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Influence of the hydrostatic pulpal pressure on droplets formation in current etch-and-rinse and self-etch adhesives: A video rate/TSM microscopy and fluid filtration study

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ABSTRACT

Objectives. The aim of this study was to investigate the droplet formation using a real-time/confocal microscopy technique, when different self-etching and etch-and-rinse adhesives were applied in the presence or absence of pulpal pressure. Resin–dentin permeability (%P) was also evaluated.

Methods. Optibond FL, Silorane adhesive, Scotchbond 1XT, G-Bond and DC-Bond were bonded in the presence or in absence of simulated pulpal pressure. A fluid-transport model was used to measure the water permeability through resin-bonded dentin. Half of the specimens bonded in the presence of the hydrostatic pulpal pressure (20 cm H_2O) were light cured, whereas the remnant half received no light curing. The same was done with the half of the specimens bonded under no pulpal pressure. The specimens were investigated under a confocal TSM.

Results. Optibond FL and G-Bond had the lowest dentin permeability. Optibond FL adhesive showed few water droplets on the polymerized external surface and within the resin–dentin interface. G-Bond showed static interfacial globular-like droplet formation. DC-Bond and Scotchbond 1XT were the most water permeable adhesives both in the presence and in absence of pulpal pressure. A dynamic interfacial non-globular-like droplet formation was observed. Severe droplet formation was observed on the polymerized external surface.

Significance. The presence of the pulpal pressure may cause increasing in fluid filtration and droplet formation in simplified adhesives containing HEMA. The adhesives containing 4-META (G-Bond) may be affected by static phase separation but by very low osmotic droplets formation and water permeability. The three-step adhesives are less affected by these problems.

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1. Introduction

Dentin–enamel bonding adhesives (DBAs) have been simplified by combining all ingredients in a single bottle (i.e. self-etching all-in-one or one-bottle/etch-end-rinse systems) in order to offer a reduction in technique sensitivity, application difficulties and chair-time [1,2]. Nevertheless, many observations have been raised against the efficacy and bonds longevity of these simplified adhesives [3–6].

One explanation for the lower efficacy of the simplified DBAs is the presence of interfacial droplets at the formed hybrid layers [7–9]. The origin of these droplets and microporosities need further investigation. However, some reasons have been advocated: (1) water entrapment due to osmotic processes and water sorption [8,9], which may be increased by the presence of pulpal pressure [10,11], after long-term water immersion [12,13], or due to the rapid evaporation of acetone (when used as solvent) exceeding that of water [14]; (2) permeability of formed hybrid layers to the fluids after DBA polymerization [10,15,16]; (3) phase separation reaction due to the complex mixture of hydrophobic and hydrophilic monomers, which may be major in DBAs free of 2-hydroxyethylmethacrylate (HEMA) [17,18] or after rapid solvent evaporation [7]; and (4) the existence of an acid–base reaction between acidic monomers and tertiary amines included as chemical initiators for resin polymerization [19].

The aim of this study was to investigate the droplet formation using a real-time/TSM confocal microscopy technique, when different self-etching and etch-and-rinse adhesives were applied in the presence or absence of hydrostatic pulpal pressure. Fluid filtration (dentin permeability: P%) throughout formed hybrid layers was also evaluated. The null hypotheses tested were that there is no difference in the water permeability or in the droplets formation between the tested DBAs when applied in the presence or absence of an hydrostatic pulpal pressure.

2. Materials and methods

2.1. Sample preparation

Human molars (age 20–40), extracted for surgical reasons under a protocol approved by an institutional review board of the King's College London Dental Institute (London, UK), were used in this study. The teeth were stored in deionized water at 4 °C for no more than 1 month. Crown segments were obtained by first removing the roots 1.5 mm beneath the cementum-enamel junction (CEJ) using a slow-speed water-cooled diamond saw (Labcut, Agar Scientific, Stansted, UK). The occlusal enamel of each crown segment was subsequently removed with a parallel cut to expose the deep dentin. Pulpal tissue was carefully removed from the exposed pulp chamber without altering the pre-dentin surface by using thin tissue forceps. A pincer-type calliper was used for measuring the remaining dentin thickness (RDT) from the surface to the highest pulpal horns (0.7 and 0.9 mm). Each tooth section was attached to a Perspex$^{\text{TM}}$ (Perspex Distributions Ltd., London, UK) platform (2 cm × 2 cm × 0.5 cm) that was perforated by an 18 gauge stainless steel tube using cyanocrylate adhesive (ROCKET Heavy DVA, Corona, CA, USA). Each specimen was connected to a hydraulic fluid filtration able to deliver an hydrostatic water pressure (Fig. 1).

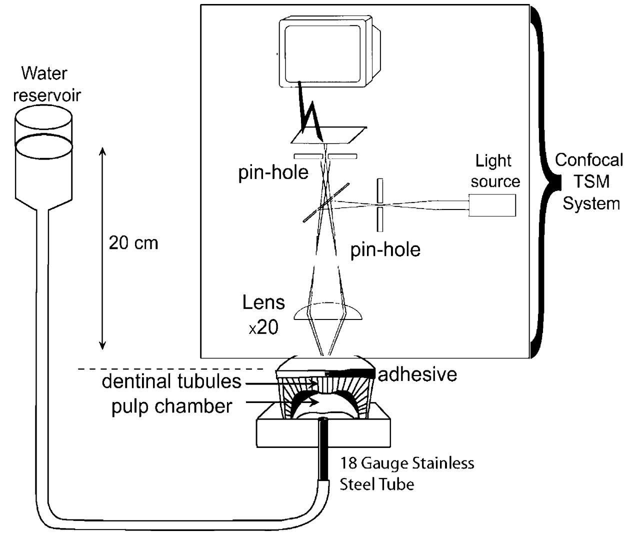

Fig. 1 – schematic illustration of how the dentin specimens were connected to the fluid filtration device and submitted to real-time TSM investigation under hydrostatic pulpal pressure.

