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"Evaluation of acidic functional monomers with spacer chains with diferente hydrophilicities and lengths"

"Avaliação de monômeros funcionais ácidos com cadeias espaçadoras de diferentes hidrofilias e comprimentos"

Piracicaba
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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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"Evaluation of acidic functional monomers with spacer chains with diferente hydrophilicities and lengths"

Orientador: Prof. Dr. Américo Bortolazzo Correr
Co-Orientador: Prof. Dr. Mário Alexandre Coelho Sinhoreti

"Avaliação de monômeros funcionais ácidos com cadeias espaçadoras de diferentes hidrofilias e comprimentos"

TESE DE DOUTORADO APRESENTADA À FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP PARA OBTENÇÃO DO TÍTULO DE DOUTOR EM MATERIAIS DENTÁRIOS.

DOCTORATE THESIS PRESENTED TO THE PIRACICABA DENTAL SCHOOL OF UNICAMP TO OBTAIN THE Ph.D. GRADE IN DENTAL MATERIALS.

Este exemplar corresponde à versão final da Tese defendida pelo aluno Victor Pinheiro Feitosa e orientada pelo Prof. Dr. Américo Bortolazzo Correr

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A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 23 de Agosto de 2013, considerou o candidato VICTOR PINHEIRO FEITOSA aprovado.

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EPÍGRAFE

"A vida sem ciência é uma espécie de morte"

(Sócrates)

RESUMO

O objetivo neste estudo foi avaliar o efeito da cadeia espaçadora (comprimento e polaridade) de cinco monômeros funcionais fosfatados, MEP (dois carbonos), MDP (dez carbonos), MDDP (doze carbonos), MTEP (cadeia mais hidrófila) e CAP-P (cadeia com hidrofilia intermediária) sobre a união química com dentina e cálcio, as propriedades físico-químicas e a adesão à dentina. Este estudo foi separado em 3 Capítulos. No Capítulo 1 foi verificada a influência da cadeia espaçadora de monômeros funcionais no grau de conversão, resistência coesiva, sorção de água e molhamento de adesivos experimentais de passo único. A fotoativação em todos os capítulos foi realizada com o aparelhos de luz de lâmpada halógena com irradiância mínima de 600mW/cm². Todos os dados foram submetidos à análise de variância de um fator e teste de Tukey ($\alpha=0.05$). Os resultados mostraram que os diferentes monômeros promoveram grau de conversão semelhante quando adicionado em porcentagens molares iguais. Entretanto, a resistência coesiva foi diferente, com maiores valores para monômeros capazes de propiciar interações intermoleculares (MTEP, MDP e MDDP). A sorção de água e o molhamento sobre superfícies de dentina foram maiores utilizando os monômeros mais hidrófilos (polares). No Capítulo 2, o objetivo foi avaliar a interação química dos monômeros funcionais com cálcio e dentina. Foi analisada a formação de sais monômero-cálcio através da concentração de cálcio livre (Ca^{2+}) em espectroscopia de absorção atômica (AAS), a interação de soluções aquosas dos monômeros com fatias de dentina antes e após a lavagem com água e etanol através de espectroscopia infravermelho transformada de Fourier (FTIR) e a presença dos monômeros sobre as fatias de dentina também foi observada em microscopia eletrônica de varredura. Os resultados mostraram que a união iônica com cálcio é maior com monômeros contendo cadeias espaçadoras longas e menos polares (MDP e MDDP). A estabilidade dessas ligações frente à lavagem foi afetada somente para o monômero com cadeias curta. A hidrofilia da cadeia não afetou a estabilidade da união química. No Capítulo 3, o objetivo foi avaliar a formação da ligação entre monômeros e cálcio e adesão de sistemas adesivos de passo único usando os monômeros estudados. Foi analisada a resistência de união à microtração após 24 horas e um ano de armazenagem em água, a absorbância de cálcio livre em AAS, a micropermeabilidade e a nanoinfiltração dos adesivos. Os resultados mostraram que a resistência de união inicial e a formação de ligações com cálcio foram menores para os monômeros com cadeias espaçadoras curtas e polares. A resistência de união foi reduzida após armazenagem somente para o monômero com cadeia espaçadora mais hidrófila (MTEP), provavelmente devido a maior micropermeabilidade e sorção de água. Houve pouca nanoinfiltração somente para monômeros com cadeias longas e hidrófobas. Como conclusão, observou-se que cadeias espaçadoras mais hidrófilas e mais curtas prejudicam a união química, a adesão e as propriedades físico-químicas dos monômeros ácidos funcionais.

Palavras-chave: adesivos, dentina.

ABSTRACT

The aim of this study was to evaluate the effects of spacer chain (length and polarity) of five phosphoric functional monomers, MEP (two carbons), MDP (ten carbons), MDDP (twelve carbons), MTEP (more hydrophilic spacer chain) and CAP-P (intermediate hydrophilicity spacer). It was assessed the chemical bonding with dentin and calcium, the physicochemical properties of one-step self-etch adhesives and the adhesion to dentin. This investigation was separated in 3 chapters. Chapter 1 verified the influence of spacer chain of functional monomers on the degree of conversion, ultimate tensile strength (UTS), water sorption and wettability of experimental all-in-one adhesives. Light-activation in all chapters was undertaken using halogen-lamp with minimal irradiance of 600mW/cm². All data were submitted to one-way ANOVA and Tukey's test ($p<0.05$). The outcomes showed that different monomers promote similar degree of conversion when added in same molar percentage. Nevertheless, their UTS were distinct, with high values for monomers able to promote intermolecular interactions. The water sorption and dentin wettability were greater with more hydrophilic (polar) monomers. In chapter 2, the purpose was to assess the chemical interaction of studied functional monomers with calcium and dentin. It was analyzed the formation of monomer-calcium salts through evaluation of free-calcium (Ca^{2+}) in atomic absorption spectroscopy (AAS), the interaction of aqueous monomer-containing solutions with dentin slabs before and after rinsing with water and ethanol by means of Fourier-transform infrared spectroscopy (FTIR) and the presence of monomers onto the dentin slabs was imaged in scanning electron microscopy. The results displayed better ionic bond with calcium with monomers based on long and less polar spacer chains. The stability of such linkages was affected only for the monomer with short spacer chain. The hydrophilicity of spacer chain did not affect the stability of the chemical bond. In chapter 3, the aim was to evaluate the formation of bonds between monomers and calcium as well as the bonding performance of all-in-one adhesives using the investigated monomers. It was analyzed the microtensile bond strength after 24h and one year of water storage, the AAS absorbance of free-calcium, the micropermeability and the nanoleakage of adhesives. The outcomes showed that initial bond strength and formation of linkages with calcium were lower for monomers with short and polar spacer chains. The bond strength was reduced after aging only for the monomer with highly hydrophilic spacer chain, likely due to higher micropermeability. There was little nanoleakage only for monomers with long and hydrophobic spacer chains. As conclusion, it was observed that more hydrophilic and short spacer chains jeopardize the chemical bonding, the adhesion and the physicochemical properties of acidic functional monomers.

Key words: adhesives, dentin.

SUMÁRIO

INTRODUÇÃO

Os materiais poliméricos são utilizados de alguma forma na aplicação dos materiais restauradores odontológicos em sua maioria. As cerâmicas figuram como o principal material restaurador indireto (6), quase sempre necessitando de cimentações adesivas com cimentos resinosos. Já na técnica restauradora direta, as resinas compostas representam o material de escolha na maioria dos casos (2). Recentemente, foram desenvolvidos cimentos resinosos autoadesivos (10) que facilitam o protocolo de cimentação de próteses e também diminuem o tempo clínico desses procedimentos. Nos últimos dois anos, as resinas compostas restauradoras autoadesivas foram introduzidas no mercado após preparos químicos similares (11) aos utilizados com os cimentos resinosos autoadesivos. Os componentes-chave para o desenvolvimento desses dois materiais inovadores foram os monômeros funcionais ácidos. Esses monômeros propiciam o condicionamento dos tecidos dentais e a simultânea infiltração da resina (7).

Os monômeros ácidos funcionais surgiram na Odontologia Adesiva no início da década de 80 (18). Eles são compostos por um ou mais radicais polimerizáveis (acrilato, metacrilato, metacrilamida, etc) e por um ou mais radicais ácidos (carboxílicos, fosfórico, fosfônico, etc). Esses radicais são separados por uma cadeia espaçadora que pode ter diferentes características (7) como hidrofilia e comprimento. Tais monômeros reduzem o pH do meio (17) tornando-o ácido durante a aplicação dos materiais (adesivos autocondicionantes, cimentos resinosos e resinas compostas autoadesivas), promovendo a desmineralização do substrato (normalmente esmalte e dentina) e criando micro retenções as quais são preenchidas pelas resinas. Dessa forma, a união micromecânica em que se baseia a camada híbrida é obtida similarmente aos adesivos de técnica úmida, mas sem a necessidade de condicionamento e lavagem em passos separados. Além da união micromecânica tradicional, os monômeros funcionais geram também outro mecanismo de adesão baseado na interação química com cálcio e hidroxiapatita na interface de união (21). Tal união química se baseia na ligação iônica de ânions fosfatos e cátions de metais, principalmente com o cálcio (17).

Os monômeros funcionais são utilizados principalmente em adesivos autocondicionantes. Nessa categoria de material odontológico, eles exercem uma função essencial de promover condicionamento e penetração dos monômeros e, com um bom monômero funcional empregado, a durabilidade das restaurações adesivas pode ser aumentada (4, 9). O monômero MDP (10-metacriloxi-decil-dihidrogeno fosfato) demonstrou ser o padrão ouro dentre todos os monômeros funcionais ácidos utilizados na Odontologia (16, 17, 19, 22). Tal monômero é composto por um radical polimerizável metacrilato e por um radical fosfórico separados por uma cadeia espaçadora longa e relativamente hidrófoba. Esta cadeia espaçadora é a maior justificativa para o MDP possuir ótimas propriedades físico-químicas e união química que geram maiores e mais duráveis resistências de união (4, 16, 19). A cadeia longa composta por dez carbonos permite o equilíbrio necessário em relação à polaridade da molécula, compensando a hidrofilia do radical fosfórico (16, 19). Isso mostra a importância da cadeia espaçadora nas propriedades dos monômeros funcionais.

Diversos monômeros foram comparados com o MDP em estudos prévios (4, 16, 19, 21). Como por exemplo, três monômeros fosfonatados a base de acrilamida foram sintetizados com grupos anexos ao radical polimerizável (16, 21). Esses grupos ligados aos monômeros possuíam diferentes hidrofilias e através desses estudos foi comprovado que monômeros mais hidrófobos possuem melhor ligação química e maior resistência de união que os monômeros com radicais mais hidrófilos (polares) (16, 21). Dessa forma, é possível observar que diversas características dos monômeros funcionais foram avaliadas. Entretanto, o papel da cadeia espaçadora nunca foi testado com radicais polimerizáveis e ácidos padronizados iguais aos do MDP.

Diante do exposto, é importante estudar o efeito de cadeias espaçadoras com diferentes comprimentos e polaridades em monômeros funcionais na adesão à dentina e propriedades físico-químicas de adesivos autocondicionantes experimentais. Com os resultados de tais análises, o desenvolvimento de novos monômeros funcionais ácidos pode ser orientado para a obtenção de monômeros que promovam maior durabilidade de restaurações que empreguem materiais que os utilizarem, sejam eles sistemas adesivos, cimentos resinosos autoadesivos ou resinas compostas autoadesivas. A hipótese testada foi que as diferentes cadeias espaçadoras não induzem diferenças nas propriedades físico-químicas e na adesão à dentina.

CAPÍTULO 1

The role of spacer carbon chain in acidic functional monomers on the physicochemical properties of self-etch adhesives

ABSTRACT

Objectives: To evaluate the effects of acidic functional monomers with different hydrophilicity and spacer carbon chain length on the degree of conversion (DC), wettability (contact angle), water sorption (WS) and ultimate tensile strength (UTS) of experimental one-step self-etch adhesives (1-SEAs).

Methods: A series of standard resin blends was prepared with each formulation containing 15 mol% of a different acidic monomer. The structural variations of the acidic monomers were MEP (spacer chain with two carbons), MDP (ten carbons), MDDP (twelve carbons), MTEP (more hydrophilic polyether spacer chain) and CAP-P (intermediate hydrophilicity ester spacer). The DC was assessed by FTIR. Dumbbell-shaped and disc specimens were prepared and tested for UTS and WS respectively. Moreover, the wettability of each 1-SEA was evaluated on glass slides and flat dentin surfaces. Results were analyzed with one-way ANOVA and Tukey's test ($\alpha=0.05$).

Results: The outcomes showed lower UTS for CAP-P, control blend (no functional monomer) and MEP than MTEP, MDDP and MDP ($p<0.05$). The degree of conversion was statistically similar for all resins ($p=0.122$). On dentin, the wettability was higher (lower contact angle) with the most hydrophilic monomer MTEP. Higher wettability and WS was attained in presence of the MTEP. However, the length of the spacer carbon chain did not result in different wettability and WS ($p>0.05$).

Significance: At similar molar percentage, different acidic functional monomers induced similar degree of conversion and different UTS when included in a 1-SEA. However, the inclusion of highly hydrophilic monomer may increase the wettability on dentin and the WS.

Key Words: contact angle, wettability, ultimate tensile strength, degree of conversion, functional monomers, self-etching adhesives.

1. INTRODUCTION

Most of the resin monomers used for the formulation of dental bonding agents (DBAs) present two terminal groups separated by a spacer chain [1,2]. One of the terminal groups constitute the polymerizable site of the monomer [1,3], which is predominately represented by methacrylates and the other terminal group represents some functionalities. Dental restorative resin composites are mainly formulated using cross-linking di-methacrylates, whereas DBAs may be formulated using a variety of different monomers which can also contain specific functional groups [1]. Functional monomers accomplish specific roles such as dentin/enamel etching, phase separation stabilization, improvement of the penetration of cross-linking monomers and antibacterial effects [3]. The spacer carbon chain of a monomer may be composed by hydrophobic alkanes as in the 10-methacryloxy-decyl-dihydrogen-phosphate (MDP) or by relatively hydrophilic polyethylene glycols as in the tri-ethylene-glycol-di-methacrylate (TEGDMA).

Acidic functional monomers play an essential role on the bonding performance and on the physico-chemical properties of self-etch adhesives, as they may be capable of conditioning enamel and/or dentin substrates. MDP may be considered nowadays as a gold-standard monomer [4-6] in the formulation of high-performance DBAs due to its effectiveness in chemical interaction and durability with hard dental tissues [4-6]; the excellent performance of MDP is also attributed to its hydrophobic spacer carbon chain [4-6]. Indeed, its chemical structure relies on a polymerizable methacrylate group separated from an acidic di-hydrogen-phosphate functionality by a relatively hydrophobic ten-carbon spacer chain [1].

However, self-etching acidic functional monomers in DBAs should also fulfill some specific requirements [3,7] such as high degree of conversion, optimal wetting on the tooth surface, minimal water sorption and adequate mechanical strength. The length and composition of the spacer chain between the polymerizable and the functional/acidic groups may influence these physicochemical properties [3].

