested that the polymerization of CQ/amine-based adhesives may be negatively affected by the interaction between functional monomers such as 10-MDP and tertiary amines.(9) Therefore it is important to evaluate how the incorporation of 10-MDP can influence the properties of the adhesive systems.

The dental adhesives are hydrophilic to strengthen their connection to intrinsically moist dentin. The hydrophilic monomers are usually dissolved in water and/or organic solvents such as acetone and ethanol. These organic solvents present in adhesive has the function of interacting with the water present in the network of collagen exposed after the etching to allow infiltration of the resin monomers.(13) Although the solvents are important for an appropriate bonding procedure, part of these solvents may remain trapped in the hybrid layer and damage the adhesive bond.(25, 26) In addition, the evaporation of solvents/water during the adhesive protocol can significantly influence the strength of the adhesive interface.(8) Thus, the present study aimed to evaluate the influence of the evaporation of solvents or water addition on the degree of conversion (% DC) of experimental dental adhesives containing a three-component photoinitiator (CQ/DABE/DPIHP) and different concentrations of 10-MDP as compared to a commercially available material. The following null hypotheses were evaluated:

- Different adhesives would not influence the % DC;
- The evaporation of solvents or the inclusion of water into adhesives would not influence the % DC;
- The amount of 10-MPD in the experimental adhesive would not influence the % DC.

2 ARTICLE

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Introduction

The adhesive dentistry has been increasingly used in restorative treatments, whether for direct or indirect procedures, because it allows the preservation of tooth structure since classical retention forms based on cavity configurations and prosthetic preparations are no longer essential for restoration to be maintained in position. Thus, understanding the use of adhesive systems has become critical to a good practice of Restorative Dentistry. However, the characteristics of the materials available are essential to provide a good performance and longevity to the restorations.

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Various adhesive systems with different characteristics are available in the market. Depending on the prior application or not of phosphoric acid on the dental substrate, adhesives can be classified into conventional or self-etching. Conventional adhesives require the prior conditioning with phosphoric acid. These adhesives can be further classified according to the number of operative steps in three steps (phosphoric acid, primer and adhesive) and two steps (phosphoric acid and adhesive). The self-etching adhesives have acidic monomers in their composition and dispense acid etching and can be classified into two steps (acidic primer and adhesive) and one step self-etching adhesives (all components into a single solution).[5] It has been observed that some etch systems with moderate pH (pH ≈ 2) are able to dissolve only a part of the dentin surface, causing a substantial amount of HAp crystals remain within the hybrid layer. Thus, the carboxylic groups and phosphate acid monomers of these adhesives can chemically interact with this residual HAp.[27] In terms of durability of the adhesive interface, the micromechanical and chemical adhesion mechanism could be advantageous. The 10-metacryloxydecyl dihydrogen phosphate (10-MDP) is a typical functional phosphate-based acid monomer, that has the potential to interact with hydroxyapatite and can contribute significantly to the bond durability of dentin-resin interface [6, 28]. Thus, considering the differences between the adhesive system available in the market, different behaviors are expected.[3, 7, 17, 21-23]

It is well known that light-cured resin-based materials commonly contain camphorquinone (CQ) and tertiary amines as a two-component-photoinitiator system for allowing visible-light-initiated free-radical polymerization. On the other hand, it has been suggested that the inclusion of a third component to the CQ/amine photoinitiating system such as iodonium salts could improve the photo-activation induced by visible light sources.[4] However, it has been suggested that the polymerization of CQ/amine-based adhesives may be negatively affected by the interaction between functional monomers such as 10-MDP and tertiary amines.[9]

Therefore it is important to evaluate how the incorporation of 10-MDP can influence the properties of the adhesive systems.

The dental adhesives are hydrophilic to strengthen their connection to intrinsically moist dentin. The hydrophilic monomers are usually dissolved in water and/or organic solvents such as acetone and ethanol. These organic solvents present in adhesive has the function of interacting with the water present in the network of collagen exposed after the etching to allow infiltration of the resin monomers.[13] Although the solvents are important for an appropriate bonding procedure, part of these solvents may remain trapped in the hybrid layer and damage the adhesive bond.[25, 26] In addition, the evaporation of solvents/water during the adhesive protocol can significantly influence the strength of the adhesive interface.[8] Thus, the present study aimed to evaluate the influence of the evaporation of solvents or water addition on the degree of conversion (% DC) of experimental dental adhesives containing a three-component photoinitiator (CQ/DABE/DPIHP) and different concentrations of 10-MDP as compared to a commercially available material. The following null hypotheses were evaluated:

- Different adhesives would not influence the % DC;
- The evaporation of solvents or the inclusion of water into adhesives would not influence the % DC;
- The amount of 10-MPD in the experimental adhesive would not influence the % DC.