2.2. Bonding procedures

Five dentin bonding agents (DBAs) were examined in this study. They included a three-step adhesive (Optibond FL, Kerr, Orange, CA, USA), a two-step self-etching primer adhesive system (Silorane adhesive, 3M ESPE, St. Paul, MN, USA), a two-step etch-and-rinse adhesive (Scotchbond 1XT, also known in USA as Single Bond Plus, 3M ESPE) and two one-step self-etch adhesive systems, G-Bond (GC Corp., Tokyo, Japan); DC-Bond (Kuraray Medical Inc., Tokyo, Japan). Their compositions and pH values are listed in Table 1. Each DBA was applied as per manufacturer's instructions. Light activation of the DBAs was performed using a halogen light-curing unit for 20 s with a blue light source with a 10 mm long tip (470 nm, $\sim$600 mW cm^{-2} Optilux VLC, Demetron Research Co., CT, USA) when required in the experimental project. The tested DBAs were bonded while connected to the fluid filtration apparatus in the presence (20 cm H_2O) or in absence (0 cm H_2O) of pulpal pressure.

2.3. Permeability measurement

Ten crown segments were used for each DBA. Halves of the tested specimens were bonded with the selected adhesives in the presence of a pulpal pressure (20 cm H_2O) [20] and the other half part of the specimens were bonded when the pulpal pressure was absent (0 cm H_2O). Prior to bonding procedures, a smear layer was created on dentin surface using 180 SiC-grit-paper for 30 s under water irrigation. The smear layer was subsequently removed by treating the dentin surface with 37%

Table 1 – Chemical composition, adhesive type, pH and source of tested adhesives.

DBAs	Chemical composition	Adhesive type	pH value
Clearfil DC-Bond, Kuraray Medical Inc., Japan	2-Hydroxyethylmethacrylate (HEMA) Dibenzoyl peroxide Bisphenol A diglycidylmethacrylate (BIS-GMA) 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) Ethyl-alcohol Colloidal silica DL-Camphorquinone Initiators	Self-etching, all-in-one (dual-curing)	2.7
G-Bond, GC Corp., Japan	Phosphorylated methacrylates 4-Methacryloxyethyltrimellitate anhydride (4-META) Triethylene glycol dimethacrylates (TEGMA) Urethane dimethacrylates (UDMA) Acetone	Self-etching, all-in-one	~2
Scotchbond 1XT, 3M ESPE, USA	2-hydroxyethylmethacrylate (HEMA) Bis-GMA Ethyl-alcohol Dimethacrylates Polyacrylic acid methacrylated (PAM) Urethane-dimethacrylates (UDMA) Fluoride complex Camphoroquinone Tripheylantimony Phosphine Water Quartz	Etch-and-rinse, one-bottle	35%H_3PO_4, <1
Silorane 3M ESPE, USA	Primer: Phosphorylated methacrylates Vitrebond copolymer BisGMA HEMA Water Ethanol Silane treated silica filler Initiators Stabilizers Bond: Hydrophobic methacrylates Triethylene glycol dimethacrylates (TEGMA) Silane treated silica filler Initiators Stabilizers	Self-etching-primer and bond, two-steps	<2.7
Optibond FL, Kerr, USA	Primer: 2-Hydroxyethylmethacrylate (HEMA) Glycerophosphate-dimethacrylate (GPDM) Mono(2-methacryloyloxy)ethylphthalate (MMEP) Ethanol Water Initiators Bonding: Bisphenol A diglycidylmethacrylate (BIS-GMA) 2-Hydroxyethylmethacrylate (HEMA) Glycerophosphate-dimethacrylate (GPDM) Barium–aluminum borosilicate glass Disodium hexa-fluoro-silicate Fumed silica	Etch-and-rinse, three steps	35%H_3PO_4, >1, Primer pH 1.7

H_3PO_4 for 30 s to evaluate the maximum fluid filtration of each specimen, to which was arbitrarily assigned a value of 100% permeability.

Subsequently, a smear layer was re-created on the dentin surface in the manner previously described, before the application of the self-etching DBAs. The permeability of each resin-bonded dentin segment was measured and expressed as a percentage of the maximum fluid flow obtained in each respective unbonded, 37% H_3PO_4-etched dentin specimen. The percentage of dentin permeability (%*P*) was calculated

Table 2 – Relative adhesive permeability, fluid flow rate of the and resin-bonded dentin.

DBAs	%P/20 cm H_2O	%P/0 cm H_2O	% of increase 0 cm vs. 20 cm H_2O
G-Bond	^{A1} 15.8	^{a1} 13.3%	+15.8%
DC-Bond	^{B1} 33.7	^{b2} 26.7%	+20.8%
Scotchbond 1XT	^{B1} 37.3	^{c1} 34.1%	+8.6%
Optibond FL	^{A1} 13.2	^{a1} 11.5%	+12.8%
3M Silorane	^{C1} 19.4	^{d1} 17.8%	+7.9%

Relative water permeability represented as a percentage of the maximum permeability of the smear layer-depleted dentin for each crown segment. Letters indicate differences ($p < 0.05$) in columns and numbers in rows. The % of increase indicates the percentage difference between the %P obtained at 0 *vs.* those obtained at 20 cm H_2O in each single adhesive system.