To date, there is little information regarding the effects of different spacer carbon chains on the physicochemical properties of self-etch adhesives. Furthermore, there is no investigation showing the effect of different spacer carbon chains with standardized polymerizable methacrylate and acidic dihydrogen-phosphate groups similar to MDP. In other words, the role of the spacer carbon chain of MDP on the physicochemical properties of self-etch adhesives is not clear and would be of high interest to investigate.

This study aimed to assess the influence of length and hydrophilicity of the spacer chain in acidic functional monomers on the degree of conversion, ultimate tensile strength (UTS), water sorption (WS) and wetting of experimental self-etch adhesives. Two null hypotheses were tested: 1) The monomers composed by more hydrophilic spacer chains display no differences on the selected physicochemical properties; 2) Monomers composed by spacer chains with different lengths attain no differences on the selected physicochemical properties.

2. MATERIALS AND METHODS

2.1 Synthesis of functional monomers

The functional monomers were synthesized as described by Ogliari et al. [8]. Briefly, 1,10-decanediol [$HO(CH_2)_{10}OH$], 1,12-dodecanediol [$HO(CH_2)_{12}OH$] and tetra ethylene glycol [$HO(CH_2CH_2O)_3CH_2CH_2OH$] (all from Sigma Aldrich, St. Louis, USA) were esterified using methacrylic acid in order to attach the methacrylate group in one extremity of the molecule. Caprolactone 2-methacryloyloxy-ethyl ester [$HO(CH_2)_5CO_2CH_2CH_2O_2CC(CH_3)=CH_2$] was used after this process as it is available as the methacrylate-functionalized intermediate. The synthesis of 10-methacryloyloxy-decyl-dihydrogen phosphate (MDP), 12-methacryloyloxy-dodecyl-dihydrogen phosphate (MDDP), methacryloyloxy-tetraethylene-glycol-dihydrogen phosphate (MTEP) and methacryloyloxy-caprolactone dihydrogen phosphate (CAP-P), respectively was accomplished by reaction of methacrylate-attached intermediates with phosphorus pentoxide and methylene chloride in an ice bath for 48 h. Subsequent to reactions and purification [8], the isolated products were characterized using FTIR to confirm the synthesis of the MDP, MDDP, MTEP and CAP-P. The monomer referred as HEMA-P, 2MP or 2-methacryloyloxy-ethyl-dihydrogen phosphate (MEP) was purchased from

Esstech (Essington, PA, USA) and used without further purification. The chemical structures of the monomers evaluated in the present investigation are displayed in Figure 1.

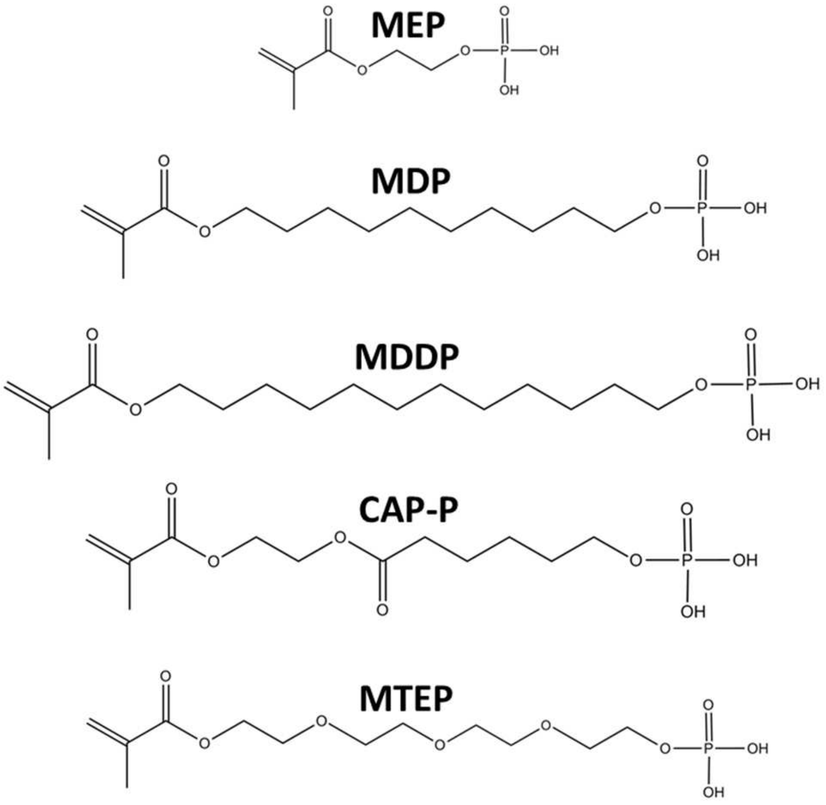

Figure 1. Chemical structures of the five acidic functional monomers evaluated. 2-methacryloyloxy-ethyl-dihydrogen phosphate (MEP), 10-methacryloyloxy-decyl-dihydrogen phosphate (MDP), 12-methacryloyloxy-dodecyl-dihydrogen phosphate (MDDP), methacryloyloxy-caprolactone dihydrogen phosphate (CAP-P), and methacryloyloxy-tetraethylene-glycol-dihydrogen phosphate (MTEP).

2.2 Formulation of experimental adhesives

A base resin blend (control) was prepared by mixing 30 wt% urethane-dimethacrylate (UDMA), 10 wt% bisphenol-A-diglycidyl-methacrylate (BisGMA), 7 wt% triethylene-glycol-dimethacrylate (TEGDMA), 5 wt% hydroxyethyl-methacrylate (HEMA), 15 wt%

deionized water, 30 wt% absolute ethanol and 3 wt% photoinitiation system. The photosensitive molecule used was camphoroquinone (CQ, 0.5 wt%). The ethyl 4-dimethylaminebenzoate (EDAB, 1 wt%) was the coinitiator and the onium salt [9,10] was the diphenyliodonium hexafluorophosphate (DPIHP, 1.5 wt%).

The acidic functional monomers were added in 15 mol% to the base resin blend to create a series of experimental one-step self-etch adhesives (1-SEA). Therefore, the number of functional monomer molecules was standardized rather than to standardize their weight percentage which would afford differences due to strikingly different molar masses.

2.3 Degree of Conversion

The degree of conversion (DC) of the experimental 1-SEAs and base resin blend (control) was undertaken following a protocol previously described [11]. Briefly, a small drop (3 µL) of each adhesive resin (n=3) was analyzed using the Attenuated Total Reflection Fourier-transform Infrared spectrophotometer (Nicolet 5700, Thermo Fisher Scientific, Loughborough, UK). The spectra were assessed before and subsequent to light-activation (40s; > 600 mW/cm^2, Optilux VLC, Demetron Kerr, Orange, USA). All spectra were obtained in a range of 1800-1500 cm^{-1}, with 12 scans at 4 cm^{-1} resolution in transmission mode and 2.8 mm/s mirror speed. The residual unreacted carbon-carbon double bond content (% C=C) in the polymer film (thickness 800±200µm) was determined from the ratio of absorbance intensities of aliphatic C=C (peak at 1637 cm^{-1}) against an internal standard (aromatic carbon-carbon bond peak at 1608 cm^{-1}) before and 120 s after starting the light-activation. Degree of conversion was determined by subtracting the C=C% from 100 %. Data were statistically analyzed using one-way ANOVA (α=0.05).

2.4 Ultimate tensile strength

Dumbbell-shaped specimens of the 1-SEAs containing the different functional monomers and the base resin blend (control) were created using silicone molds (n=10). The specimens had the dimensions of 0.5 mm thickness, 10 mm length, 1 mm constriction and 8 mm width as shown in Figure 2A. The UTS assessment was realized following a protocol similar to that of Hosaka et al. [12]. The resins were poured into the moulds without solvent evaporation and gently air-blasted for 5 s. A Mylar strip covered the resins and the light-activation using the Optilux VLC (Demetron) was performed for 40 s with the light tip in contact with strip. The light tip diameter was 10mm; thus, it covered the entire specimen allowing a single light-activation for each specimen. Thereafter, the specimens were carefully removed from the moulds and stored in a dark environment with 100 % relative humidity for 24 h.

The specimens were fixed to a metal jig using cyanoacrylate glue (Super Bonder Gel, Loctite, Henkel Co., Rocky Hill, USA) and stressed to failure in a universal testing machine (EZ-test, Shimadzu Co., Kyoto, Japan) with a 500-N load cell and a cross-head speed of 0.5 mm/min. The exact cross-sectional area of each tested specimen was measured after fracture using a digital caliper. The UTS data was transformed to MPa by dividing the tensile force at failure (N) by the cross-sectional area of the specimen (mm^2). The results were statistically analyzed using one-way ANOVA and Tukey's test (α=0.05).

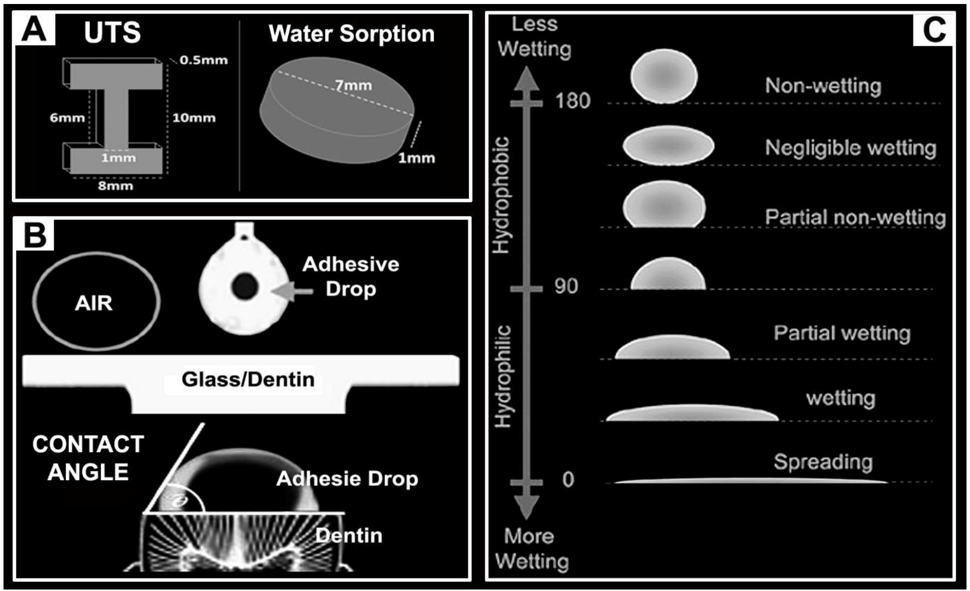

Figure 2. Schematic representation of the specimens used for ultimate tensile strength (A, left) and water sorption (A, right). (B) The lateral view of the FTA software. (C) The sorts of wetting and spreading of the resins and water droplets onto the dentin surface.

2.5 Water Sorption

The water sorption evaluation was undertaken according to protocols previously described [12, 13], following the method outlined in ISO specification 4049 except for specimen dimensions. The self-etch adhesives and the control resin blend had the solvents completely evaporated using a 3 bar air-stream from an oil-free triple syringe until a constant mass was achieved. Ten disc-shaped specimens with 7 mm diameter and 1 mm thickness (Figure 2A) were prepared for each adhesive using standard silicone moulds. A polyester strip covered the resins poured into the moulds and the light-activation for 40 s using the halogen lamp (Optilux) was performed with the light tip in contact with the strip. The specimens were removed from the moulds and weighed on an analytical balance (JK-180: Chyo, Tokyo, Japan) with 0.00001 g precision up to the stabilization of the mass.

The specimens were subsequently stored in a silica-containing desiccator at 37 °C and weighed after 24 h intervals up to the stabilization of the constant mass (M1), (variation less than 0.2 mg in three weight measures). The volume (V) of the specimens (mm^3) was calculated by measuring the thickness and diameter with a digital caliper (± 0.01 mm). The specimens were immersed in 1.5 mL of distilled water at 37 °C and weighed after 14 days storage (M2). Subsequently, the specimens were dried in the desiccator and weighed daily until a final constant mass was obtained (M3). Water sorption (WS) was calculated using the equation: $WS=(M2-M3)/V$ [13]. Data was statistically analyzed by one-way ANOVA and Tukey's test ($\alpha=0.05$).

2.6 Wettability/Contact Angle

The wettability survey followed a similar protocol to that published by Grégoire et al. [14]. The 1-SEAs' contact angle as a function of time was assessed using a FTA Drop shape instrument (FTA Instruments, Cambridge, UK). A highly hydrophobic resin blend (negative control) with 30 wt% BisGMA, 10 wt% TEGDMA, 20 wt% UDMA and 40 wt% ethoxylated bisphenol-A-diglycidyl-methacrylate (BisEMA) was prepared for this survey. The wettabilities of each 1-SEA, the negative control, and the control blend were evaluated.

One small calibrated drop (3 µL) of each resin was carefully applied onto an untreated glass slide using a micropipette. The transverse contact angle (direct lateral view) [14] of the droplet on the glass slide was measured after 3 s (initial accommodation) by the

FTA equipment during 120 s with 1 picture (analysis) per second (Figure 2B and 2C). The right and left angles were measured and averaged by the FTA software which automatically calculates the tangent of the droplet shape and the mean contact angle. Five droplets per group (n = 5) were tested in different glass slides. The real-time analysis during 120 s allowed the detection of the spreading which was considered as the percentage reduction (%Red) from the maximum contact angle (Max) at time 0 s to the minimum contact angle (Min) at 120 s. The wettability analysis on the glass slides was performed in order to evaluate the contact angles on a relatively inert substrate which has no water content unlike dentin.

For the dentin wettability/contact angle measurements, ten extracted human molars were selected after extraction. The teeth were used after approval by the appropriate institutional ethics committee (protocol 127/2011). They were sectioned longitudinally with two parallel cuts (1.5 and 3 mm above the cemento-enamel junction) in the occlusal crowns. One slab per tooth was obtained with a flat medium dentin surface. In a pilot study, the slabs were wet-polished with 600-grit silicon carbide papers for 30 s in order to create a clinically relevant smear layer. One droplet (3 μL) of distilled water was applied onto the smear layer covered dentin surface and the contact angle measured as aforementioned. This procedure was realized in triplicate for each dentin slab. One-way ANOVA and Tukey's test ($p<0.05$) were used in this pilot study to select the five dentin slabs with most similar wettability. These results led to selection of standardized dentin slabs. The five selected slabs with similar water contact angles (hydrophilicity) were selected in order to standardize the dentin substrate. They obtained statistically similar ($p = 0.874$) wettability.

The contact angle of each resin (five 1-SEAs, negative control and control blend) was assessed as described with the glass slides with resin drops of 3 μL onto the dentin slabs (n=5). The dentin slabs were vigorously rinsed with acetone for 30s in order to completely remove the resins. The dentin surfaces were re-abraded using a 600-grit silicon carbide paper to re-create the smear layer. The results of adhesive wettability on the glass slide were statistically analyzed with three separate one-way ANOVA (maximum angle at 0 s, minimum angle at 120 s and percentage reduction from the maximum to the minimum angles) and Tukey's test at ($\alpha=0.05$). Similarly, further three one-way ANOVA tests (Max, Min, and %Red) and Tukey's tests (p<0.05) were used to analyze the outcomes of adhesive wettability on the dentin surfaces.