Materials and methods

The present study was designed to evaluate two factors: adhesives (in seven levels) and manipulation protocol (four levels). Experimental adhesives (EA) were manipulated containing five different concentrations of 10-MDP added to a basic monomer composition. The basic composition (A1) of the adhesive consisted in weight of: camphorquinone - CQ (0,50%); 1,2-diaminobenzene - DABE (1,00%); butylhydroxytoluene - BHT (0,20%); iodonium salt diphenyliodonium hexafluorophosphate - DPIHP (0,45%); 2-hydroxyethyl methacrylate - HEMA (10%); triethylene glycol dimethacrylate - TEGDMA (15%); ethoxylated bisphenol A glycol dimethacrylate - Bis-EMA (25%); ETANOL (10%); urethane dimethacrylate - UDMA (25%); bisphenol A diglycidyl methacrylate - Bis-GMA (12,85%). To this basic composition was added 3% (A2), 6% (A3), 9% (A4), 12% (A5), or 15% (A6) by weight of 10-MDP. They were compared with one commercially available adhesive (Ambar, FGM, Joinville, SC, Brazil). This adhesive contains 10-MDP, UDMA, HEMA, methacrylates monomers acids, monomers hydrophilic methacrylates, silanized silicon dioxide, camphorquinone, ethyl 4-dimethylamino benzoate and ethanol.

Four manipulation protocols (MP) for each adhesives were evaluated: I) Control (without any manipulation); II) 5 seconds of evaporation; III) 20 seconds of evaporation; and IV) Addition of water to the adhesive. The addition of water in protocol IV was conducted to simulate the amount of water present between the collagen fibrils after acid etching of the dentin. For this purpose, for each 12 μl of adhesive 0.3 μl of water was added.

The evaluation of the % DC was conducted with a Fourier Transform Infrared Spectrophotometer - FTIR (IRPrestige-21, Shimadzu Corporation, Tokyo, Japan), with a resolution of 4 cm^{-1} and 32 scan in the range of 4000-400 cm^{-1}. An attenuated total reflection unit (ATR) was coupled to the spectrophotometer (Miracle ATR, Pike Technologies, Madison, USA). Twelve replicates for each condition were made (n = 12).

The amount of double bonds was determined using the absorption peaks of the double bonds of methacrylates before and after photo polymerization. The initial reading was performed by placing approximately 12 µl of the uncured material to cover the entire surface of the ATR crystal with the aid of a micropipette (Transferpette S, BRAND GMBH + CO KG, Wertheim, Germany). Immediately after putting on the crystal of the ATR, the dental adhesive was covered with a dark cap to prevent further evaporation of the solvent. After the initial reading of the non-polymerized material, the adhesive was cured with a LED device (Valo Cordless, Ultradent, South Jordan, UT, USA), operating at 1000 mW/cm^2 and 395 to 480 nm. A new reading was then performed. The temperature and relative humidity was monitored and controlled at 21°C ± 1°C and 30% ± 5%, respectively. The light-curing device was positioned with the aid of a support made with a condensation silicone material in order to standardize at approximately 5 mm the distance between the light source and the material.

For groups with evaporation of the solvent 0.05 ml of adhesive were inserted on a glass dappen dish and the air jet directed at standardized distance and pressure of 20 cm and 40 psi, respectively. Then 12 µl of this pre-evaporated adhesive was deposited over the ATR crystal and readings proceeded in the same manner already described. For the group of water addition (protocol IV), 0.3 µl of distilled water was added to 12 µl of adhesive and the mixture was deposited over the ATR crystal and readings proceeded in the same manner already described. The absorption peaks of the aromatic bonds were recorded in 1608 cm^{-1} (Abs. 1608), and the peaks of the aliphatic double bonds (C = C) were registered in 1636 cm^{-1} (Abs. 1636). The peak of aliphatic bonds decreases with curing, while the peak of the aromatic bonds remains unchanged, serving as a reference.

The percentage of remaining double bonds (% RDB) was determined according to the formula (1):

$$\% \, RDB = \left[\frac{(Abs\ 1636 / Abs\ 1608\ _{cured})}{(Abs\ 1636 / Abs\ 1608\ _{uncured})} \right] \times 100 \qquad (1)$$

The % DC of double bonds from FTIR-ATR was calculated by adopting the following formula (2):

$$\% \, DC = 100 - \% \, RDB \qquad (2)$$

Data were analyzed with two-way Anova considering adhesives and manipulation protocols as independent variables. The multiple comparisons were made with Tukey's HSD test. A global level of significance of 5% was adopted.

