UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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How 10-MDP functional phosphate monomer and 2% digluconate chlorhexidine solution interact on sound, artificial carious or eroded dentin substrates

Como o monômero funcional fosfatado 10-MDP e a solução de digluconato de clorexidina a 2% interagem em substratos dentinários hígido, cariado e erodido artificialmente

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“Treine seus olhos a enxergar detalhes, pois as mãos só serão capazes de reproduzir o que a mente foi capaz de enxergar”

Baratieri et al., 2002

ABSTRACT

How 10-MDP functional phosphate monomer and 2% digluconate chlorhexidine solution interact on sound, artificial carious or eroded dentin substrates

Objective: Carious and eroded dentin represent common modified dental substrates, which mostly require restorative procedures. MDP-based dentin bonding system (DBS) and digluconate chlorhexidine (CHX) are presented as promising agents to minimize intrinsic degradation of the resin-dentin interface by interaction with calcium (Ca). Therefore, the purpose of this study was to explore the possible interaction between MDP in a universal adhesive system with 10-MDP in self-etching mode and CHX on substrates artificially modified by caries and erosion, through microtensile bond strength (µTBS). Additional characterization of the adhesive interface was performed by SEM/EDS analyses.

Material and methods: Flat dentin surfaces were obtained from 120 specimens (n=20/group) prepared from extracted sound human third molars and randomly divided into three groups according to the dentin substrate: sound-control (S), artificial carious (C) and artificial eroded (E). Half of these specimens were pre-treated with distilled water (W) and other half with 2% CHX, constituting 6 groups: S-W, S-CHX, C-W, C-CHX, E-W, E-CHX. After, all the specimens were restored with a universal adhesive system (Apder Single Bond Universal) using self-etching mode and two increments of composite resin (Filtek Z-350), following manufacturer's instructions. Slices (0.8mm) were obtained to SEM analysis and beams (0.64mm²) were obtained and evaluated by EDS analysis and µTBS in universal testing machine (500N/ 0.5mm/min) after 24 hours and 6 months. Data was statistically analyzed by two-way ANOVA and Tukey tests ($p<0.05$).

Results: Substrate type was a statistically significant factor ($p<0.0001$), whereas the pretreatment ($p=0.189$), time ($p=0.337$) and the interaction between three factors ($p=0.452$) were not significant.

Conclusion: Carious and eroded dentin substrates negatively interfered on the bond strength of an MDP- based universal adhesive systems, regardless its use with CHX. Likely, the reduction of available calcium from these substrates impaired the effectiveness of this system.

Key words: Dentin-Bonding Agents. Dental Caries. Erosion.

RESUMO

Como o monômero funcional fosfatado 10-MDP e a solução de digluconato de clorexidina a 2% interagem em substratos dentinários hígido, cariado e erodido artificialmente

Objetivo: Substratos dentinários cariados e erodidos representam os substratos modificados mais comuns na prática clínica. Sistemas adesivos baseados em MDP e CHX são apresentados como promissores agentes para minimizar a degradação intrínseca da interface resina-dentina devido a suas interações com cálcio. O objetivo deste estudo foi explorar a possível interação entre MDP em um sistema adesivo universal com 10-MDP em modo autocondicionante e CHX em substratos artificialmente modificados por cárie e erosão através da resistência de união (μTBS). Caracterizações adicionais das interfaces adesivas foram realizadas por análise em MEV/EDS.

Material e métodos: Cento e vinte terceiros molares hígidos foram preparados e randomizados em três grupos de avaliação de acordo com a condição dentinária (n=20/grupo): hígida (controle), cariada e erodida. Metade dos espécimes foram tratados com água (controle) e a outra metade com solução aquosa de CHX a 2%. Todos os espécimes foram restaurados com sistema adesivo Adper Single Bond Universal no modo autocondicionante e resina composta Filtek Z250, seguindo as instruções do fabricante. Fatias (0,8mm) foram obtidas paras as análises em MEV e, na sequência, palitos (0,64mm²) para as avaliações de EDS e μTBS em máquina de ensaios universal (500N/0,5mm/min) depois de 24 horas e 6 meses. Os dados foram submetidos à análise estatística (ANOVA e teste Tukey ($p<0,05$).