3. RESULTS

The outcomes (means and standard deviations) of the degree of conversion analysis are presented in Figure 3a. The statistical results generated by the comparison of the experimental adhesives showed no difference (p = 0.122). The mean values varied from the lowest (82.7 %) degree of conversion (DG) for MEP to the highest (91.5 %) DG for MDDP.

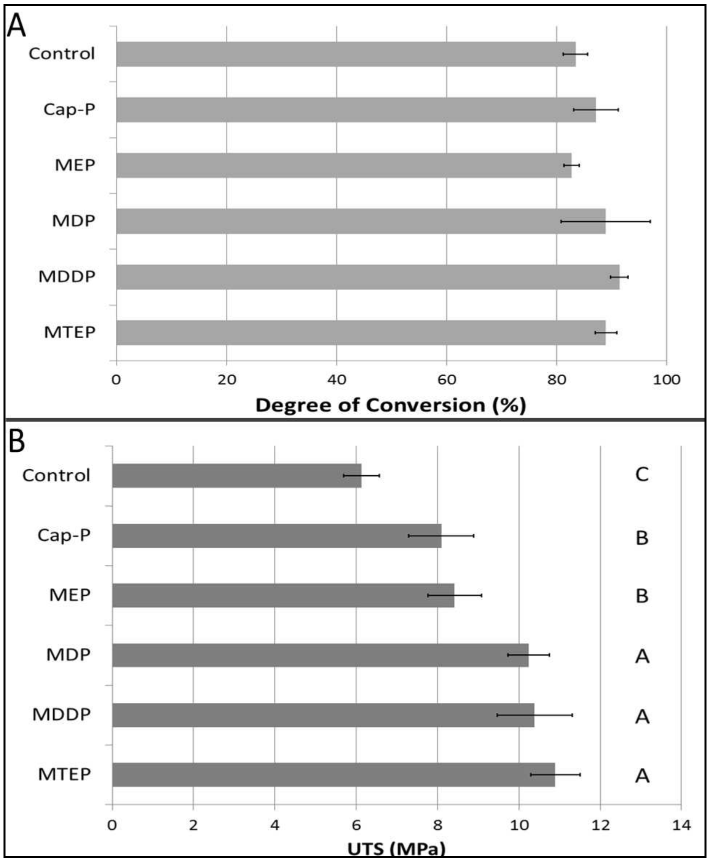

Figure 3. (A) Outcomes of the degree of conversion (%) analysis represented by means and standard deviations. No statistical difference was detected among all groups (p=0.122). (B) Means and standard deviations obtained from the ultimate tensile strength (UTS) survey. Different letters at right position represent statistically significant different UTS outcomes (p<0.05).

The UTS results are depicted in Figure 3b. Statistically significant differences ($p<0.001$) were found between the different monomers tested in this study. The control blend obtained the lowest UTS (mean 6.1 MPa) whereas MEP (8.4 MPa) and CAP-P (8.1 MPa) presented intermediary outcomes. The adhesives containing MTEP (10.9 MPa), MDP (10.2 MPa) and MDDP (10.3 MPa) achieved the highest ultimate tensile strengths ($p < 0.01$).

Significant differences ($p < 0.001$) were also attained subsequent to the water sorption study (Figure 4). The most hydrophilic functional monomer MTEP (mean 100.9 µg/mm³) and the intermediate hydrophilic monomer CAP-P (mean 85.5 µg/mm³) obtained significantly higher water sorption than the other functional monomers and the control blend. The water sorption of CAP-P and MTEP were similar ($p = 0.093$). The results of Control (mean 62.7 µg/mm³), MDP (mean 61.0 µg/mm³), MEP (mean 67.1 µg/mm³) and MDDP (mean 59.4 µg/mm³) were statistically similar.

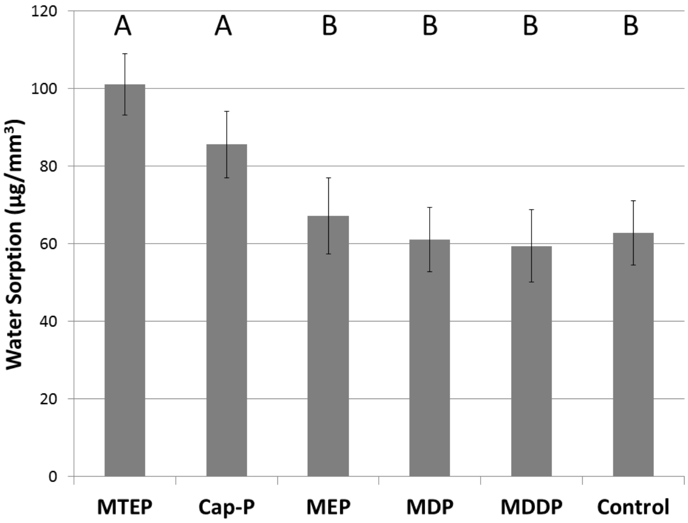

Figure 4. Graph showing the outcomes (means and standard deviations) of water sorption examination. Same letters above the columns depict statistical similarity ($p>0.05$).

Representative images of the droplets (contact angle measurement) on the glass slide or dentin surface are shown in Figure 5a and 5b respectively. The contact angle was notably higher on the dentin surface than the glass slide (Figure 5) for the control blend, negative control and all functional monomers, except for the MTEP (mean 22.1° maximum angle on glass slide; mean 24.1° maximum angle on dentin). The wettability results for the glass slide and dentin are shown in Figure 6a and Figure 6b respectively. The statistical analysis of contact angles (Max and Min) and spreading (%Red) on the glass slides showed highest maximum angle for the negative control (mean 37.6º) and the lowest with the 1-SEA containing CAP-P (mean 17.7º). The highest minimum angle after 120 s was obtained also with negative control (mean 33.2º) and the lowest with CAP-P (mean 12.0º) which was statistically similar to MTEP (mean14.3º, $p = 0.309$) and MDP (mean 14.5º, $p = 0.236$). The percentage reduction was similar between the control blend and all functional monomers ($p > 0.05$) but the negative control (11.6 %) presented lower percentage reduction than all other resins ($p < 0.001$).

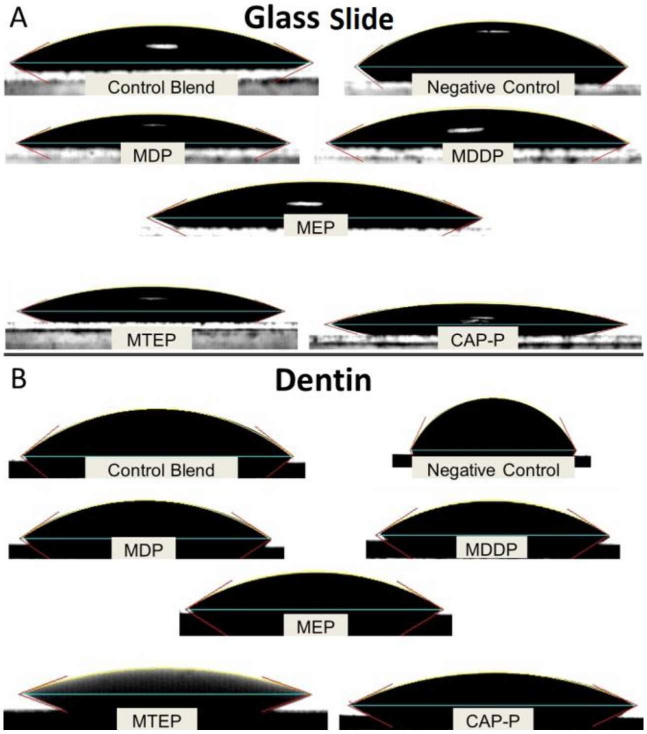

Figure 5. Representative images yielded by the FTA software. All images are related to droplets with the maximum contact angles (time 0s) on glass slide (A, upper board) and dentin (B, lower board). The left and right margins of the droplets in contact with the substrates may not be observed due to reflective effects of the equipment. Note the higher

contact angle (lower wettability) on dentin than on the glass slide except for MTEP which obtained similar wettability on both substrates.

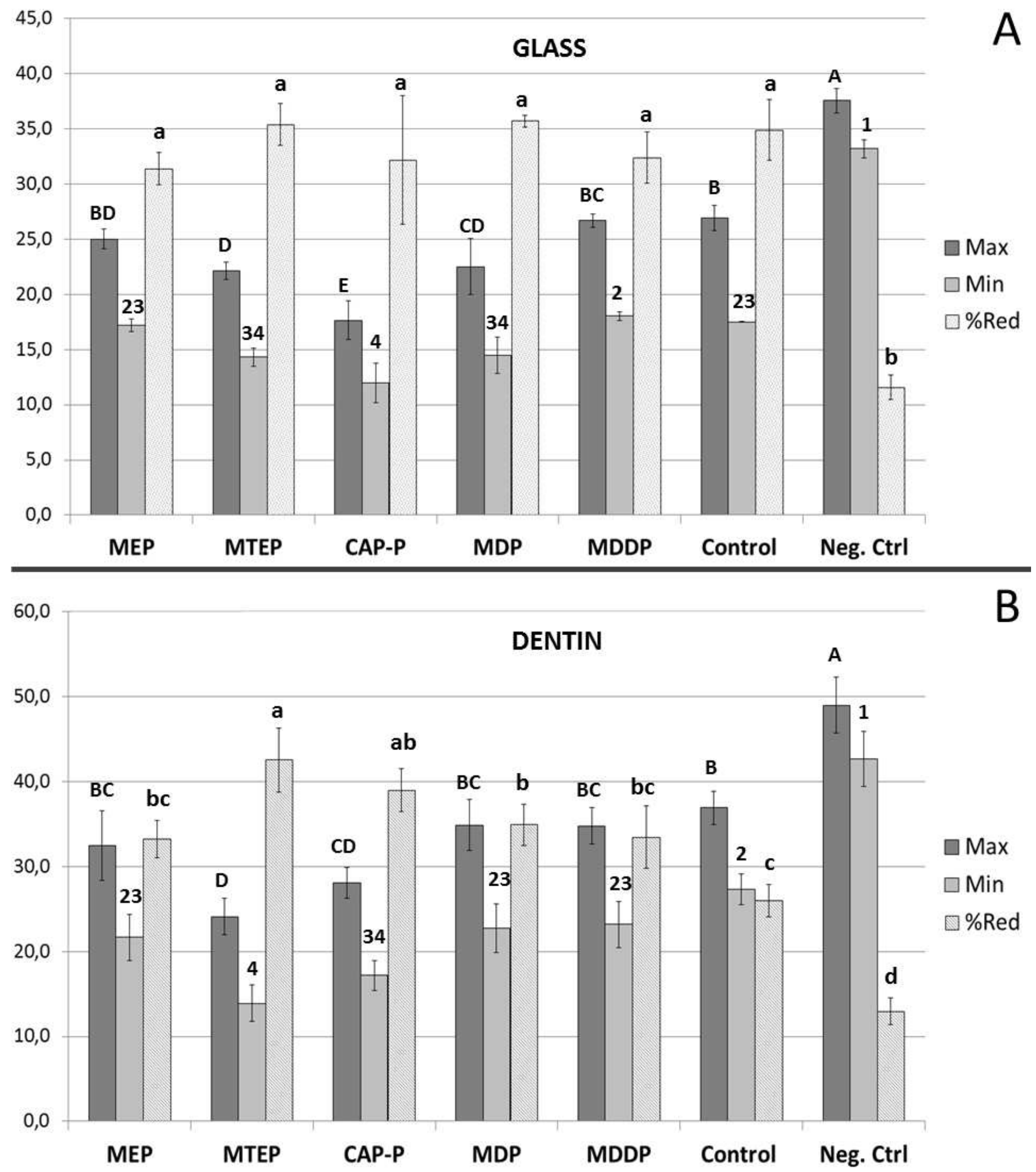

Figure 6. (a) Graph depicting the results of the wettability assessment on glass slide. The means and standard deviations of maximum (Max), minimum (Min) contact angles and percentage reductions (%Red) are presented with the statistical outcomes above the columns. Different lower-case letters indicate statistical difference ($p<0.05$) on the spreading (%Red). (b) Graph showing the outcomes of the dentin wettability. Means and

standard deviations of Max and Min angles as well as the spreading (%Red) are exhibited with the statistical results above the columns. Different capital letters indicate statistical difference ($p<0.05$) on the maximum contact angles. Same numbers (i.e. 34 and 23) obtained statistically similar ($p>0.05$) minimum contact angles and different lowercase letters indicate statistical difference ($p<0.05$) on the percentage reduction.

The wettability on dentin showed higher maximum angle for the negative control (mean 49.0º) than all the other resins ($p<0.001$). MTEP and CAP-P obtained the best initial dentin wettability (lower maximum contact angle). Similarly, the final contact angle was higher for negative control (mean 42.7º) and lower for MTEP (mean 13.9º), which was similar to CAP-P (mean 17.2º, $p = 0.749$). The spreading (%Red) on dentin was statistically higher for the most hydrophilic functional monomer MTEP (mean 42.6 %) than all other resins, except CAP-P (mean 39.0 %, $p = 0.778$). The percentage reduction of the negative control (mean 12.9 %) was lower than all other resins ($p < 0.001$). No statistical differences were observed among MEP, MDP and MDDP ($p > 0.05$) on the maximum and minimum contact angles as well as on the spreading (percentage reduction) both onto the glass slide and the dentin.

4. DISCUSSION

The present results demonstrated that the five functional monomers tested in this *in vitro* study induced remarkable differences in terms of ultimate tensile strength, water sorption and dentin wettability. Furthermore, this study also demonstrated that the degree of conversion was similar regardless the functional monomer included within the formulation of a standard one-step self-etch adhesive.

The first null hypotheses must be rejected as the UTS of CAP-P was lower than MDP's UTS, the water sorption of both MTEP and CAP-P were higher than that of MDP, and the glass slide and dentin wettabilities of MTEP and CAP-P presented significant differences compared to the 1-SEA containing MDP. As MEP showed lower UTS than MDP and the degree of conversion, water sorption, glass slide wettability and dentin wettability were similar for MEP, MDP and MDDP, the second null hypotheses must be partially rejected.

In the present investigation, the tested functional monomers were added to a standardized resin blend in the same mole percentage; in other words, the number of molecules of each functional monomer added to the resin blend was identical, and since all monomers are mono-methacrylates, the number of polymerizable groups was the same in all the experimental DBAs formulated in this study. In fact, the differences in monomer structures would be expected to contrast differences in the limiting conversion as the short chain monomers could afford early vitrification with lower level of conversion. For instance, if MEP was homopolymerized, the polymer would certainly have higher glass transition temperature than MDP and the other longer chain monomers. Furthermore, 15mol% of each functional monomer might be low to provide such differences among the DBAs' conversion.

The degree of conversion of DBAs is generally related to the photo-initiation system [15] and the light-curing exposure times [16]. The light-curing unit (LED or halogen lamp) may also play an important role depending on the wavelengths and irradiance emitted during the procedure [17,18]. The solvent content and rate of evaporation also promote discrepancies in the degree of conversion. However, the standardized amounts of both solvents added prevented such effects. The present degree of conversion outcomes showed that different spacer carbon chains in acidic functional monomers induced no effect on the monomer conversion when included in the same molar percentage. Nevertheless, most of the prior investigations [11] did not use equivalent molar concentrations but rather they add the acidic functional monomers in similar weight percentage which could induce differences in the polymerization and final conversion due to more polymerizable groups included with lower molecular weight monomers.