Results

Means and standard deviations of the % DC data are shown in Table 1. Significant differences were found between the adhesives ($p < 0.0001$) and between the handling protocols ($p = 0.000001$). An interaction effect between these variables ($p < 0.0001$) was also observed. When the factor adhesive was considered, the EA containing different concentrations of 10-MDP showed higher % DC values, with A5 showing the highest value (89.70% ± 4.21). The lowest values occurred with the EA without 10-MDP (76.35% ± 8.45). Ambar showed intermediate values (83.68% ± 9.46) (Figure 1A). When the factor manipulation protocol was considered, the highest % DC was observed with Control (88.50% ± 2.72), while no differences were observed for 20-s-evaporation (85.13% ± 6.95), 5-s-evaporation (85.18% ± 6.08) and addition of water (85.80% ± 10.33) (Figure 1B). Figure 2 shows mean values considering the interaction effect between adhesives and manipulation protocols. It can observed that the experimental adhesives containing 10-MDP were not influenced by the manipulation protocol.

Table 1 – Means and standard deviations of % DC. Means followed by the lowercase letters are not statistically different ($p > 0.05$)

Adhesives	Protocol			
	Control	**5 seconds**	**20 seconds**	**Water**
Ambar	85.55 ± 0.73 b	87.00 ± 4.04 bc	87.02 ± 2.27 bc	75.15 ± 15.91 a
A1	89.38 ± 3.50 bc	72.92 ± 4.28 a	70.75 ± 3.99 a	72.33 ± 3.09 a
A2	88.82 ± 2.36 bc	89.61 ± 5.95 bc	85.64 ± 2.37 b	88.42 ± 3.69 bc
A3	86.98 ± 1.02 bc	86.63 ± 1.79 bc	86.07 ± 3.89 b	90.28 ± 4.01 bc
A4	90.37 ± 3.66 bc	86.58 ± 2.46 bc	87.18 ± 1.25 bc	90.69 ± 4.02 bc
A5	88.07 ± 1.46 bc	87.30 ± 0.63 bc	90.66 ± 5.62 b	92.79 ± 4.61 c
A6	90.32 ± 1.90 bc	87.35 ± 0.85 bc	88.62 ± 2.84 bc	91.00 ± 3.90 bc