using the following equation [10]:

$$\left[\%P = \frac{\text{fluid filtration rate } (\mu\text{l/ min at } 20\text{ cm } H_2O) \text{ of resin bonded dentin}}{\text{fluid filtration rate } (\mu\text{l/ min at } 20\text{ cm } H_2O) \text{ un-bonded acid} - \text{etched dentin}} \times 100\right].$$

This represents the permeability exhibited by the resin-bonded dentin relative to its maximum H_3PO_4-treated value, with each tooth serving as its own control. Fluid transudation through the resin-bonded dentin was performed according to the hydraulic conductance protocol reported by Pashley and Depew [21]. Fluid flow was measured by following the movement of an air bubble trapped within a 25 μl capacity glass capillary tube (0.7 mm inside diameter) (Microcaps, Fisher Scientific, Atlanta, GA, USA) that was positioned between the pressure reservoir and the crown segment (Fig. 1). The permeability (%P) obtained for the adhesives bonded to dentin were statistically compared using a two-way ANOVA design with “adhesive type” and “presence/absence of pulpal pressure” as the two factors. Multiple comparisons were performed with the Fisher’s PLSD test. Statistical significance were set at $\alpha = 0.05$ [10].

The experimental design involved measurement of fluid flow across 37% H_3PO_4-treated dentin, smear layer-covered dentin before bonding and then after bonding, under 20 cm H_2O pressure for five times (2 min each) period of a total period of 10 min [10].

2.4. *Video rate confocal microscopic imaging*

A further 12 crown segments were used for each DBA for the video-rate/confocal microscopy evaluation. In half of the specimens, the adhesives were applied in the presence of pulpal pressure (20 cm H_2O) [$n = 6$] and in the other half the pulpal pressure was absent (0 cm H_2O) [$n = 6$]. Half of the specimens bonded in the presence of a pulpal pressure [$n = 3$] were light cured using a halogen light-curing unit for 20 s as previously described, whereas, the remnant half part of the specimens received no light curing. The same was done with the half of the specimens bonded under no pulpal pressure [$n = 3$], that were light cured for 20 s, whereas the remnant half part of the specimens [$n = 3$] received no light curing. The un-polymerized resin-bonded dentin specimens were covered by a thin cover-slip applied on the top of the resin–dentin surface and immediately positioned under a confocal tandem scanning microscope (TSM, Noran Instruments, Middleton, Wisconsin, USA) to evaluate in real-time any interfacial water droplet formation (Fig. 1).

The resin-bonded specimens immediately light cured were submitted to TSM investigation in order to evaluate in real-time any droplet formation on the polymerized external adhesive surface.

All specimens were illuminated with a mercury arc lamp filtered at 546 nm wavelength (green) using a oil immersion objective (20× NA 0.8; Olympus UK, London, UK) in conjunction with 10× ocular and phototube. The images with a field of view of 400 μm × 400 μm were captured and recorded using a Andor iXon^{EM}, EMCCD-Back-illuminated Range camera (Andor Instruments, Belfast, Northern Ireland), at 25 frames/s for a period of 10 min. The images and videos were processed or reconstructed with suitable computer hardware and software Software-iQ, Andor iXon^{EM} (Andor Instruments).

3. Results

Permeability data (%P) of the resin-bonded dentin are shown in Table 2, expressed as percentages of the maximum permeability in H_3PO_4 acid-etched dentin. Optibond FL and G-Bond had the lowest dentin permeability either when they were applied in the presence of pulpal pressure or in absence. The presence of pulpal pressure did not influence attained permeability values for these two adhesives.

The confocal microscopy investigation (TSM) on the external surface of the polymerized Optibond FL adhesive showed no water droplets. Whereas, when TSM investigation was performed within the un-polymerized Optibond FL showed that only very few droplets were induced by simulated pulpal pressure either immediately following the application (Fig. 2A) and after 10 min of polymerization delay (Fig. 2B). In the absence of pulpal pressure very small water droplets were detected after application with no change overtime (Fig. 3B).

G-Bond showed interfacial globular-like droplet formation during the application procedures either in the presence (Fig. 2C and D) or the absence of pulpal pressure (Fig. 3B). Indeed, these droplets preserved a globular-like shape with no radical increase over time after 10 min of polymerization delay. Very few droplets were detected on the external surface