Despite the similarities in the degree of conversion, significant differences were observed in the ultimate tensile strength (UTS) of the tested DBAs. França et al. [19] also showed similar UTS and different degree of conversion with pre-heated resin cements presenting no correlation between these two properties. Conversely, no correlation between the degree of conversion and UTS for self-etch and etch-and-rinse adhesives was also reported [18]. The control blend had higher ethanol and water percentage than the experimental DBAs containing the experimental functional monomers; hereby, this might explain the lower UTS (Figure 3b) of the control group even with similar degree of polymerization (Figure 3a). More non-evaporated solvent may have remained entrapped into the specimens compromising the mechanical strength due to reduced cross-link density and the softening effects even with not large differences in the solvent content. The findings of previous investigations [20,21] corroborate with the present outcomes showing that model adhesives may present similar degree of conversion and lower UTS when increasing the solvent content. A possible reason for increased UTS of MDP- and MDDP-based adhesives may be due to the Van der Waals forces between the long and apolar spacer carbon chains of these monomers. Similarly, the dipole-dipole interactions between two tetra-ethylene glycol chains [22] of MTEP may also have contributed with the higher UTS of this monomer. The short spacer chain of MEP and the ester group in the spacer chain of CAP-P may not favour such intermolecular interactions.

Water sorption survey showed similar outcomes for control blend, MEP, MDP and MDDP, whereas the more hydrophilic functional monomers (CAP-P and MTEP) obtained higher results. The most rational explanation for the higher water sorption attained with CAP-P and MTEP may be attributed to the higher hydrophilicity of their spacer carbon chains [23]. The length of the spacer carbon chain provided no difference in water sorption. The hypothetical addition of similar weight percentage of functional monomers instead of similar molar percentages would have included more hydrophilic phosphate groups for the lower molecular weight monomer MEP and triggered higher water sorption. However, with the present experimental design, this effect was avoided and only the hydrophilicity of the spacer carbon chain could affect the water sorption. It is important to take into account that the water sorption and the resin hydrophilicity are negatively correlated with the bonding durability [23].

Another physicochemical property strongly correlated with the bonding performance is the wettability [3,14], which is often measured by the contact angle [14]. Figure 5 illustrates the initial wettability of the experimental self-etch adhesives, the control blend and the highly hydrophobic negative control blend. By comparing Figures 5a and 5b, one may observe the noteworthy higher contact angle (lower wettability) of most of the resins applied onto dentin surfaces in comparison to the glass slide. However, the most hydrophilic monomer MTEP presented similar contact angles on both substrates (Figures 6a and 6b). Therefore, one may conclude that the smear layer covered dentin surface possess hydrophilic features [24] in comparison with the relatively inert glass slide substrate. It is well known that the polishing procedure performed on the dentin under running water may create the standardized smear layer and promote water uptake within the smear debris yielding to a relatively high wet/hydrophilic surface [24].

The use of a high hydrophobic negative control resin was advocated in order to assess the wettability of a solvent-free hydrophobic solution. This allows contrasting differences in terms of substrate hydrophilicity and spreading non-related to the solvent evaporation. It is possible to note that the spreading ability of the negative control resin (Figures 6a and 6b) on the glass slide (mean 11.6 % reduction) was very near to that on the dentin surfaces (mean 12.9 % reduction). In addition, the spreading of the negative control was very much lower than the spreading of solvated resins whilst the maximum and minimum contact angle of the negative control was higher than those of control blend and 1-SEAs in both substrates. This might be explained by the notable difference in the viscosity as the negative control resin is far more viscous than the other resins due to absence of solvent and low-viscosity hydrophilic monomer such as HEMA.

On the glass slides, the maximum and minimum contact angle varied following the viscosity of the functional monomers used within the formulation of the standardized blend. The hydrophilicity of the spacer carbon chain exhibited significant changes in the contact angles whereas few differences were observed with spacer carbon chains with different length (Figure 6a). However, the spreading represented by the percentage reduction was similar for all the tested monomers and control blend due likely to the similar solvent content and solvent evaporation rate.

Onto the smear layer covered dentin, the maximum and minimum contact angle of MTEP (most hydrophilic monomer) was lower than the other tested monomers, whilst the spreading ability of MTEP was higher (Figure 6b). The unique functional monomer statistically similar (Figure 6b) to MTEP in the three parameters was CAP-P (intermediary hydrophilic spacer chain) which presented outcomes that fall between the hydrophilic and hydrophobic monomers. Indeed, the hydrophilicity of the smear layer covered dentin contributed to the better outcomes of MTEP. Furthermore, the higher contact angles onto the dentin surface with the hydrophobic monomers in comparison with the glass slide show the hydrophilic nature of the smear layer covered dentin surface [24]. This may have jeopardized the wettability of more hydrophobic monomers. Therefore, the contact angles and spreading are proportional to the interaction between the adhesive and the dentin substrate [14]. Furthermore, the surface energy of substrates and the superficial tension of the experimental adhesives are strictly correlated with the wettability and spreading [14]. Indeed, the more hydrophilic spacer chains may reduce the surface tension of experimental adhesives, improving their wetting ability.

The different length of spacer carbon chains plays no apparent role on the dentin wettability (Figure 6b) due to the similar hydrophilicity of the functional monomers and respective DBAs (Figure 4). The smear layer produced by different burs (i.e cross-cut carbides, finishing carbides and diamond rotary burs) present different surface roughness which may influence the dentin wettability [25]. The smear layer plays an important role in the self-etch adhesive dentistry [26] and in the dentin wettability of 1-SEAs [27]. Therefore, the caries removal, excavation procedures and cavity finishing should be regarded as important factors that may affect the dentin wettability [25].

Overall, important findings were observed in the present investigation regarding the physicochemical behavior of self-etch adhesives related to the spacer carbon chain of acidic functional monomers. The monomers with hydrophilic spacer chains presented better wetting but also increased water sorption. Indeed, the wetting behavior of self-etch adhesives may afford different interactions with the dental hard tissues. Several commercial adhesives employ more hydrophilic functional monomers in order to improve the dentin wettability; on the other hand, they may induce more water uptake and accelerate the degradation processes within the resin-dentin interface. Therefore, the higher the hydrophilicity of the adhesive resins generally the lower the bonding durability [13, 23]. Indeed, different composition and length of the spacer group can additionally affect the pH and steric ability of the acidic functionality to interact with the dentin and any remaining mineral. This might change the formation and stability of the monomer-calcium salts. Further experiments are already in progress in order to evaluate the microtensile bond strength, micropermeability and chemical interaction of these monomers.

5. CONCLUSION

Different spacer chains of acidic functional monomers do not afford different degrees of polymerization conversion when the monomers are included in same molar percentage. Nevertheless, both the length and hydrophilicity of the spacer chain provide different mechanical properties whereas only more hydrophilic spacer carbon chain induces more water sorption and better dentin wettability.

6. ACKNOWLEDGEMENTS

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CAPÍTULO 2

Effects of spacer chain on the chemical interaction of functional monomers

ABSTRACT

The number of the carbons and/or ester/polyether groups in spacer chains may influence the interaction of functional monomers with calcium ions (Ca^{+2}) and dentin. The present study assessed the chemical interaction of five standard-synthesized phosphoric functional monomers with different spacer chain characteristics similar to 10-methacryloyloxi-decyl-dihydrogen-phosphate (MDP) through Atomic Absorption Spectroscopy (AAS), ATR-FTIR, Thin-Film X-Ray Diffraction (TF-XRD) and Scanning Electron Microscopy (SEM). The tested functional monomers were MEP (two carbons spacer chain), MDP (ten carbons), MDDP (twelve carbons), MTEP (more hydrophilic polyether spacer chain) and CAP-P (intermediate hydrophilicity ester spacer). The formation of monomer-calcium salts (detected by AAS) depicted MDDP=MDP>CAP-P>MTEP>MEP. FTIR and SEM analyses of monomer-treated dentin surfaces showed permanence of the monomer onto the dentin after rinsing procedures in all groups, except for MEP. TF-XRD confirmed the weaker interaction of MEP. The shorter the spacer carbons chain (MEP) of phosphoric functional monomers, the higher the risk to jeopardize the chemical interaction and formation of stable monomer-calcium salts in dentin. The presence of ester or ether bonds within longer spacer carbons chain (CAP-P and MTEP) may affect the hydrophobicity and also the formation of monomer-Ca linkages.

INTRODUCTION

The bonding mechanism of self-etch adhesives is based on a micro-mechanical interlocking along with a chemical interaction (i.e. ionic bond) of functional monomers with calcium ions (Ca^{+2}) (Van Meerbeek *et al*. 2011). Several types of functional monomers (e.g. phosphoric, phosphonic, carboxylic) are currently available as they represent the foremost components of self-etch adhesives (SEAs). They allow simultaneous etching of the smear layer/dentin and priming hard dental substrates with inward diffusion of comonomers (Nishiyama *et al.* 2007; Van Meerbeek *et al.* 2011). The interaction between functional monomer and hydroxyapatite generates monomer-calcium salts with definite hydrolytic stability (Van Landuyt et al. 2008) depending the monomer used (Yoshihara et al. 2010). SEAs containing a phosphoric functional monomer MDP (10-methacryloyloxi-decyl-dihydrogen-phosphate) create a nano-layered structure within the resin-dentin interface (Yoshihara *et al.* 2011a, Yoshihara *et al.* 2011b) with outstanding bonding integrity and clinical longevity (Peumans *et al.* 2010, Van Meerbeek *et al.* 2011). Moreover, other investigations (Yoshida *et al.* 2004, Yoshihara et al. 2010) reported a greater chemical interaction of MDP compared to the carboxylic monomer 4-MET and the phosphate monomer Phenyl-P.

Further investigations compared the effectiveness of MDP to three different phosphonated functional monomers with different hydrophilicity (Van Landuyt *et al.* 2008, Yoshihara *et al*. 2011b) showing that the higher the number of hydrophobic appended radicals, the greater the bonding performance. Yet, it was reported that the length of spacer chain of functional monomers notably influences the pH and the bond to enamel (Ikemura *et al*. 2006).

The structure of the spacer chain is very important as it influences specific properties, such as solubility, viscosity, wetting and penetration. Moreover, subsequent to polymerization the structure of the spacer chain also influences the properties of the polymers, such as hydrophilicity, swelling and stiffness (Moszner et al. 2005).

Although the number of carbons (Nishiyama *et al.* 2007) or ester/polyether groups in spacer chains may influence the hydrophilicity of functional monomers, no investigation specifically tested the effect on the chemical affinity to calcium ions and dentin of standard-synthesized functional monomers with similar methacrylate and phosphate functionalities but with diversity in the spacer chain.

This investigation assessed the chemical interaction of five functional monomers with spacer chains with different length and hydrophilicity. The two null hypotheses tested were that the chemical bonding of the functional monomers is not influenced by: i) spacer chains with different lengths (number of carbons); ii) spacer chains with different hydrophilicity (presence of ether or ester groups).

MATERIALS AND METHODS

Synthesis of the functional monomers

The functional monomers were synthesized as described by Ogliari *et al.* (2008). In brief, 1,10-decanediol [$HO(CH_2)_{10}OH$], 1,12-dodecanediol [$HO(CH_2)_{12}OH$] and tetra ethylene glycol [$HO(CH_2CH_2O)_3CH_2CH_2OH$] (Sigma Aldrich, St. Louis, USA) were esterified using methacrylic acid. Caprolactone 2-methacryloyloxy-ethyl ester [$HO(CH_2)_5CO_2CH_2CH_2O_2CC(CH_3){=}CH_2$] (Sigma Aldrich) was not submitted to this process as it contains the methacrylate radical. 10-methacryloyloxy-decyl-dihydrogen phosphate (MDP), 12-methacryloyloxy-dodecyl-dihydrogen phosphate (MDDP), methacryloyloxy-tetraethylene-glycol-dihydrogen phosphate (MTEP) and methacryloyloxy-caprolactone dihydrogen phosphate (CAP-P) were finally synthesized by the reaction of methacrylate-attached byproducts and caprolactone using phosphorus pentoxide and methylene chloride for 48h. Subsequently, the monomers were submitted to a purification procedure (Ogliari *et al.* 2008) and the concentrate products were characterized using FTIR. The 2-methacryloyloxy-ethyl-dihydrogen phosphate (MEP) was purchased by Esstech (Essington, PA, USA) and used without further purification. The chemical structures of the monomers are shown in Figure 1a.

Atomic Absorption Spectroscopy (AAS)

Each monomer (0.1 mM) was diluted in ethanol (50/50 vol%), while a calcium-containing solution was prepared by dissolving calcium chloride in deionized water (0.1 mM). The monomers were mixed (30s) with the calcium solution in equimolar ratios equivalent to 4 ppm of calcium to form monomer-calcium salts. Thereafter, the solutions were analyzed using an atomic absorption spectrophotometer AA240FS (Varian, Palo Alto, USA) to detect the concentration of the unbounded calcium ions; the final concentration of calcium was inversely related to the formation of monomer-calcium salts. Pilot studies were undertaken in order to ensure that only the free-calcium ions (Ca^{++}) were detected by AAS.

All solutions were prepared in triplicate (n=3). Data were analyzed with one-way ANOVA and Tukey's test ($p<0.05$).

Infrared Spectroscopy (ATR-FTIR)

Extracted human third molars were obtained under approval of institutional Ethics Committee (protocol 127/2011) and stored in 0.5% chloramine/water solution at 4°C no longer than two months after extraction. Each tooth was sectioned to expose a flat mid-coronal dentin surface using a slow-speed water-cooled diamond saw (Isomet, Buehler, Lake Bluff, USA) and ground with 600-grit SiC papers under constant water irrigation (30s) to create a standard smear layer. Each monomer (15 mol%) was first dissolved in ethanol/water solution (50/50 vol%) and applied onto the dentin surfaces for 30s followed by air-drying (5s). Fourier transform infrared spectroscopy (ATR-FTIR) was performed using the FTIR Nicolet 5700 (Thermo Fisher Scientific, Loughborough, UK) equipped with an ATR crystal. The spectra of the dentin and each neat monomer were also attained as well as the spectra of the dentin specimens treated with the monomers and subsequently rinsed for 1 min using distilled water (30s) and absolute ethanol (30s) as described by Yoshihara *et al.* (2011b). All spectra were obtained in a range of 1800-800cm^{-1}, with 8 scans at 4cm^{-1} resolution in transmission mode and 2.8 mm/s mirror speed. The FTIR spectra were obtained in triplicate for each specimen and subsequently processed for baseline correction and normalization.

Thin-Film X-Ray Diffraction (TF-XRD)

Five crown-dentin specimens were prepared and treated with each tested functional monomer/ethanol/water solution as previously described. The surface of the "monomer + dentin specimens" and "monomer + water/ethanol rinsed dentin specimens" were assessed using a x-ray diffractometer (XPert Pro MPD, PANalytical, Westborough, USA) operated under 40kV acceleration, 200mA current and with a scanning rate of 0.02º per second for $2\theta/\theta$-scan with the incident x-ray beam angle fixed at 1.0º. This experimental protocol was performed in accordance with those of Yoshihara *et al.* (2011a and 2011b). By using this protocol, a depth of 5-10µm was analyzed.