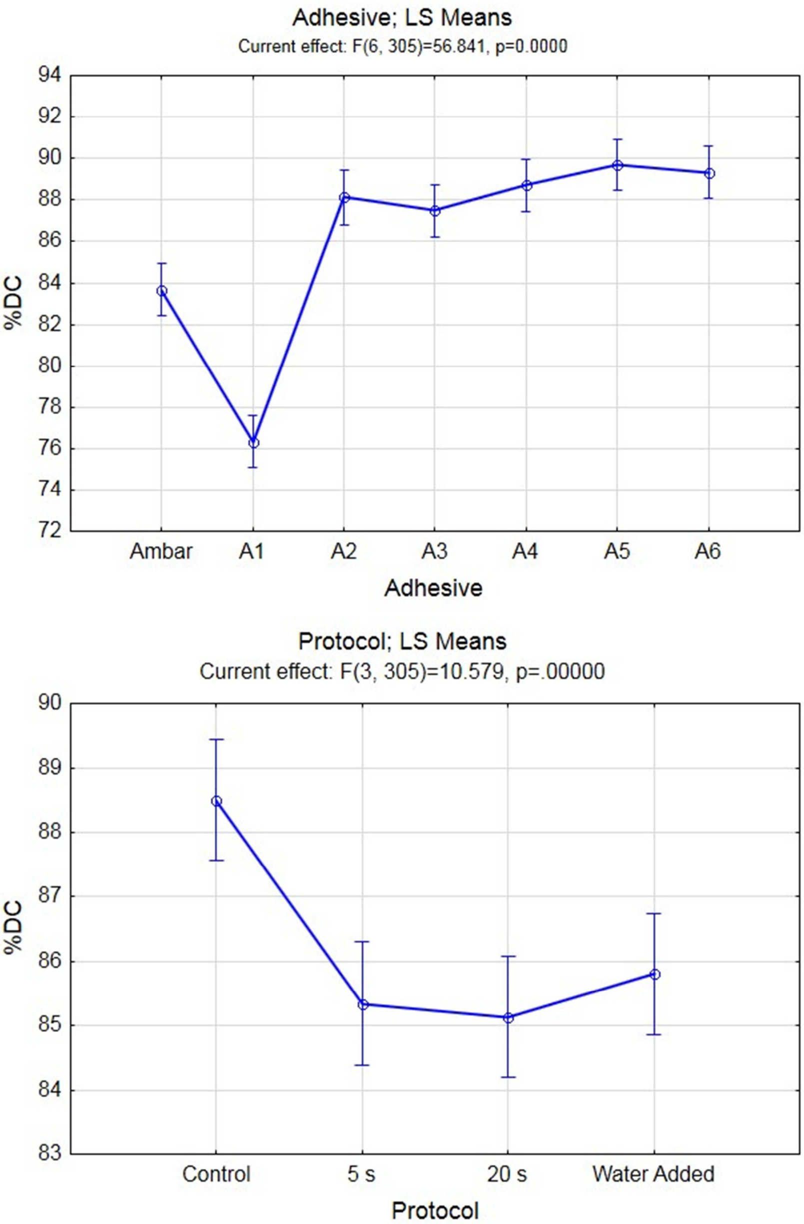

Figure 1 – Mean values considering independent variables adhesives (A) and manipulation protocols (B). Vertical bars denote 0.95 confidence intervals.

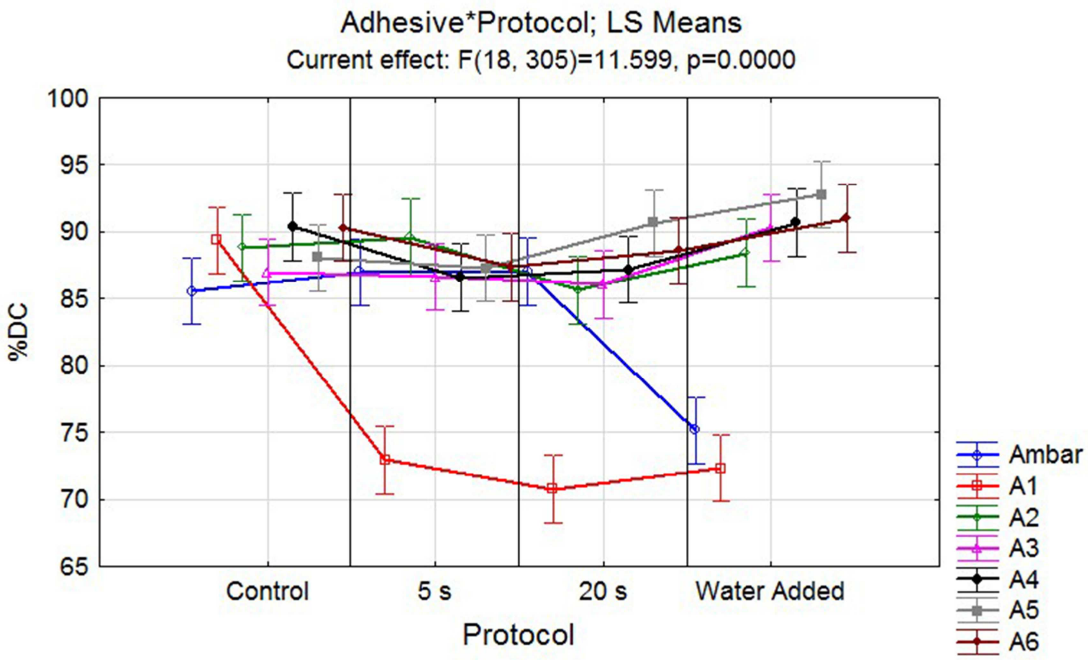

Figure 2 – Mean values considering the interaction effect between adhesives and manipulation protocols. Vertical bars denote 0.95 confidence intervals.

Discussion

The null hypothesis that different adhesives would not influence the % DC was rejected, as there were differences between adhesives. At the same time, the null hypothesis that the amount of 10-MDP added to the experimental adhesives would not influence the % DC was also rejected in a way that, the lowest % DC was observed without 10-MDP while the addition of 10-MDP significantly increased the % DC. This result is in disagreement with a recently published manuscript in which the % DC a 10-MDP-containing adhesive was not significantly different from the % DC a 10-MPD free adhesive.[9] A possible explanation may be related to the addition of the iodonium salt DPIHP in the composition of the evaluated experimental adhesives. It has been shown that iodonium salts when added to CQ/Amine systems enhance both mechanical properties and final conversion due to the production of active phenyl radicals with regeneration of the CQ, with the effect of increasing the compatibility between monomers and initiators.[18] However, when only the 10-MDP containing adhesives were considered, the % DC was not influenced by the concentration. The null hypothesis that the evaporation of solvents or the inclusion of water into adhesives would not influence the % DC was rejected as the control group showed higher % DC values. This result can be observed on Figure 1B in which the groups of 5 seconds, 20 seconds, addition of water showed lower % DC when compared with the control. The evaporation of solvents may increase the viscosity of the adhesive; decreasing the velocity of polymerization reaction and consequently the % DC.[2] Another explanation is related to the idea that the evaporation could trap oxygen into the material reducing the % DC.[15] Although the evaporation of solvents has been known and preconized by the manufactures to promote the evaporation of solvents further work is required. In the present study, 10 wt % of ethanol were added to the experimental adhesives. Maybe commercially available adhesives have more solvents in their composition, which may result in a higher evaporation.