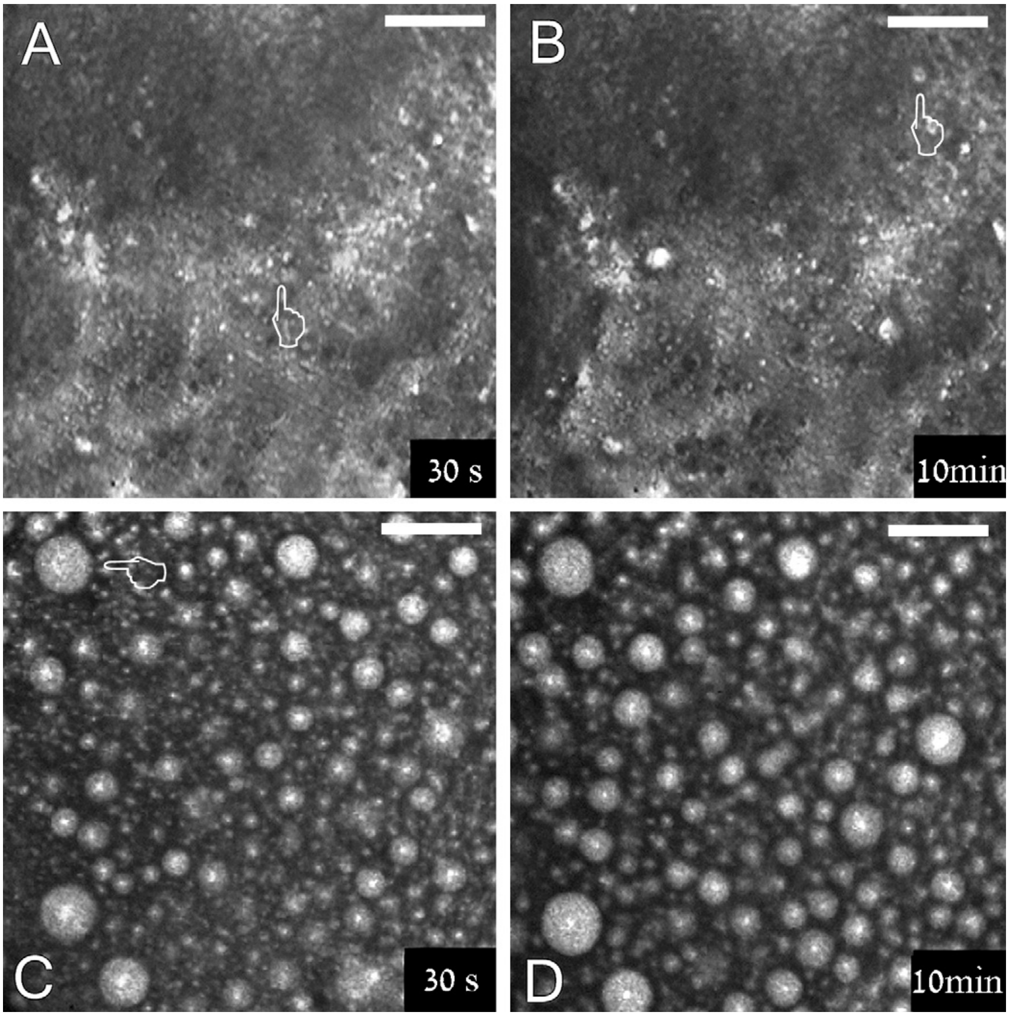

Fig. 2 – TSM images (20× NA 0.8; FV: 400 μm × 400 μm/scale bar: 100 μm) performed 10 min before resin polymerization and under simulated pulpal pressure. (A) Very few and small droplets occurred in Optibond FL, immediately following the resin application (Pointer). (B) Simulated pulpal pressure did not induce radical droplets formation (Pointer), and no dynamic phase separation was evidenced, after 10 min. (C) Phase separation characterized by globular-like shape droplets and/or water flowing is observed after G-Bond application (Pointer). (D) A slight increase in water droplet formation was observed after 10 min but no dynamic shape and size change (D).

of the specimens bonded with G-Bond both in the presence and in the absence of pulpal pressure.

The resin–dentin interfaces created by Clearfil DC-Bond exhibited high relative water permeability of 33.7% in presence of pulpal pressure and 26.7% in absence of pulpal pressure (Table 2). A statistical difference ($p < 0.05$) was found between these two groups. The TSM investigation reported severe interfacial droplet formation over time when submitted to simulated pulpal pressure. Droplets were irregular with a non-globular morphology, different from those observed in the group of the G-Bond. These droplets changed in number, dimension and shape over time (Fig. 4A). In the absence of pulpal pressure, the droplets had an irregular non-globular morphology since the beginning of the investigation and mainly occurred during the application of the adhesive. Slight change in dimension and shape was observed over time (Fig. 3C). TSM microscopy investigation of the polymerized external surface showed an increasing appearance of smaller droplets over time (Fig. 5). This droplet formation was also observed in the absence of pulpal pressure, but in a slower manner.

Scotchbond 1XT was one of the most water permeable adhesives between the four DBAs examined both in the presence and in the absence of pulpal pressure. No statistical difference was found when this adhesive was applied in the presence or in the absence of the pulpal pressure (Table 2). TSM investigation showed in the presence of pulpal pressure, formation of numerous and large non-globular shaped droplets within the un-polymerized adhesive layer that increased in number and dimension over time (Fig. 4B). When the pulpal pressure was absent, of the droplets had a non-globular shape and occurred during the application of the adhesive with slight change in dimension and shape over time (Fig. 3D). TSM microscopy investigation of the external surface of the polymerized adhesive showed also in this case an increasing appearance of smaller droplets over time, compare to these