Ultra-morphology assessment (SEM)

The SEM Ultra-morphology assessment of chemical interaction was undertaken in accordance with a protocol described by Fujita *et al.* (2011) and Yoshihara *et al.* (2011b). In brief, further 600-grit SiC paper-ground dentin specimens (n=3) were treated using each monomer (ethanol/water solutions) tested in this study. The "monomer + dentin specimens" and "monomer + water/ethanol rinsed dentin specimens" were mounted on aluminum stubs, dehydrated in silica gel, gold-sputter coated and observed in SEM (JSM-5600LV, JEOL, Tokyo, Japan), operated at 15 kV.

RESULTS

The AAS survey demonstrated the ability of the tested monomers in forming monomer-calcium salts (Figure 1B). MDP and MDDP showed the lowest ($p<0.001$) free calcium concentration between the groups (2.28±0.01 and 2.26±0.01 ppm, respectively). The highest free-calcium concentration was observed with MEP (2.86±0.01), while the free calcium concentration observed in CAP-P and MTEP was 2.55±0.01 and 2.79±0.02, respectively.

FTIR outcomes are presented in Figure 2. The CO-stretching vibration ($1718 cm^{-1}$) of acrylate/methacrylate carbonyl groups indicated the remaining presence of monomer on the dentin surface; this peak was observed in the spectra of neat monomers, monomer-treated dentin specimens and after rinsing procedures in all monomers except MEP.

TF-XRD assay confirmed monomer-Ca salts on dentin for all monomers (Figure 3). The characteristic TF-XRD peaks assigned to MEP-Ca salts were $2\theta= 2.9^o$, 5.1^o, 10.0^o and 15.5^o, whilst the peaks $2\theta= 30.2^o$ and 37.9^o were ascribed to dentin hydroxyapatite. Only a small peak ($2\theta= 3.2^o$) describing the MEP/dentin interaction was found subsequent to rinsing procedures, while the peaks of dentin increased along with the formation of dicalcium phosphate dehydrate (DCPD: $2\theta= 11.7^o$). Conversely, MDP-Ca salts were observed in the range at $2\theta= 2.8^o$, 6.3^o and 8.1^o, while the MDDP-Ca salts showed characteristic peaks at $2\theta= 2.0^o$, 4.3^o and 8.4^o; these remained notably high after rinsing procedures together with DCPD. CAP-P-Ca salts were detected at $2\theta= 1.9^o$, 3.0^o, 4.9^o, 6.6^o

and 8.5º, while those created with MTEP were found in the range of 2θ= 1.5º, 1.9º, 4.1º, 5.4º and 8.9º. These peaks were visible also after rinsing procedures together with DCPD peaks.

The SEM results in Figure 4 supported those attained in FTIR and TF-XRD analyses. Indeed, all monomer-treated dentin specimens showed a layer of monomer before rinsing procedures (Figure 4A), while after rinsing procedures, only those treated using MDP, MDDP, MTEP and CAP-P showed a remaining thin layer still covering the dentinal tubules (Figures 4B, 4C, 4E and 4F). Conversely, MEP was completely rinsed off from and the dentin surface showed exposed patent tubules (Figure 4D).

DISCUSSION

Several contemporary self-etch and etch-and-rinse dental adhesives have been recently formulated with MPD to improve the chemical bonding to calcium/hydroxyapatite of dentin and enamel (Yoshida *et al.* 2012). The unique chemical structure of MDP with a stable phosphoric radical plays the key role both on the initial bonding performance and durability of resin-dentin interfaces (Inoue *et al.* 2005, Van Meerbeek *et al*. 2011).

The present study provided relevant information regarding the influence of the spacer chain on the monomer-calcium chemical interaction generated by standard-synthesized phosphoric functional monomers. Indeed, despite similar polymerizable and acidic phosphate radicals, the functional monomers tested in this study displayed remarkable differences in terms of chemical interaction. The main feature highlighted in this study was that the length of spacer chain (number of carbons) in spacer chains with similar nature plays a much more important role on the chemical ionic bond than its hydrophilicity. Indeed, MEP (shorter chain), MTEP and CAP-P (hydrophilic spacer) provided a significant lower formation of monomer-calcium salts than MDP and MDDP (Figure 1B). However, although MDDP afforded numerically higher formation of monomer-calcium salts compared to MDP, no statistical significant difference was encountered; the subtle difference of two carbons between the two monomers was not sufficient to promote differences.

The FTIR survey confirmed that a short spacer chain (MEP) may influence the chemical interaction and the generation of monomer-Ca salts with low hydrolytic stability (Figure 2). However, all the other tested monomers remained attached to the dentin surface after rinsing procedures. Surprisingly, the more hydrophilic monomers MTEP and CAP-P, which present ester and ether groups within the spacer chains, were both able to remain on the dentin surface subsequent to the rinsing procedures. The FTIR analysis was performed considering the representative peak of the double bond of methacrylate groups (1718 cm^{-1}), although the peak of phosphate (i.e. 1034 cm^{-1}) were also selected in a previous investigation (Yoshihara *et al.* 2011b) to evaluate the presence of monomer onto the dentin surface; this latter peak was discarded as both the dentin and the monomers contain phosphate groups.

Likewise the outcomes of previous studies (Yoshihara *et al.* 2010, Yoshihara *et al.* 2011a), the present TF-XRD results disclosed the formation of monomer-calcium salts in the range of 2θ= 0-10º. In agreement with FTIR outcomes, the stability of MEP chemical interaction (TF-XRD) was compromised (Figure 3) and the monomer layer was washed out (SEM) after rinsing procedures (Figure 4). Remarkably, more hydrophilic functional monomer formed less, but hydrolytically stable monomer-Ca.

The null hypotheses that the length of the spacer chain (numbers of carbons) has no effect on the chemical interaction of the tested functional monomers must be rejected. Conversely, as the more hydrophilic polyether-containing spacer chain of MTEP showed lower ability to form monomer-Ca salts, although these showed acceptable hydrolytic stability, the second null hypothesis must be only partially rejected.

According to the Adhesion-Decalcification (AD) concept (Yoshida *et al*. 2004, Van Meerbeek et al. 2011), monomers that rather etch than chemically interact with calcium ions attain lower bonding to dentin. Moreover, despite we had standardized the pH of monomer/ethanol/water solution in 2.0, MEP presented lower initial pH than other monomers. Indeed, this means, in other words that this monomer rather decalcifies than adheres to dental hard tissues and it may be correlated with its deeper penetration into dental substrates during the application due to their lower molecular weight. This might explain the more evident etching patterns observed in dentin during SEM analysis in the specimens treated using MEP (Figure 4D). Furthermore, the increased decalcification might be correlated with the reduced stability of the ionic interaction (Figure 2) according to the AD concept (Yoshihara et al. 2010). However, it is important to consider that ester groups in monomers such as CAP-P are hydrolytically unstable under strong acidic conditions (Moszner et al. 2005).

The stability of the chemical bonding of MTEP and CAP-P may have been improved by their better wettability onto dentin (unpublished data) and due to a relatively long spacer chain. It has been demonstrated that monomers having weak interaction with hydroxyapatite are less prone to produce high initial bond strength and durability compared to those with higher chemical interaction (Van Landuyt *et al.* 2008).

In conclusion, the formation of monomer-calcium salts is influenced by the length and hydrophilicity of the spacer chain of functional monomers. Nevertheless, the latter characteristic may have no effect on their hydrolytic stability. These findings are essential to guide the formulation of future functional monomers which should be structured by long and hydrophobic spacer in order to get more stable chemical interaction and longer resin-dentin interface durability. Further studies are already in progress to evaluate long-term durability of resin-bond created using SEAs containing the tested monomers. Future studies should focus in assessing the effects of other chemical structures such as the acidic functionality (phosphoric, carboxylic, and phosphonic) with standardized polymerizable group and spacer chain.

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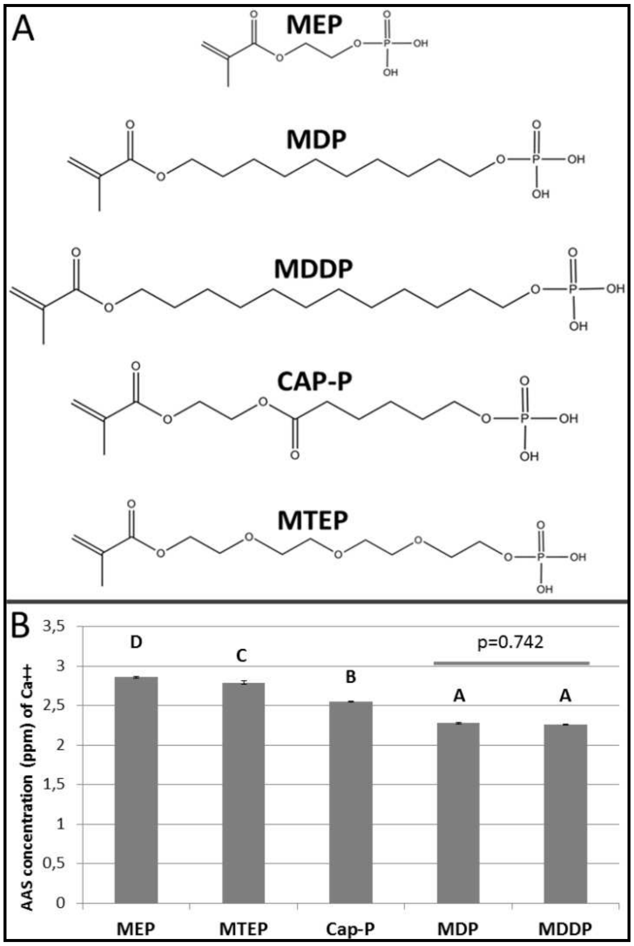

Figure 1. (A) The chemical structures of the acidic functional monomers tested. (B) Atomic absorption spectroscopy (AAS) concentration of free-calcium. The lower is the concentration of free-calcium, the higher is the formation of monomer-calcium salts. MDP and MDDP afforded the highest formation of monomer-calcium salts whereas MEP provided the lowest formation among all monomers. The initial concentration of free-calcium was 4 ppm.

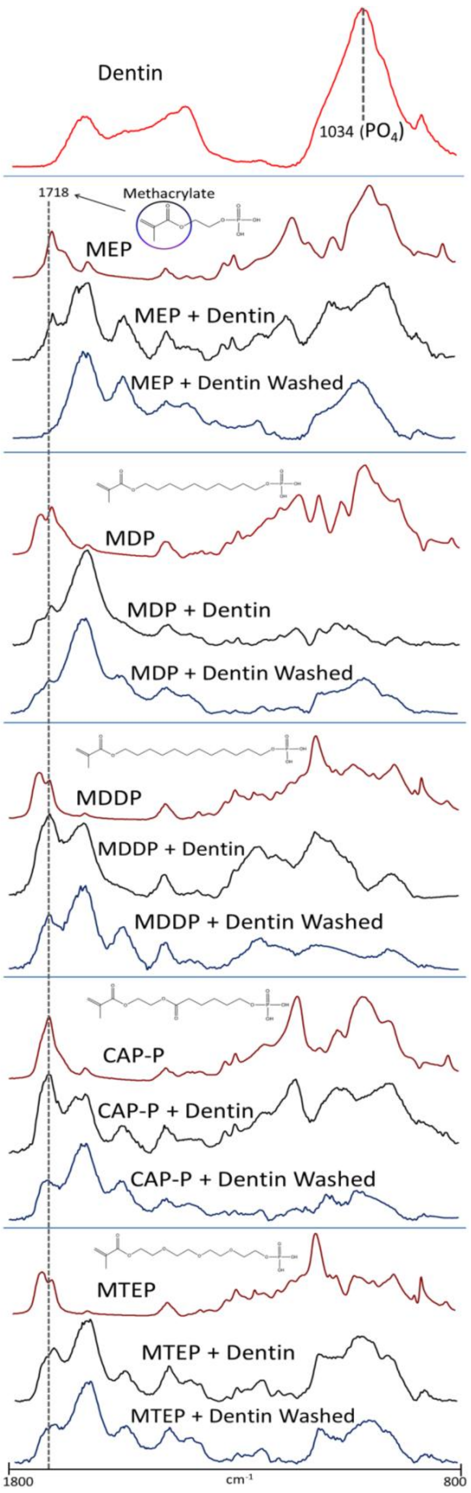

Figure 2. The FTIR spectra of dentin (top) showing the presence of the peak at 1034cm^{-1} related to dentin phosphate and absence of methacrylate peak at 1718cm^{-1}. Note that all

monomers (methacrylate carbonyl peak) remained on the dentin surface after washing with distilled water except MEP which, in turn, showed increased peak of dentin phosphate.

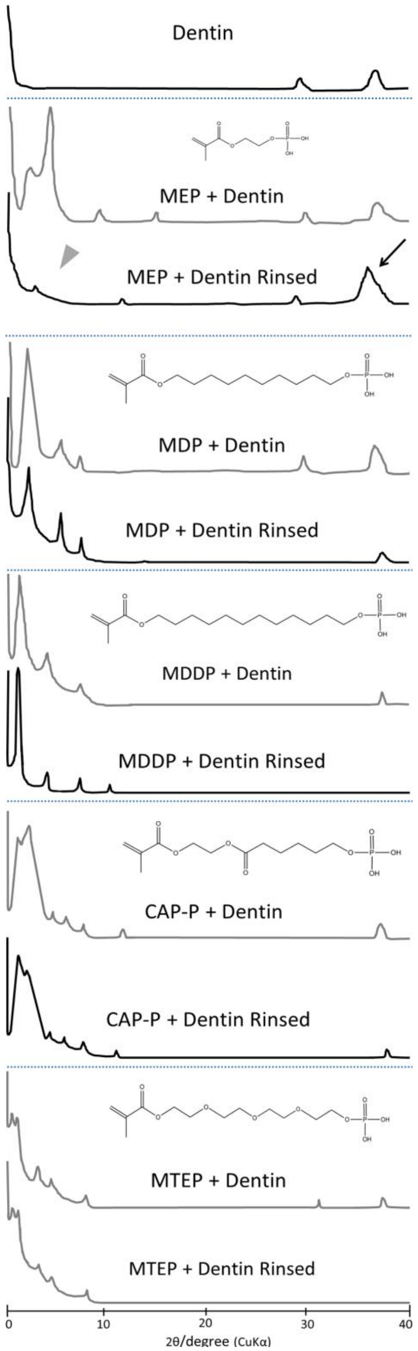

Figure 3. TF-XRD patterns of the interaction of the five functional monomer/ethanol/water solutions with dentin. The pattern of untreated dentin is shown in the upper position. Similar peaks were observed before and after rinsing with water and ethanol for all monomers except for 2-MEP. This monomer disclosed a striking reduced intensity (gray arrowhead) of the peaks 2θ = 2.5º, 5.1º, 10.0º and 15.5º which are ascribed to the ionic

bonding between 2-MEP and dentin along with an increased intensity (black arrow) of the peak $2\theta = 37.9^{\circ}$ assigned to dentin hydroxyapatite.