Before the acid-etching process, the dentin is composed of approximately 20 vol.% of water and after the acid-etch procedure the mineral is replaced by water, turning into 70 vol.% of water.[16] This intrinsic water cannot be all replaced by monomers,[19] producing a hybrid layer with water into the collagen fibril matrices, [10] turning into a weak hybrid layer. The addition of water in protocol IV was conducted to simulate the amount of water present between the collagen fibrils after acid etching of the dentin. It has been suggested that an average hybrid layer has 5 µm of depth [20], an area of 78.5 mm^2 and a thickness of 150 µm of adhesive. [1] This results in 12 µl of adhesive. Thinking in the same condition, the hybrid layer would have a 0,4 µl of volume and the quantity of water inside of demineralized dentin after the acid-etching process should be 0,3 µl (70%).[16] Therefore, in the present study for each 12 µl of adhesive, 0,3 µl of water was added. The water between the collagen fibrils is very important for the maintenance of those fibrils to promote a good primer infiltration.[12] It has been suggested that when there is 20% of water the % DC of adhesive decreases in 50% [11]. The post hoc test showed that in present study the addition of water reduced the % DC. This result is in agreement with another study.[9] On the other hand, as observed in Figure 2 the experimental adhesives containing 10-MDP were not influenced by the MP, with the water showing a borderline behavior tendency of increasing % DC. It has been shown that the iodonium salt can benefit from the presence of water,[18] which is in agreement with the results of the present study. However, it should be noted that when the % DC of self-etching adhesives is evaluated the water already present in the composition of the material may negatively affect the apparent value of % DC measured.[14] For this reason, the subtraction of unwanted overlapping water band in the FTIR spectrum to calculate the apparent DC has been previously suggested.[14] In order to avoid due to the overlapping water peak at 1640 cm^{-1} when evaluating the aliphatic C=C bond for determining % DC heavy water (deuterium oxide, 99.9% D_2O) could also be used.[18] Since no material evaluated in the present study, contain water on its composition this subtraction was not done in the groups in which the water was added to standardize all analyses.

An interaction effect between adhesives and MP has been observed. This means that adhesives do not show the same behavior depending the manipulation. When the adhesive does not have 10-MDP and was submitted to the evaporation of solvent for 5 and 20 seconds, or when water added the % DC lowered. This result can be better observed on Figure 2. In fact, the MP influenced the % DC only for A1 and Ambar.

The present study was conducted at controlled room temperature and humidity. Care was taken to avoid the influence of the external light during the % DC evaluation; such as room lights were turned-off during the experiments. The results, however, could be different if the temperature and humidity could be manipulated to simulate oral conditions.

It is well-known that when etch-and-rinse adhesives are used all HAp is demineralized while dentin collagen fibrils are exposed. Self-etch adhesives, instead, do not demineralize all dentine leaving a residual HAp to interact with the functional monomers such as 10-MDP producing a strong ionic bonds with calcium and consequently a more stable reaction than others functional monomers.[27]

During clinical procedures, 10-MDP plays an important role when in contact with calcium present in HAp. Yoshida et al. [27] showed an stable chemical reaction with the adhesives that contain 10-MDP with HAp been less soluble in the presence of water. In the present study the results showed that the increase in the concentration of 10-MDP has not shown an increase in the % DC of EA. Despite the results of the present study, it is still necessary to establish minimun and maximun thresholds of 10-MDP-content for the chemical bonding to HAp that can contributes to that stable union. It also could be hypothesized that an over concentration of 10-MDP monomers added to the adhesive could jeopardize the chemical reaction as acidic monomers needs to be neutralized in the chemical reaction. Considering the limitations of the present study, it may be concluded that 10-MDP can enhance the %DC of the three-component-photoinitiator system experimental adhesives evaluated.

Acknowledgements

The authors gratefully acknowledge FGM (Joinville, SC, Brazil) for providing the materials used in this study.

Clinical relevance

In the future, adhesives with 10-MDP and DPIHP the iodonium salt could be a good suggestion for adding to dentin adhesives.

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