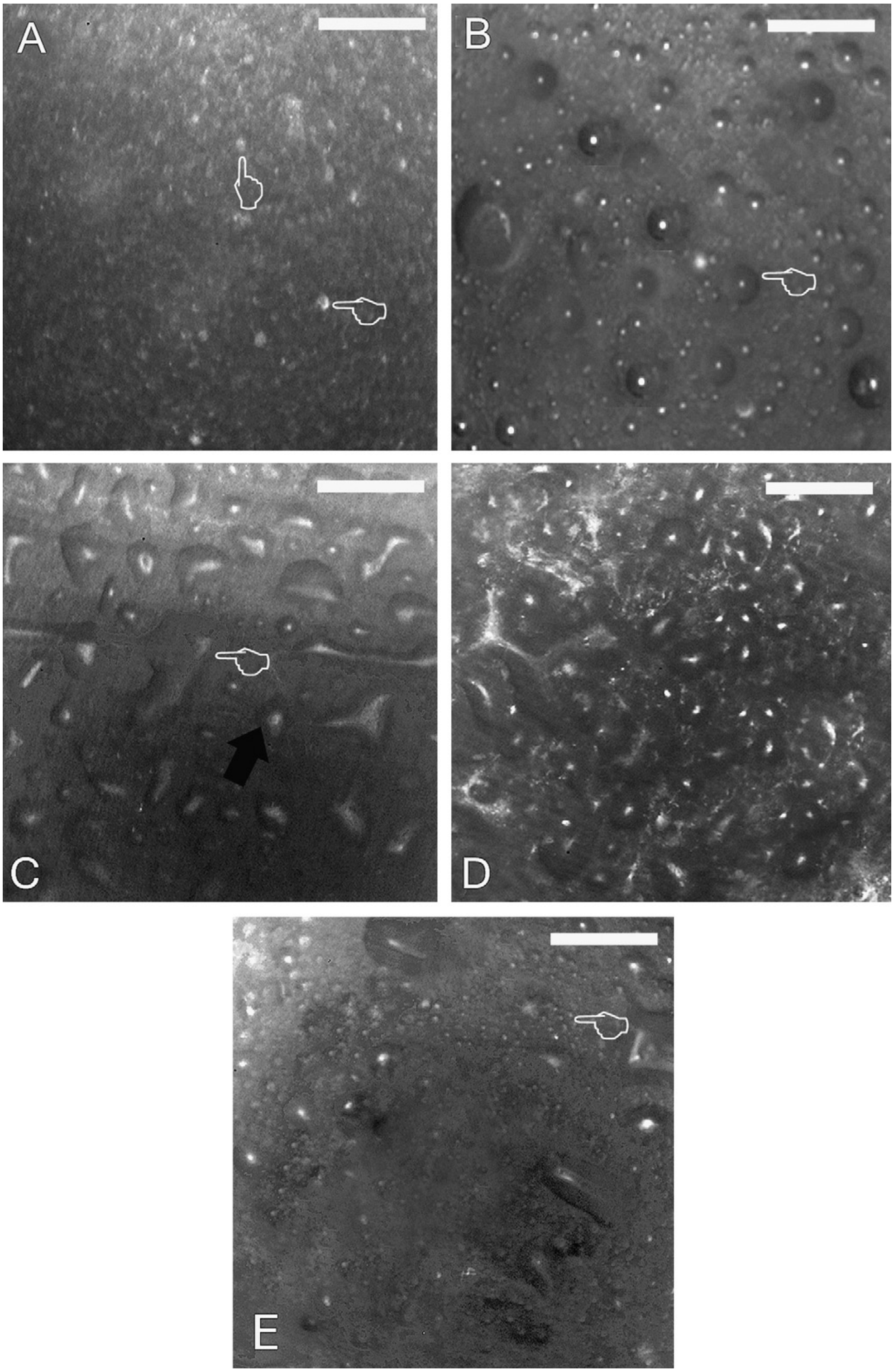

Fig. 3 – TSM images (20× NA 0.8; FV: 400 μm × 400 μm/scale bar: 100 μm). Pictures were performed immediately after adhesive application for 10 min and previous to resin polymerization, without simulated pulpal pressure. (A) Few small droplets are evident after Optibond FL application (pointer). (B) Phase separation, characterized by the droplet globular-like shape, is observed when G-Bond was applied onto the dentin surface (pointer). (C) DC-Bond showed mainly water droplet formation, morphologically different to those observed in G-Bond (arrow). These droplets slightly increased in dimensions after 10 min (pointer). (D and E) Water droplet formation is observed after Scotchbond 1XT the Silorane primer application, respectively. Very small droplets may be also evidenced in Silorane primer (pointer).