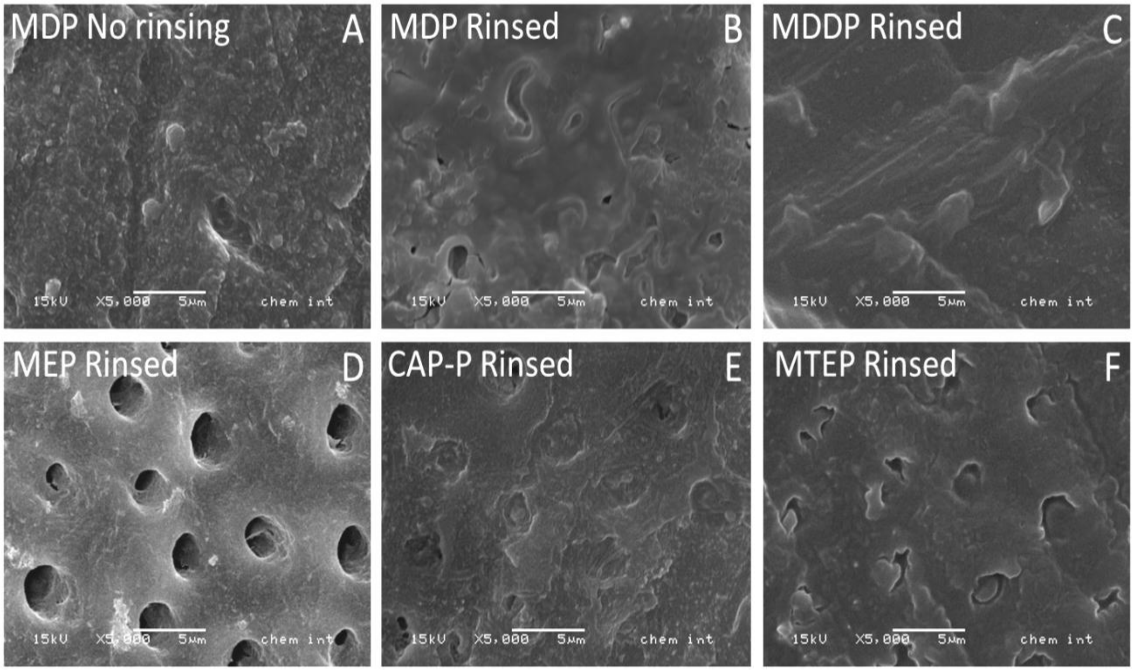

Figure 4. SEM micrographs depicting the chemical interaction of the functional monomers with dentin. All dentin specimens treated with the monomer/water/ethanol solutions before rinsing with distilled water showed the same pattern observed in Figure A for the MDP specimens. The following micrographs were taken from treated specimens after rinsing, Figure B shows MDP-treated specimens, figure C shows MDDP-treated specimens, figure D shows MEP-treated specimens, figure E shows CAP-P-treated specimens and figure F shows MTEP-treated specimens. Note that all specimens remained covered with remnants of the monomer layer after water rinsing except MEP-treated specimens.

CAPÍTULO 3

Bond durability of functional monomers with different spacer chains

ABSTRACT

Studies have proved that MDP (10-methacryloyloxi-decyl-dihydrogen-phosphate) is an acidic functional monomer that provides long-lasting durability for self-etch adhesives. Its long and relatively hydrophobic spacer chain plays a role in this regard. The present study evaluated the dentin bonding performance of MDP and other four correlated functional monomers containing spacer chains with different length and hydrophilicity. Microtensile bond strength (µTBS) after 24h and one year, atomic absorption spectroscopy (AAS), micropermeability using confocal-microscopy, fracture analysis and nanoleakage assessment through scanning electron microscopy (SEM) were the experiments undertaken. The structural modifications of the acidic monomers were MEP (spacer chain with two carbons), MDP (ten carbons), MDDP (twelve carbons), MTEP (more hydrophilic polyether spacer chain) and CAP-P (intermediate hydrophilicity ester spacer). More monomer-calcium salts (AAS survey) were formed as follows MDDP = MDP > CAP-P > MTEP > MEP. The initial bond strength was higher for MDP and MDDP whereas only the bond strength of MTEP dropped significantly after aging. The latter also attained the highest micropermeability. MEP, CAP-P and MTEP presented more nanoleakage after water aging. These findings demonstrated that hydrophilic or shorter spacer chains in functional monomers negatively affect the chemical interaction with calcium and particularly the higher hydrophilicity impairs the dentin bond durability.

INTRODUCTION

Several components of dentin bonding agents (DBAs) may interfere with the dentin bonding performance. For instance, the solvent percentage and evaporation rate may induce different conversions (Faria-E-Silva et al. 2013; Cadenaro et al. 2009) which can affect the bond strength. Moreover, the photoinitiator system and its solubility parameters are strictly correlated with the adequate polymerization of the DBAs (Loguercio et al. 2013). This also concerns the bonding performance (Hayakawa et al. 2005) and interfacial nanoleakage (Loguercio et al. 2013).

In the self-etch approach, the removal of separate acid etching step is feasible due to the acidic functional monomers (Moszner et al. 2005). They also affect the initial and long-term bonding performance (Van Meerbeek et al. 2011). The partially demineralized and monomer-infiltrated dentin constitutes the hybrid layer which is created after the polymerization and entanglement of polymer and collagen (Van Meerbeek et al. 2003). Overall, the functional monomers in self-etch adhesives are responsible for the twofold dentin bond mechanism relying on micromechanical interlocking and further ionic interaction (Van Landuyt et al. 2008, Yoshihara et al. 2011). 10-methacryloyloxi-decyl-dihydrogen-phosphate (MDP) is currently the best functional monomer in terms of chemical interaction features and dentin adhesion (Peumans *et al.* 2010, Van Meerbeek *et al.* 2011). This is generally explained by its long and relatively hydrophobic spacer chain (Yoshida et al. 2004, Inoue et al. 2005, Van Landuyt et al. 2008). Nonetheless, no study has to date assessed specifically the role of the spacer chain on the self-etch bonding performance.

Therefore, the goal of this investigation was to evaluate the chemical interaction and dentin bond of MDP and other four functional monomers containing spacer groups with different length and polarities through atomic absorption spectroscopy (AAS), microtensile bond strength (µTBS), nanoleakage and micropermeability analyzed by confocal microscopy. The null hypotheses tested were that spacer chains with: 1) different lengths or 2) different hydrophilicity do not attain different chemical interaction and dentin bonding performance.

MATERIALS AND METHODS

Syntheses of functional monomers

The functional monomers were synthesized as described by Ogliari et al. (2008). In brief, 1,10-decanediol [$HO(CH_2)_{10}OH$], 1,12-dodecanediol [$HO(CH_2)_{12}OH$] and tetra-ethylene-glycol [$HO(CH_2CH_2O)_3CH_2CH_2OH$] (all from Sigma Aldrich, St. Louis, USA) were esterified using methacrylic acid (Sigma) to attach the methacrylate functionality. Caprolactone 2-methacryloyloxy-ethyl ester [$HO(CH_2)_5CO_2CH_2CH_2O_2CC(CH_3)=CH_2$] was used after this process as it is available as the methacrylate-functionalized intermediate. The synthesis of 10-methacryloyloxy-decyl-dihydrogen phosphate (MDP), 12-methacryloyloxy-dodecyl-dihydrogen phosphate (MDDP), methacryloyloxy-tetraethylene-glycol-dihydrogen phosphate (MTEP) and methacryloyloxy-caprolactone-dihydrogen phosphate (CAP-P) was accomplished by reaction of methacrylate-byproducts with phosphorus pentoxide and methylene chloride in an ice bath for 48h. After reactions and purifications, the isolated products were characterized using FTIR to confirm the synthesis of the MDP, MDDP, MTEP and CAP-P. The monomer 2-methacryloyloxy-ethyl-dihydrogen phosphate (MEP) was purchased from Esstech (Essington, PA, USA) and used without further purification. The chemical structures of monomers studied are shown in Figure 1A.

Atomic Absorption Spectroscopy (AAS)

The neat monomers were mixed with water/ethanol (50/50v%) in 0.1mM. The free-calcium (Ca^{2+}) was solubilized by dissolution of CaCl in deionized water. The solubilized monomers were mixed with the free-calcium solution in equimolar ratios equivalent to 3 ppm of Ca^{2+} and the monomer-calcium salts were formed by the ionic bond between anionic functional monomers and Ca^{2+}. The resultant solutions were analyzed to detect the absorbance related to the presence of free-calcium using atomic absorption spectroscopy (AAS) with the spectrophotometer AA240FS (Varian, Palo Alto, USA). The observed absorbance of Ca^{2+} was inversely proportional to the formation of monomer-calcium salts. All solutions were prepared in triplicate (n=3) and data were analyzed with one-way ANOVA and Tukey's test ($\alpha=0.05$).

Formulation of adhesives

A base resin blend (control) was prepared by mixing 30 wt% urethane-dimethacrylate (UDMA), 10 wt% bisphenol-A-diglycidyl-methacrylate (BisGMA), 7 wt% triethylene-glycol-dimethacrylate (TEGDMA), 5 wt% hydroxyethyl-methacrylate (HEMA), 15 wt% deionized water, 30 wt% ethanol and 3 wt% photoinitiation system. The photoinitiator used was camphoroquinone (0.5 wt%) whilst ethyl 4-dimethylaminebenzoate (1 wt%) and diphenyliodonium hexafluorophosphate (1.5 wt%) were the co-initiators. Acidic functional monomers were added in 15 mol% to the base resin blend to create a series of experimental one-step self-etch adhesives (1-SEAs). All adhesives had their pH standardized in 2 using sodium hydroxide powder when necessary.

Microtensile Bond Strength

Extracted human third molars were used after approval of the Institutional Ethics Committee (protocol 127/2011) from Piracicaba Dental School and stored in 0.5% chloramine/water solution at 4°C for no longer than two months.

Each tooth was sectioned in the occlusal crown to expose a flat middle dentin surface using a slow-speed water-cooled diamond saw (Isomet-1000, Buehler, Lake Bluff, USA) and at the cemento-enamel junction with a parallel cut to remove the roots. The dentin surfaces were wet-abraded using 600-grit SiC papers to create standardized smear layers and immediately bonded using the experimental 1-SEAs. They were actively applied for 30s, gently air-dried and light activated for 20s using the halogen-lamp XL-2500 (>600mW/cm², 3M-ESPE, St. Paul, USA). The resin composite Filtek Z250 (3M-ESPE) was used for build-ups.

Bonded-teeth (n=10) were immersed in distilled water for 24h at 37ºC and subsequently sectioned into 0.8±0.01mm² sticks using a slow-speed water-cooled diamond saw (Isomet-1000, Buehler, Lake Bluff, USA). Sticks from the periphery presenting residual enamel were excluded. Half of the sticks from each bonded-tooth were tested immediately whilst the remaining half was stored for one year in distilled water which was monthly exchanged. The sticks were fixed to jigs with cyanoacrylate glue and tested to failure under tension in a universal testing machine EZ-test (Shimadzu, Kyoto, Japan) with a crosshead speed of 1.0 mm/minute. Bond strengths of sticks from the same bonded-tooth were averaged and the mean was used as a statistical unit. Sticks that failed prematurely were included as 0MPa. The data were statistically analyzed using two-way ANOVA (functional monomer and storage period) and Tukey's test (α=0.05).

Analysis of fracture mode

The fractured sticks were imaged for failure mode by stereomicroscopy at 60x magnification. Representative de-bonded sticks, exhibiting the most frequent failure pattern and the µTBS close to the mean were processed for scanning electron microscopy (SEM). The parts of the fractured sticks were paired and mounted on aluminum stubs. Thereafter, they were dehydrated in silica gel, gold-sputter coated and examined using SEM, JSM-5600LV (JEOL, Tokyo, Japan), operated at 15 kV. The fractures were classified as adhesive, cohesive in dentin, cohesive in composite or mixed.

Micropermeability assessment

Three teeth per functional monomer were bonded as previously described with the 1-SEAs doped with 0.1wt% rhodamine-B (Sigma) and assessed by confocal-laser scanning microscopy (CLSM) (Sauro et al., 2013). Briefly, the micropermeability evaluation of resin-dentin interfaces was performed using a 0.3 wt% aqueous fluorescein (Sigma) solution. This fluorescent dye was perfused for 3h under 20 cmH_2O simulated pulpal pressure (Feitosa et al. 2012) to trace the water-filled spaces and test the sealing ability of 1-SEAs. The specimens were subsequently cut into 1 mm slabs, slightly polished with 2000-grit polishing paper and ultra-sonicated for 5 minutes.

The microscopy examination was performed using a confocal laser scanning microscope (Leica SP2 CLSM, Heidelberg, Germany) equipped with a 63×/1.4 NA oil immersion lens using a 488 nm (fluorescein excitation) and a 568 nm (rhodamine excitation) laser illumination. CLSM reflection and fluorescence images were obtained with 1 µm z-step to section optically the specimens to a depth of up to 20 µm below the surface. The z-stack scans were compiled into single projections. Each resin-dentin interface was completely investigated and 5 optical images were randomly captured representing the most common features of micropermeability observed along the bonded interfaces.

Nanoleakage evaluation

Five bonded-sticks from each group were submitted to a nanoleakage evaluation as previously described (Tay *et al.* 2002) using a 50 wt% ammoniacal silver nitrate solution [$Ag(NH_3)_2NO_3$]. The specimens were immersed in the tracer solution for 24h and then and immersed in photo-developing solution for 8h under a fluorescent light to reduce silver ions into metallic silver grains. Following this, the specimens were rinsed thoroughly in distilled water, included in epoxy resin stubs and polished using wet 600-, 1200-and 2000-grit SiC papers and diamond pastes (Buehler, Lake Bluff, IL, USA) 3, 1 and 0.25 µm; they were ultrasonically cleaned (5 min) after each abrasive/polishing step. The specimens were dehydrated in silica gel, coated with carbon and examined using SEM JSM-5600LV (JEOL) in backscattered electron mode.

RESULTS

The AAS evaluation showed the ability of the tested monomers at forming monomer-calcium salts (Figure 1B). MDP and MDDP showed similar outcomes which were the lowest ($p<0.001$) free calcium absorbance among all monomers (0.1731±0.0008 and 0.1719±0.0004 Arbitrary ABS, respectively). The higher free-calcium absorbance was observed with MEP (0.2202±0.0004), while the free calcium absorbance observed with CAP-P and MTEP was 0.1931±0.0007 and 0.2121±0.0009, respectively.

The µTBS outcomes are shown in Figure 2A. At 24h, the µTBS of MDDP (41.2±5.3MPa) and MDP (39.7±5.9MPa) were statistically higher than those of MEP (27.9±3.9MPa), CAP-P (31.4±4.0MPa) and MTEP (30.4±3.9MPa). This latter functional monomer was the unique to provide significant µTBS reduction (23.2±3.1MPa) after 1-year water storage ($p=0.014$). Three or less pre-test failures occurred in each group.

Fractures were prevalently adhesive (Figure 2B), except for MDDP-24h which presented more mixed failures. The SEM micrographs depicted in Figure 3 showed signs of hydrolytic degradation for 1-SEAs containing CAP-P (Figures 3G and 3L) and MTEP

(Figures 3H and 3M) with the latter monomers disclosing porosities within the adhesive layers after aging. MEP also showed some degradation at the bottom of hybrid layer (Figure 3O) after water storage.