Resultados: O tipo de substrato foi o único fator estatisticamente significante ($p<0,0001$), enquanto o pré-tratamento ($p=0,189$), tempo ($p=0,337$) e a interação entre os três fatores ($p=0,452$) não foram significantes.

Conclusão: Substratos dentinários cariados e erodidos interferem negativamente na resistência de união de sistemas adesivos universais baseados em MDP, independente do uso de CHX. Provavelmente a redução da disponibilidade de cálcio nesses substratos prejudica a efetividade deste sistema.

Palavras-chave: Adesivos dentinários. Cárie. Erosão.

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DBS	Dentin bonding system
MDP	10-methacryloyloxy-decyl-dihydrogen phosphate
CHX	Digluconate chlorhexidine
Ca	Calcium
μTBS	Microtensile bond strength
SEM	Scanning electron microscopy
EDS	Dispersive energy spectroscopy
S	Sound-control
C	Artificial carious
E	Artificial eroded
W	Distilled water
S-W	Sound-water group
S-CHX	Sound-chlorhexidine group
C-W	Carious-water group
C-CHX	Carious-chlorhexidine group
E-W	Eroded-water group
E-CHX	Eroded-chlorhexidine group

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1 Introduction

1 INTRODUCTION

Since the introduction of adhesion in Dentistry, advances in scientific evidences have driven for technological development on the production of new categories of materials and strategies. In consequence, the widespread use of these systems has been supporting clinical procedures based on extremely conservative treatments to allow greater preservation of dental structures (INNES et al., 2016, SCHWENDICKE et al., 2016, BJORNDAL et al., 2017). However, even with favorable clinical performance there still are some drawbacks, mainly involving dentin substrate as nanoleakage and degradation (PEUMANS et al., 2005; HEINTZE; RUFFIEUX; ROUSSON, 2010), resulting in sensitivity and secondary caries (TAY; PASHLEY; YOSHIYAMA, 2002).

Today, it is of common sense that the bonding agent must balance to interact with substrate dynamic and restorative material to seek for ideal bonding interface. Therefore, the most challenge relies to a better comprehension of different dental substrate conditions to establish effective technical strategies (WANG; SPENCER; WALKER, 2007, KOMORI et al., 2009, GIACOMINI et al., 2017).

Even sound, morphological and compositional characteristics of dentin determine a complex and heterogeneous tissue. The radial arrangement of the dentinal tubules varies according to each zone and this distribution interferes with the interaction with bonding materials (NAKABAYASHI; TAKARADA, 1992, MARSHALL et al., 1997).

A great variety of procedures based on different materials and strategies has been proposed, depending on the etiological factor and the level of dental compromising. Among these options, restorative treatment for reduction of hypersensitivity, prevention of pulp involvement and restoration of dental anatomic contour is indicated to recover function and/or aesthetics (VAN MEERBEEK et al., 1994, TAY; PASHLEY, 2004, WANG; LUSSI, 2010, PEUTZFELDT; JAEGGI; LUSSI, 2014).

In clinical practice routine, dentin substrates are frequently altered due to events as caries and erosion, which implies in a vulnerable substrate (ISOLAN et al., 2018, KOMORI et al., 2009, OLIVEIRA et al., 2017, SIQUEIRA et al., 2018).

However, bonding investigations are usually performed using sound dentin (ISOLAN et al., 2018).

Dental caries is the most common and challenging clinical dental issue condition of the population (KIDD, 2011), especially in low socioeconomic communities, although it has reduced significantly (MARTHALER, 2004, PETERSEN, 2005). Caries corresponds to a biofilm and sugar-dependent disease that causes destruction of hard dental tissues due to the action of acids derived from bacterial metabolism that generate an unbalance in which demineralization domains remineralization process (KIDD, 2011). As carious dentin is chemical and structurally modified compared to sound substrate (HAJ-ALI, 2006, WANG; SPENCER; WALKER., 2007), multiple complex factors compromise the sucess of adhesion overtime (ISOLAN et al., 2018, de ALMEIDA NEVES et al., 2011).