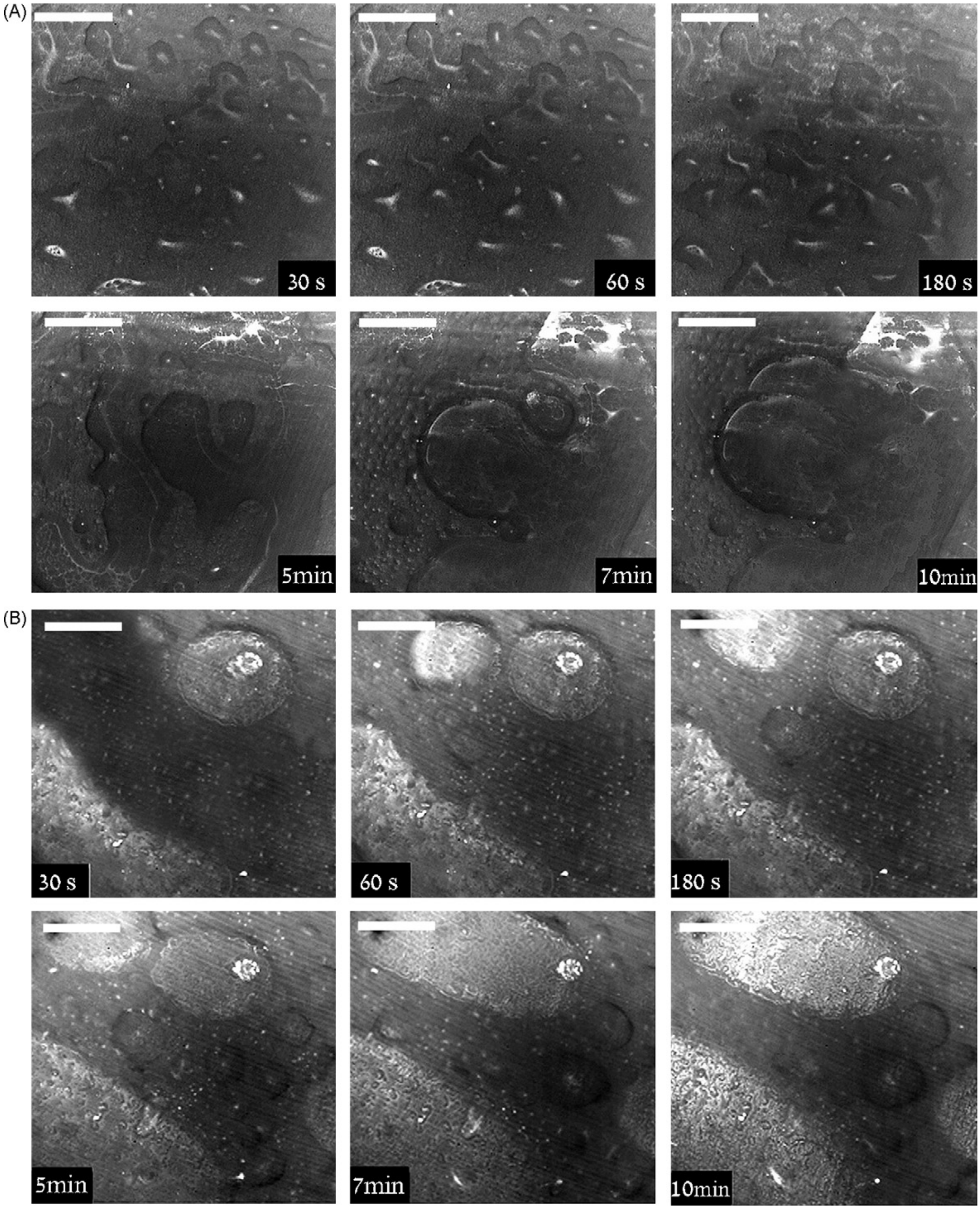

Fig. 4 – TSM microscopy image (20× NA 0.8; FV: 400 μm × 400 μm/scale bar: 100 μm) performed within the un-polymerized resin layer applied under simulated pulpal pressure. A sequence of pictures captured in a real-time of 10 min is shown. (A) Phase separation induced by simulated pulpal pressure in DC-Bond immediately following the application. Water flow coming from the tubules is evidenced, causing a severe phase separation over time. (B) Phase separation in Scotchbond 1XT under simulated pulpal pressure after 10 min. Also in this case pulpal pressure induced a severe phase changing.

observed at the resin–dentin interface, eventually covering completely the adhesive surface. This phenomenon was also present, but manifested more slowly, when this adhesive was applied in the absence of pulpal pressure.

The resin–dentin interfaces created by 3M Silorane showed no statistical difference when applied either in the presence and in absence of pulpal pressure. The TSM investigation showed that the pulpal pressure induced also in this case the formation of non-globular droplets in the primer of the Silorane that increased over time. When the pulpal pressure was absent, the presence of droplets was lower than in that observed in the presence of pulpal pressure; in this case the

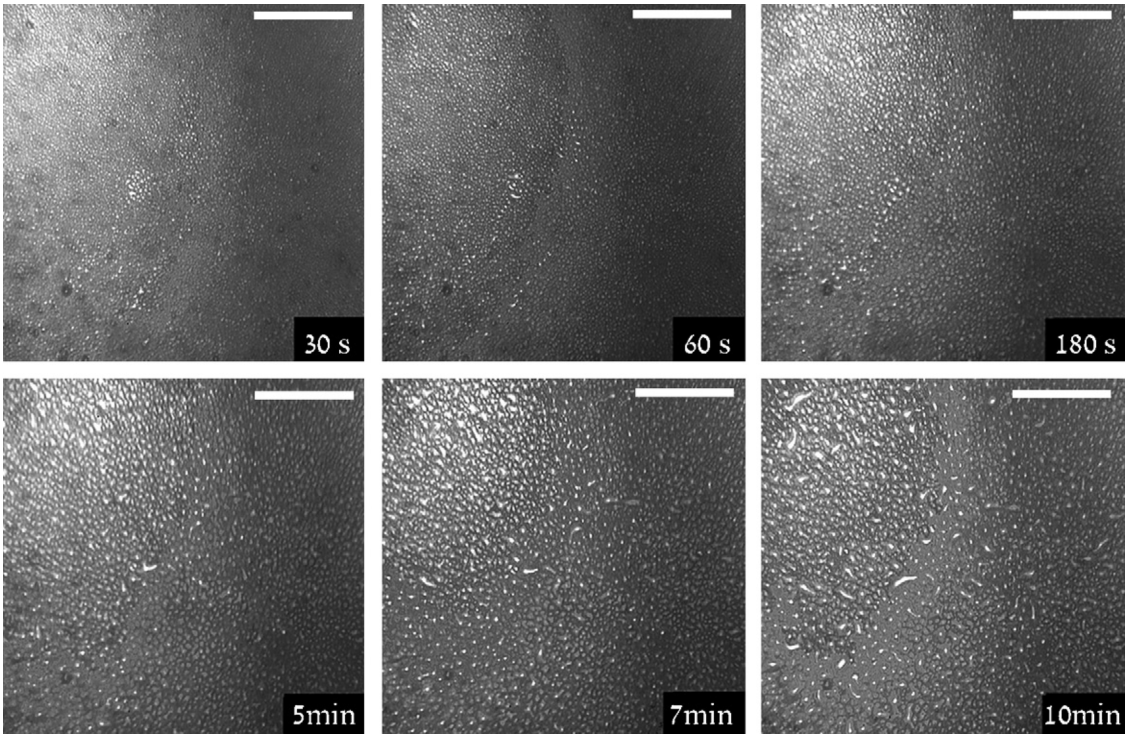

Fig. 5 – TSM microscopy investigation (20× NA 0.8; FV: 400 μm × 400 μm/scale bar: 100 μm) performed on the polymerized external surface of DC-Bond applied under simulated pulpal pressure of 20 cm H_2O. A sequence of pictures captured in a real-time of 10 min is presented. It is possible to observe how the presence of water bubbles increased over time. The low dimension of the bubbles indicates that the permeability is induced by osmosis.

droplets were created only during the adhesive application, slight changes in dimension were detected over time (Fig. 3D). No droplets were observed on the polymerized external surface of this adhesive both in the presence or the absence of pulpal pressure.