Fluorescein uptake in the micropermeability assessment was notably more intense in interdiffusion zones of CAP-P and MTEP (Figures 3B and 3C respectively) and in the adhesive layer of latter monomer with a yellow hue provided by simultaneous emission of red (rhodamine) and green (fluorescein). Intense fluorescein uptake was also observed at bottom of interdiffusion zones created using MEP (Figure 3E). Resin-dentin interfaces of MDP- and MDDP-adhesives (Figures 3A and 3D respectively) were almost devoid of fluorescein, representing very low micropermeability.

Nanoleakage micrographs are shown in Figure 4. MDP- and MDDP-based 1-SEAs created interfaces with few small silver deposits at the bottom hybrid layers initially (Figures 4A and 4D respectively) and after aging (Figures 4F and 4H respectively). Contrariwise, the nanoleakage of CAP-P, MTEP and MEP was strikingly increased after aging represented by large silver deposits (Figures 4F, 4G and 4I respectively). Some water channels within the adhesive layers were found with the more hydrophilic monomers CAP-P and MTEP (not shown).

DISCUSSION

Remarkable differences were observed in the present outcomes. Both the length and the hydrophilicity of the spacer chain attained different chemical interactions represented by the formation of monomer-calcium salts (Figure 1B) as well as different initial and long-term dentin bonding performance (Figure 2A). Thus, the two null hypotheses must be rejected.

The acidic functional monomers play a key role on the self-etch/self-adhesive approach used by dentin adhesives (Van Meerbeek et al. 2011), self-adhesive luting agents (Peumans et al. 2013) and self-adhesive resin composites (Poitevin et al. 2013). This sort of monomer allows the simultaneous etching and resin infiltration to form the resin-dentin interdiffusion zones which are able to provide adequate bond strength. Indeed, new functional monomers are currently been synthesized and tested in order to improve the bonding performance of the materials and, more importantly, their clinical longevity. Beyond the self-etching purpose to create the micro-mechanical interlocking, the functional monomers are able to ionically interact with calcium and hydroxyapatite (Yoshihara et al. 2011). Such interaction provides an additional mechanism for the dentin bond. Whether this chemical bond is intensely formed and hydrolytically stable, the restoratives' longevity is notably improved (Peumans et al. 2010, Van Meerbeek et al. 2011).

In this investigation, five acidic functional monomers (Figure 1A) with slight differences in their chemical structures were tested. Only the spacer chain was modified in order to test the effects of its length and polarity. Monomers with long and apolar spacer chains (MDP and MDDP) attained higher formation of monomer-calcium linkages (Figure 1B) which is in agreement with previous investigations (Van Landuyt et al. 2008, Yoshihara et al. 2011). The more hydrophilic monomers (MTEP and CAP-P), in turn, formed less monomer-calcium salts by increasing the hydrophilicity of the spacer group. MEP, the monomer with short spacer chain, achieved statistically the least formation of monomer-calcium interaction. Interestingly, the outcomes of AAS survey (monomer-calcium salts formation) were strictly correlated with the initial dentin bond strength (Figure 2A) once MDP and MDDP provided higher bond strength than CAP-P, MTEP and MEP. This correlation was also found in a previous study (Van Landuyt et al. 2008).

Although the length of the spacer group played a major role concerning the formation of monomer-calcium salts (Figure 1B), the durability of dentin bonds was affected mostly by the spacer chain hydrophilicity since MTEP was the unique monomer that attained bond strength reduction between 24h and 1-year (Figure 2A) and also the highest incidence of adhesive failures (Figure 2B). In fact, these findings might be correlated with the higher micropermeability of resin-dentin interfaces created with the MTEP-based adhesive (Figure 3C). Among the test monomers, MTEP achieved the higher water sorption (unpublished results) which could be observed by the intense fluorescein uptake within its adhesive layer. Several water channels were found at this zone in fractured specimens after aging (Figure 3M). Nishitani et al. (2006) disclosed the effects of resin hydrophilicity on dentin bonds. Indeed, resin-dentin interfaces created with more hydrophilic monomers are more prone to hydrolytic degradation (Feitosa et al. 2012) and water contamination detected by micropermeability studies (Sauro et al. 2009).

The micropermeability of CAP-P and MEP was also high inside (Figure 3B) and at the bottom (Figure 3E) of the interdiffusion zones respectively. These areas also showed signs of degradation in fracture analyses (Figures 3L and 3O) and more silver-uptake after aging (Figure 4). Despite these noteworthy signs of degradation, the bond strength of CAP-P and MEP remained stable after aging. After longer periods of storage, their bond strength could likely drop significantly. This would not be expected for MDP and MDDP which attained very low micropermeability and nanoleakage.

According to the adhesion-decalcification (AD) concept (Van Meerbeek et al 2011), the monomers that rather chemically bond to hydroxyapatite than decalcify it, such as MDP, promote more durable dentin bonds. Yet, a recent investigation (Yoshihara et al. 2010) showed that functional monomers that etch deeper and form thicker hybrid layers provide less chemical interaction and consequently lower initial bond strength (Van Landuyt et al. 2008). Similarly, the hybrid layer of MEP (Figure 3E) was thicker than that created using MDDP (Figure 3D). Hence, MEP decalcified more the dentin than MDDP. This ratifies the AD concept once MDDP interacted much more than MEP with calcium (Figure 1B).

In conclusion, the length and polarity of spacer chain remarkably influences the formation of monomer-calcium salts, dentin bonding performance, micropermeability and nanoleakage. Functional monomers with long and hydrophobic spacer chains are able to better interact with calcium, to attain stable dentin bond, low micropermeability and low nanoleakage. Future studies should aim in evaluating the influence of other chemical functionalities such as the acidic group (phosphoric, carboxylic, phosphonic etc) with standardized polymerizable group and spacer chain.

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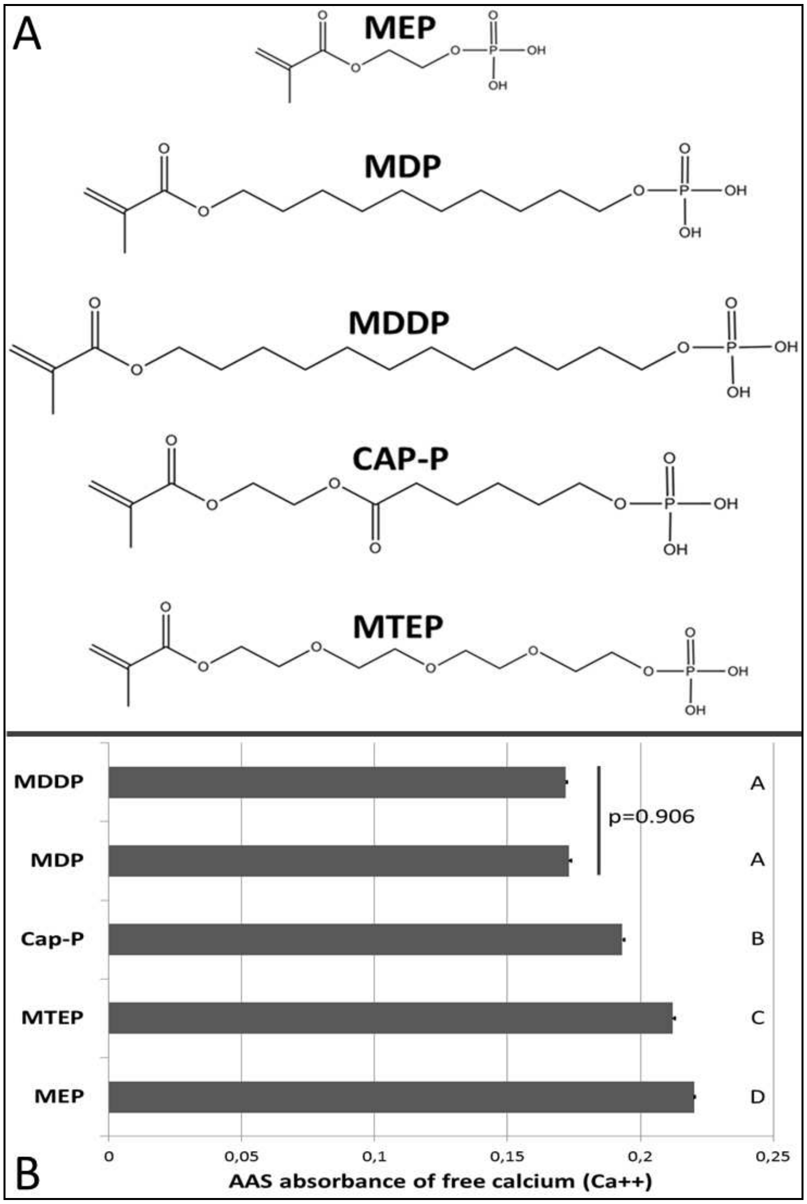

Figure 1. (A) Chemical structures of the phosphoric functional monomers evaluated with spacer chains with different length and hydrophilicity. (B) Atomic absorption spectroscopy (AAS) absorbance of free-calcium attained after treatment with studied monomers. The lower is the absorbance of free-calcium (Ca^{2+}) the higher formation of monomer-calcium salts. MDP and MDDP obtained the highest formation of monomer-calcium salts whereas MEP provided the statistically lowest formation among all monomers.

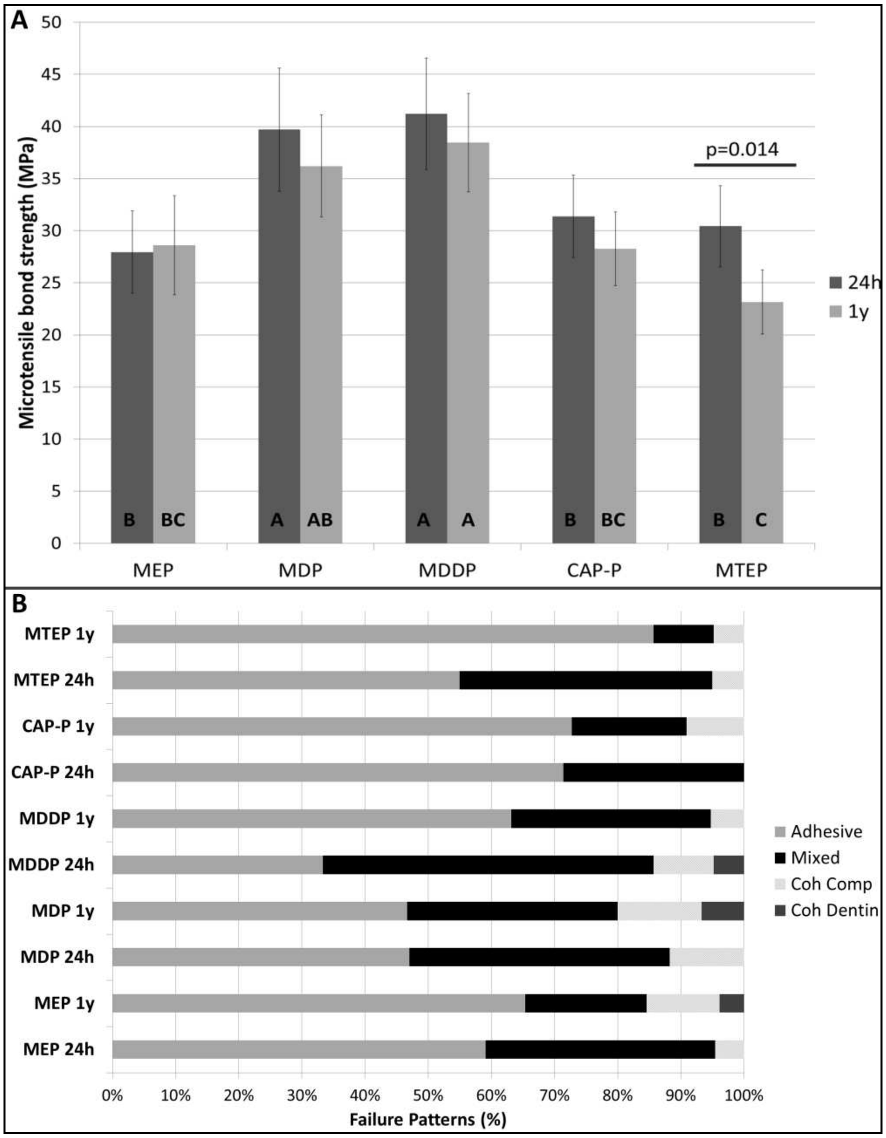

Figure 2. Graphs depicting the results of microtensile bond strength (A) and failure mode spreading (B). The outcomes of statistical analysis are presented within the bars of each group. Different capital letters depict statistically significant difference between 1-SEAs on each storage time ($p<0.05$). MDP and MDDP provided the higher initial bond strengths. These two monomers, CAP-P and MEP had no significant drop in bond strength. However, the more hydrophilic functional monomer MTEP attained significantly reduced bond strength after aging. The predominant failure mode was adhesive for all groups except MDDP-24h. Note in Figure B that fractures in group MTEP-1 year were more than 80% adhesive.

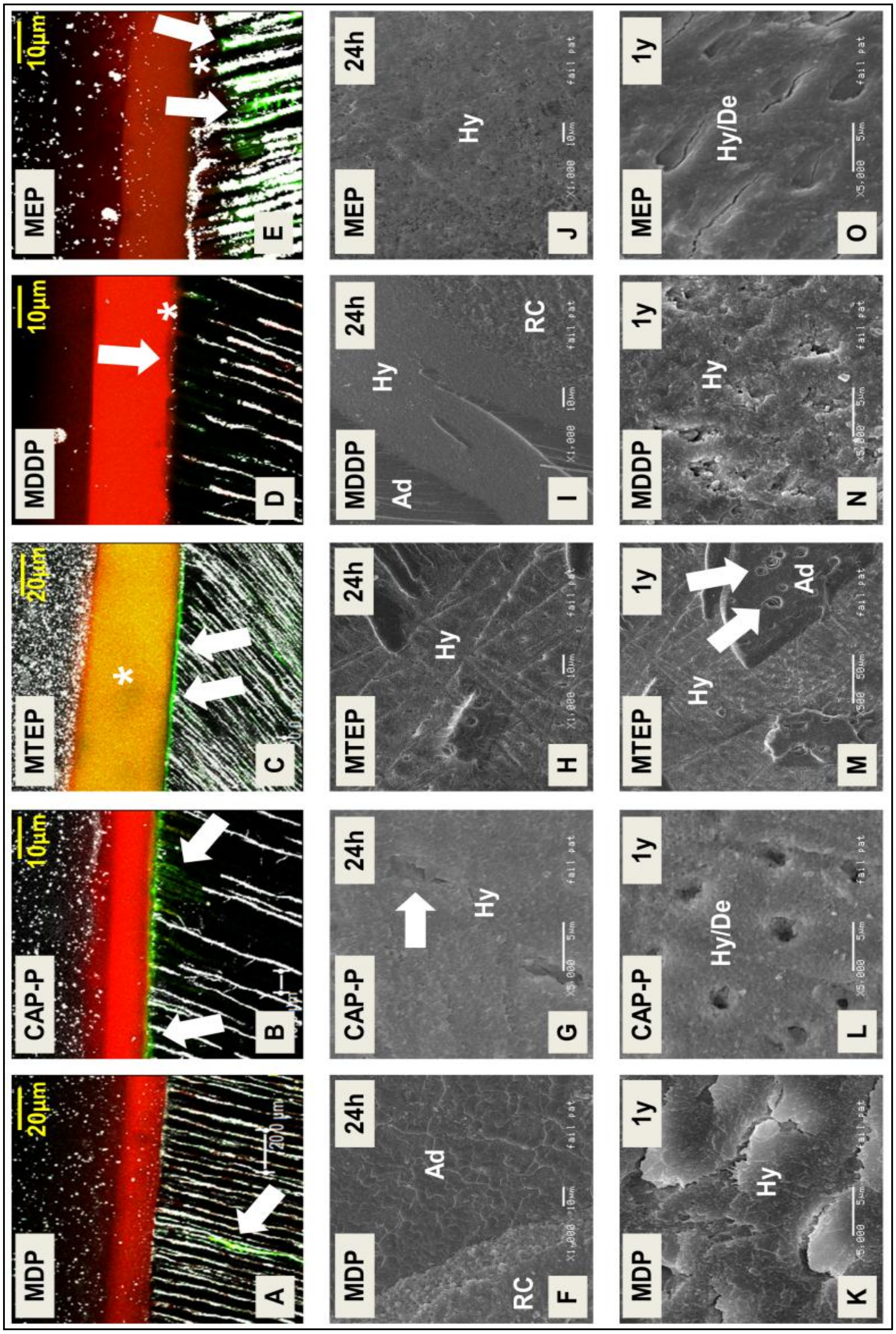

Figure 3. Confocal micrographs (upper line) of interfaces showing the main features of micropermeability (fluorescein uptake) and SEM images of fractured specimens. Note that

the resin-dentin interfaces of MDP (Figure A) and MDDP (Figure D) were almost free of fluorescein with only some deposits within dentinal tubules (arrow in Figure A). Both monomers showed low micropermeability. Conversely, the interdiffusion zones of CAP-P (Figure B) and MTEP (Figure C) interfaces were almost fully impregnated by fluorescein (arrows) whereas the adhesive layer of MTEP was also strikingly impregnated by the micropermeability dye depicting a yellow color (asterisk) attained by the mixture of red (rhodamine) and green (fluorescein) pigmentations. The interface created using MEP, in turn, showed a thicker interdiffusion zone (asterisk in Figure E) in comparison with the interdiffusion zone of MDDP (asterisk and arrow in Figure D) for instance. The fluorescein uptake with this monomer was observed only at the bottom of the interdiffusion zone (arrows). The SEM micrographs of the surface of fractured specimens (Figures F-O) showed no signs of degradation for MDP and MDDP although more adhesive failures were observed rather than mixed fractures often encountered in 24h specimens (Figures F and I). For CAP-P, evident signs of hydrolytic degradation were observed by comparing the well-hybridized smear plugs (arrow in Figure G) with the patent tubules and degraded hybrid layer after aging (Figure L). Similar event occurred with the MEP-based adhesive (Figures J and O) with signs of degradation at the bottom of hybrid layer. For MTEP, many channels in the adhesive layers were found as shown by arrows in Figure M. Water aging created these channels once they could not be observed in 24h specimens (Figure H). *RC – resin composite, Hy – hybrid layer, De – dentin, Ad – adhesive layer.*

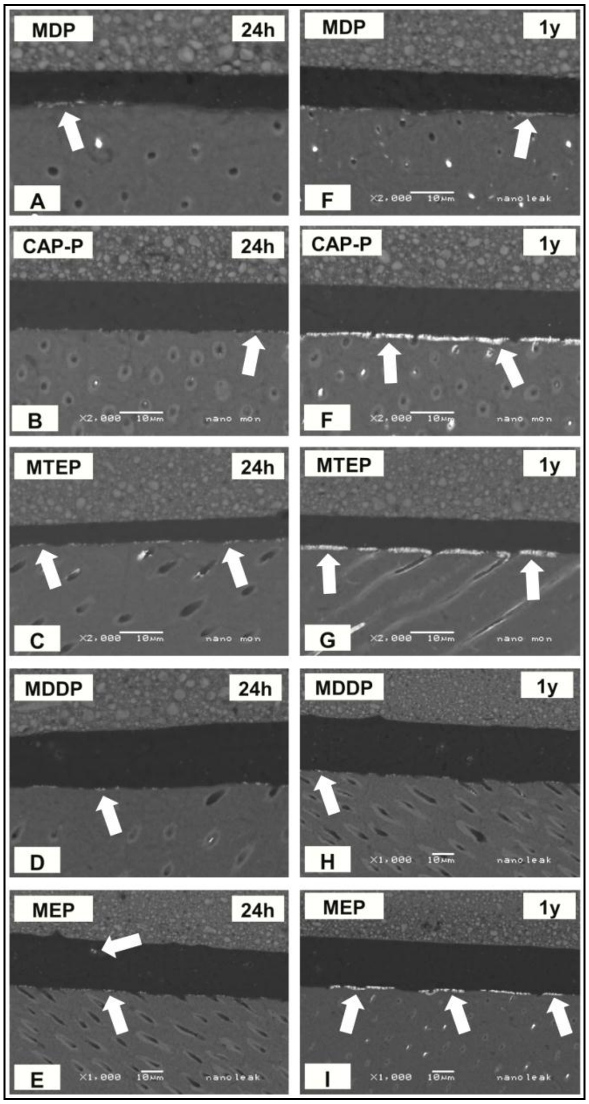

Figure 4. Backscattered SEM micrographs depicting the most common features of silver uptake (nanoleakage). Arrows indicate silver deposits within the resin-dentin interfaces. Interfaces created using MDP (Figures A and F) and MDDP (Figures D and H) were almost devoid of silver deposits before and after aging. CAP-P, MTEP and MEP showed noteworthy increase in number and thickness of silver deposits after aging (Figures F, G and I respectively). Furthermore, MTEP and CAP-P also presented some water channels (water-trees) after aging (not shown).

CONSIDERAÇÕES GERAIS

Exceto no grau de conversão, as diferentes cadeias espaçadoras promoveram diferenças notáveis em todos os experimentos testados. Assim, considera-se que a hipótese do trabalho deve ser rejeitada.

O grau de conversão de todos os adesivos experimentais e da resina controle foi alto, provavelmente devido ao sistema foto-iniciador ternário utilizado (8, 13). Não houve diferença estatisticamente significante provavelmente devido ao delineamento experimental do trabalho que adicionou os monômeros funcionais ácidos em porcentagens molares iguais em vez de porcentagens em peso, como é normalmente feito. Mesmo com conversão similar, as diferentes cadeias espaçadoras demonstraram efeito significativo na sorção de água (Figura 4, Capítulo 1), com maior sorção para cadeias mais hidrófilas, e também na resistência coesiva dos adesivos experimentais (Figura 3, Capítulo 1). O delineamento experimental (Capítulo 1) também foi importante nos resultados de resistência coesiva, pois os espécimes foram armazenados em umidade relativa, o que evitou a sorção de água com monômeros de cadeia espaçadora mais hidrófila e o possível efeito negativo dessa água na resistência coesiva dos adesivos experimentais contendo esses monômeros. Uma justificativa plausível para a maior resistência coesiva dos adesivos que utilizaram o MDP, MDDP e MTEP são as forças intermoleculares (Forças de Van der Waals, Forças de Van der Waals e Dipolo-dipolo, respectivamente) que esses monômeros são capazes de criar através das suas longas cadeias espaçadoras (5). Entretanto, a cadeia curta de MEP e o grupo funcional éster de CAP-P não propiciam tais interações intermoleculares o que pode ter sido o fator principal para que esses monômeros gerassem menores resistências coesivas.

Ainda no capítulo 1, foi interessante observar como a hidrofilia (polaridade) da cadeia espaçadora altera o molhamento (medido através do ângulo de contato) dos diferentes sistemas adesivos sobre a dentina (Figura 6, Capítulo 1) que é um substrato com característica polar. A composição mineral da dentina e a presença de água no fluido dentinário promovem tal característica que dificulta o molhamento de resinas mais hidrófobas (como o controle negativo) e ajuda na interação de monômeros mais polares como o CAP-P e o MTEP. A boa interação desses monômeros com o substrato propiciou um espalhamento mais rápido do adesivo que foi detectado através da maior diferença entre os ângulos de contato inicial e final. Sobre a lamínula de vidro, o menor ângulo de contato do adesivo autocondicionante de um passo experimental contendo o monômero funcional CAP-P (Figura 6A, Capítulo 1) pode ter sido devido ao grupo funcional éster que tem característica ácida e pode ter melhorado a interação desse monômero com a superfície de óxidos do vidro. A hidrofilia da cadeia espaçadora promoveu melhora no molhamento dos adesivos sobre a dentina (Capítulo 1) e isso pode ter influenciado a melhor interação química desses monômeros com a dentina, observada no Capítulo 2.

Em relação à interação química dos monômeros funcionais com o cálcio e a dentina (avaliada no Capítulo 2), os monômeros com características mais hidrófilas obtiveram menor formação de sais monômero-cálcio o que vai de acordo com os resultados de estudos prévios (16, 21). Entretanto, o pior resultado obtido por MEP (monômero de cadeia espaçadora curta) não era previsto por esse monômero ser menos hidrófilo que CAP-P e MTEP. Entretanto, os estudos de Ikemura e colaboradores (3) demonstraram que monômeros funcionais com cadeias espaçadoras mais curtas possuem menor resistência de união ao esmalte o que pode estar relacionado com os piores resultados obtidos por MEP. A aplicação dos monômeros sobre as superfícies de dentina induziu a formação dessas ligações com a hidroxiapatita (22) e foi observada a união de uma fina camada de monômeros quimicamente unidos à dentina (Figura 3, Capítulo 2). Tal camada foi detectada na análise em FTIR e também observada em MEV. Era esperado que após a lavagem, os monômeros mais hidrófilos (CAP-P e MTEP) fossem lixiviados da dentina e os outros três monômeros com características mais hidrófobas permanecessem ligados a ela (21). No entanto, os dois monômeros mais hidrófilos permaneceram ligados à dentina e somente o monômero de cadeia curta (MEP) foi lixiviados (Figuras 2 e 3, Capítulo 2) deixando a dentina condicionada descoberta de qualquer camada de monômero (Figura 4, Capítulo 2).

Segundo o conceito de Adesão-Descalcificação (17, 19), monômeros que condicionam mais a dentina, formando camadas híbridas mais espessas (22), descalcificam mais o substrato e se ligam quimicamente menos. Os achados deste trabalho estão de acordo com esse conceito, pois o adesivo experimental contendo MEP mostrou camada híbrida mais espessa (Figura 3, Capítulo 3) e esse monômero obteve menor formação de ligações com o cálcio (Figura 1, Capítulo 3 e Figura 1, Capítulo 2). Além disso, Van Landuyt e colaboradores (16) mostraram que monômeros que possuem menor estabilidade da ligação química com o cálcio promovem menor resistência de união inicial. De fato, a resistência de união inicial do MEP foi numericamente menor que a dos outros monômeros e estatisticamente menor que as de MDP e MDDP e também a estabilidade hidrolítica da ligação MEP-cálcio foi baixa sendo a única quebrada com a lavagem. Os resultados estão de acordo com os estudos prévios (16, 21) tanto em relação à união química como em relação à resistência de união.

Em relação a estabilidade da união dos sistemas adesivos experimentais com a dentina no capítulo 3, somente MTEP obteve redução significativa da resistência após um ano de armazenagem dos palitos em água. Correlacionando os achados do padrão de fratura e da micropermeabilidade (Figura 3, Capítulo 3), o monômero mais hidrófilo (MTEP), que também foi o que obteve maior sorção de água no Capítulo 1 (Figura 4), mostrou micropermeabilidade severamente maior que os outros monômeros. Além disso, na camada de adesivo formada por esse monômero, várias porosidades foram encontradas na análise do padrão de fratura dos palitos armazenados por um ano, o que confirma os achados da nanoinfiltração (Figura 4, Capítulo 3). A micropermeabilidade é um teste eficiente para detectar a hidrofilia e sorção de água da resina adesiva (14). É encontrado na literatura correlação entre a micropermeabilidade e a resistência de união ao longo do tempo (1, 12), justificando os achados deste estudo de que o adesivo com maior penetração de fluoresceína degradou mais rapidamente após um ano de armazenagem. A maior contaminação por água na interface adesiva (camadas híbrida e de adesivo) propiciar maior e mais rápida degradação hidrolítica do polímero e das fibrilas colágenas. É bem conhecido que monômeros hidrófilos, como por exemplo, o HEMA (15), aceleram a degradação hidrolítica da união com a dentina principalmente. A cadeia espaçadora mais polar (hidrófila) de MTEP pode ter afetado negativamente a estabilidade da união, pois permitiu a passagem de mais água, o que acelerou o processo de degradação (14).

É importante ressaltar que os monômeros que obtiveram piores interações químicas (Figura 1, Capítulo 3) com o cálcio (MEP, CAP-P e MTEP) também mostraram aumento da impregnação de prata nas interfaces adesivas (Figura 4, Capítulo 3). A união química foi então melhor com monômeros de cadeia longa e hidrófoba (MDP e MDDP) e essa união também propiciou menor degradação da união com a dentina ao longo do tempo, que está de acordo com achados prévios (22). Assim como o MDP (20), o MDDP deve formar uma estrutura em nano-camadas que aumenta a durabilidade da união. Pelos resultados de difração de raios-X de filmes finos (Figura 3, Capítulo 2), pode-se esperar que MTEP e CAP-P também formem tais estruturas. Estudos futuros com microscopia eletrônica de transmissão podem esclarecer esse assunto. Esses monômeros mais hidrófilos poderiam ser utilizados em materiais resinosos endodônticos para melhorar a união química com a dentina radicular, já que esses materiais sofrem menos os efeitos da degradação hidrolítica. Em dentina coronária, os monômeros hidrófobos (MDP e MDDP) e com cadeias longas demonstraram melhor interação química e longevidade da resistência de união quando usados em sistemas adesivos autocondicionantes.

CONCLUSÃO

Diante dos resultados encontrados neste estudo, pode ser concluído que:

1. Cadeias espaçadoras longas e relativamente hidrófobas parecem promover mais ligação química com estabilidade hidrolítica da ligação entre fosfato e cálcio.

2. Cadeias espaçadoras hidrófilas promovem união química estável entre fosfato e cálcio, apesar de induzir menor formação de ligações monômero-cálcio. Elas também promovem maior sorção de água que leva a degradação mais rápida da união à dentina.

3. Monômeros funcionais ácidos com cadeias espaçadoras curtas promovem menos interação química com menor estabilidade, menor resistência coesiva ao adesivo e menor resistência de união inicial.

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ANEXO

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Adesivos de passo único experimentais - Análise dos monômeros funcionais e efeito da densidade de energia (tempo vs. irradiância)"**, protocolo nº 127/2011, dos pesquisadores Victor Pinheiro Feitosa, Américo Bortolazzo Correr e Mário Alexandre Coelho Sinhoreti, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 11/11/2011.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Model all-in-one adhesives - evaluation of functional monomers and effect of light energy density (time vs. irradiance)"**, register number 127/2011, of Victor Pinheiro Feitosa, Américo Bortolazzo Correr and Mário Alexandre Coelho Sinhoreti, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 11/11/2011.

Livia M. A. Tenuta
Profa. Dra. Lívia Maria Andaló Tenuta
Secretária
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.