4. Discussion

According to Ciucchi et al. [20] pulpal pressure should be at 19.6 cm H_2O during the bonding process in order to reproduce the effect of the local vasoconstrictor in local anesthetics. Accordingly, the simulation of the pulpal pressure in the present study was set to 20 cm H_2O for investigating in real-time the droplet formation of current self-etching and etch-and-rinse adhesives. The resin–dentin interface may be affected by different types of droplets [7]. These droplets have different origins and may arise either from water entrapment [9–11], or phase separation [17–19]. However, there is little information regarding the influence of the pulpal pressure on the origin and manifestation of droplets at the resin–dentin interface. The results of this study showed that each class of adhesive has a different characteristic of droplet formation. The droplet formation in all the simplified adhesive systems tested in this study was constantly evident both in the presence and in the absence of pulpal pressure.

The golden standard three-step etch-and-rinse adhesive Optibond FL is affected by the formation of very small and few droplets either immediately following the application or after 10 min of polymerization delay. This feature that may be correlated to the results obtained by Sauro et al. [11] who showed low micropermeability located only at the bottom and within the hybrid layer and to those of Van Landuyt et al. [7], who showed that Optibond FL did not reduce its microtensile bond strength even when the light cuing was delayed for 20 min.

G-Bond showed a clear droplet formation during the application procedures either in the presence or in the absence of pulpal pressure. These droplets presented a globular-like shape and did not radically change over-time, but only increased in number and size very slightly. It might be classified as a reaction of static phase separation, mainly produced in HEMA-free DBAs [17], occurring mostly during the bonding procedures. A further explanations may be advocated to droplet formation due to chemical reactions between adhesive components. For instance, rapid evaporation of acetone may exceed that of water inducing the increase of aqueous fraction in the adhesive film. In this situation, the monomer-rich phase within the adhesive blend may separate from water because of the partial solubility of some resinous compounds (i.e. UDMA) [14,25]. One further factor involved in the formation of the typical globular-like shape droplets observed in the G-Bond specimens is the presence of a carboxylic acid methacrylate derivative and a phosphoric acid ester of methacrylate, along with a dimethacrylate (4-META). The 4-META resin monomer has a lower hydrophilicity compared to that of HEMA [22–25] hence, this limited affinity to water may be responsible for the static phase separation observed during delayed polymerization. It may be that once this hydrophilic monomer has reached the water sorption saturation, droplets will not increase over time anymore, both in the presence and in the absence of pulpal pressure. The phase-separation has been defined as a reaction that causes droplet formation during bonding procedures. The phase-separation has been defined as a reaction that causes droplet formation during bonding procedures. These droplets are characterized by hydrophobic BisGMA-rich particles distributed in a hydrophilic-rich matrix that compromise the structural integrity of the hybrid layer [23,26,27]. The hydrophilic-rich matrix component has low crosslink density and the resulting material is unstable in aqueous environments, degrading overtime [23]. Phase separation causes an incomplete and differential infiltration of the demineralized dentin matrix [27,28]; the collagen fibrils are not completely protected by the hydrophobic resin polymers and they will be susceptible to degradation [29,30].

It has also been stated that the chaser power of acetone in G-Bond, may be able to attract water from the underlying dentin to the surface [14,25], in the presence or absence of pulpal pressure [10,31] and produce some water droplet formation.

In the case of the DC-Bond, in the presence of pulpal pressure, the formation of many large water droplets within the un-polymerized adhesive layers is observed immediately after the adhesive application. These droplets did not presented a globular shape, and showed dynamic change due to the fast increase in the number and size of the droplets over time (10 min.) during polymerization delay. However, when the pulpal pressure was absent, these droplets occurred mainly during the application of the adhesive with a slower change in shape and size over time compared to those created in the presence of pulpal pressure. These droplets might be attributed to the chemical composition of this all-in-one adhesive (Table 1). The presence of a high concentration of hydrophilic monomer such as HEMA may be responsible for the observed non-globular droplet formation. HEMA has a relatively higher hydrophilicity and it may produce a continuing water uptake from the dentin, inducing a constant and dynamic phase separation over time when polymerization is delayed [4,32]. Furthermore, addition of self-cure activators to light-curing adhesive systems may also induce water sorption and phase changing [33].

Water entrapment is due both to osmotic processes and water sorption by hydrophilic components. The water moves from an hypotonic zone (i.e. hybrid layer) to poorly polymerized sites within the resin–dentin interface and/or to the transition zone between the adhesive layer and the resin composite (hypertonic zone: oxygen-inhibited layer) [8,16,33,34]. In general these type of droplets are small, in the order of a few μm, and detectable on the external surface of the simplified adhesive [7,35] and at the resin–dentin interface [8,9,13], whereas the results of chemical phase-separation are larger droplets than the osmotic ones, located within the resin–dentin interface [7]. These types of resin–dentin interface imperfections may be the pathway for hydrolytic degradation which would jeopardize the longevity of resin–dentin bonds over time [12,36].

Severe dynamic droplet formation was observed within the resin–dentin interface created by Scotchbond 1XT in the presence of pulpal pressure. In the absence of pulpal pressure the droplet formation within the un-polymerized adhesive occurred mainly during the application procedures and presented slower phase changing. This adhesive consists of a BisGMA/HEMA-based system containing 13% polyalkenoic acid polymer in an ethanol-water solvent. Besides the high water attraction of the HEMA, numerous studies have shown that the presence of polyalkenoic acid copolymer that resides primarily on the surface of the hybrid layer and in “islands” within the adhesive layer tends to absorb and bind a large amount of water [37] due to the multiple pendent carboxylic acids along its linear backbone. Several reports also indicate that this adhesive does not infiltrate into acid-etched dentin, creating a HEMA-rich, BisGMA-poor lower half of such hybrid layers that absorb water in the bottom half of the hybrid layer producing hydrogels [26,38]. It may be the rationale behind the numerous non-globular water droplets and the dynamic phase separation observed during the polymerization delay. The complete opening of the dentinal tubules obtained with the total-etch technique may also facilitate the water movement from dentin to the adhesive interface [39,40].

When Silorane adhesive was applied in the presence of simulated pulpal pressure non-globular water droplets within the un-polymerized adhesive and dynamic phase change were observed over time during the polymerization delay. In the absence of pulpal pressure, the droplet formation was moderate and occurred only during the application of the primer with a slower phase change. Although the manufacturer suggests the application of a second layer of a more hydrophobic layer, this step must be done after the polymerization of the primer layer, thus no beneficial effect could be offered by the adhesive layer. However, if the adhesive layer is applied on uncured primer-bonded dentin, it would offer a little reduction of the phenomenon (un-published results). Observed droplet formation may be mainly attributable to the water sorption of the hydrophilic components (such as HEMA and phosphorylated methacrylates) contained in the primer of this adhesive which increase the phenomenon of the phase separation in this adhesive [41].

Regarding the TSM investigation of droplet formation on the polymerized external surface, Optibond FL and Silorane applied both in the presence and absence of pulpal pressure showed no droplets. This issue may demonstrate that the application of a second layer of a hydrophobic resin may drastically decrease the water movement trough the resin–dentin interface induced by osmosis [11,35,41].

In the case of DC-Bond and Scotchbond 1XT, TSM investigation showed a continuous formation of small droplets on the external surface over time (0–10 min) in the presence of pulpal pressure. In the absence of pulpal pressure the formation of droplets on the surface of the polymerized adhesive was, however, present but drastically decreased and slower.

A recent paper of Van Landuyt et al. [17] demonstrated that the presence of HEMA, due to its low molecular weight and its polar properties, enhances the diffusion process of water through the adhesive layer. Moreover, they also stated that high concentration of HEMA induces the formation of a HEMA-rich oxygen-inhibition layer which may enhance the osmotic process of water movement [42–44]. It is also possible that the hydrophilicity and water attraction of HEMA and other hydrophilic monomers may cause the reduction of polymerization due to dilution of these monomers with water [32,42,43]. The incomplete polymerization of the resins may have enhanced the water diffusion [45,46] from dentin to the hypertonic region of the oxygen-inhibited layer [47,48].

G-Bond and Optibond FL were the less water permeable adhesives. The G-Bond adhesive is the only simplified adhesive tested in this study that showed reduced water permeability after polymerization. These results are in accordance with those of Sauro et al. [10] who showed that the presence of pulpal pressure did not reduce bond strength, permeability and water droplets formation on the external surface of this adhesive.

Scotchbond 1XT and DC-Bond were the most water permeable adhesives. The Scotchbond 1XT adhesive showed no statistical differences between the values obtained in the presence and in absence of pulpal pressure. This characteristic may be attributed to the complete opening of the dentinal tubules produced by 35–37% H_3PO_4 acid etching of dentin which may have facilitated the water movement through the resin-bonded dentin [39,40]. In the case of the DC-Bond adhesive, although it exhibited very high water permeability, a statistical difference was found between the two types of applications (i.e. in the presence and in absence of pulpal pressure). This significant reduction in water permeability may be due to the mode of application of the DC-Bond adhesive (i.e. self-etching) and to its pH (2.7) (Table 1). Indeed, these types of "middle" acidic adhesives demineralize the dentin surface only for few microns. The smear layer is only partially modified and part of the smear plugs may be left in the dentinal tubules [1,2,10]; this may reduce the fluid movement from the tubules to the resin–dentin interface when this adhesive is applied in the absence of pulpal pressure. However, the composition of the adhesives and the high concentration of hydrophilic components may increase the fluid movement through the microporosities within the primer layer or at the bottom of the hybrid layer [11]. Pashley et al. [49] have demonstrated that the presence of HEMA, which is a primary component in many single-bottle commercial dentin adhesives can dramatically reduce incomplete evaporation of residual water from the demineralized dentin and the diffusion of hydrophobic monomers, such as 2,2-bis[4(2-hydroxy-3-methacryloyloxy-propyloxy)-phenyl] propane (BisGMA). Moreover, the continuous fluid flow through the dentinal tubules that may have increased the presence of a linear array of connected nanometer-sized voids across the full thickness of dental adhesives to the external surface of the polymerized resin–dentin surface [12,15,50].

5. Conclusion

The presence of water transudation forced by pulpal pressure during a bonding procedure may induce a dynamic droplet formation in adhesive systems containing high concentration of HEMA. However, the application of a second more hydrophobic resin coating might reduce the effect of the pulpal pressure on the osmotic droplets and water permeability of the adhesive systems.

The adhesive systems containing 4-methacryloxyethyl-trimellitate anhydride (4-META) as hydrophilic monomer are affected by droplet formation during bonding procedures. However, the water permeability and the formation of water droplets on the polymerized external surface are very slightly influenced by the presence of the pulpal pressure.

Thus, the null hypothesises that there is no difference between the water permeability and water droplets formation when current self-etching and etch-and-rinse adhesives were applied in presence or in absence of simulated physiological pulpal pressure must be partially rejected.

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