Simultaneously, the change in lifestyle has caused premature aging of the teeth and favored an increase in the prevalence of non-carious lesions, such as erosion (HONÓRIO et al., 2008, MAGALHÃES et al., 2009, HUYSMANS; CHEW; ELLWOOD, 2011). In this case, the dental surface is demineralized by acids (extrinsic or intrinsic), without the bacterial involvement, which provokes the softening of surface followed by its wear. It can result in a complete loss of tissue specially considering mechanical removal, for instance exuberated by abrasion (HONÓRIO et al., 2008, MAGALHÃES et al., 2009, HUYSMANS; CHEW; ELLWOOD, 2011).

Both demineralized substrates can affect their interaction with the different restorative systems (CRUZ et al., 2015, GIACOMINI et al., 2017, KOMORI et al., 2009, FRANCISCONI et al., 2015a, FRANCISCONI et al., 2015b). Therefore, studies reported that the dental substrate, mainly dentin is the most challenging (KOMORI et al., 2009; GIACOMINI et al., 2017, OLIVEIRA et al., 2017, SIQUEIRA et al., 2018). Besides the mineral composition devoided of mineral from altered substrates, one still has to take the biological dynamic that involves the organic matrix into account (ISOLAN et al., 2018, SIQUEIRA et al., 2018).

The hydrolytic degradation is the issue related to the shortcomings of dentin-adhesive interface, due to the exposure of collagen fibrils that are not completely involved by resinous monomers, which are susceptible to the action of oral and tissue fluids overtime (HASHIMOTO et al., 2000, BURROW; SATOH; TAGAMI, 1996, de MUNCK et al., 2003, YANG, 2005). This effect is more exacerbated when etching-and-rinse systems are used as the acid generally promotes a discrepancy of space between demineralized dentin and infiltrated monomers (KATO; NAKABAYASHI, 1998, VAN MEERBEEK et al., 1993). Searching to solve this problem, self-etching systems emerged allowint to dispense the previous etching, which in turn decreases the discrepancy between demineralization and infiltration (TAY; PASHLEY; YOSHIYAMA, 2002, TAY et al., 2002). This modification is based on the replacement of the main monomer for an acidic functional monomer that promotes chemical adhesion. Also, it reduced the postoperative sensitivity as etching is not performed anymore (VAN MEERBEEK et al., 2011, TAY; PASHLEY; YOSHIYAMA, 2002, TAY et al., 2002). However, even advantages are offered with these systems, bonding longevity still presents clinical limitations (CARVALHO et al., 2005).

The last category of bonding system launched in the market was classified as universal systems (HANABUSA et al., 2012, PERDIGÃO; SEZINANDO; MONTEIRO, 2012). They were introduced enabling professionals to choose any technical strategy: etch-and-rinse or self-etch mode. Its friendly use conciliated with promising bonding performance seems to be interesting (VAN MEERBEEK et al., 2003, PERDIGÃO; SEZINANDO; MONTEIRO, 2012, MUÑOZ et al., 2015). Since one of the main ingredient is an acidic functional monomer, as 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), these systems also enable the advantages abovementioned (HANABUSA et al., 2012, PERDIGÃO; SEZINANDO; MONTEIRO, 2012).

10-MDP is a bifunctional organic molecule (HANABUSA et al., 2012, PERDIGÃO; SEZINANDO; MONTEIRO, 2012) able to chemically bond to dental structure, specially to calcium (Ca) resulting in a stable reaction (REIS et al., 2009). The deposition of stable MDP-Ca salts with the remaining mineral content seems to be responsible for this stability, due to the formation of a nanolayer resistant to degradation (YOSHIDA et al., 2012). Investigations show increased bond strength to dentin and aid explaining the improved longevity in terms of bonding to dentin (VAN MEERBEEK et al., 2003, YOSHIDA et al., 2004, HANABUSA et al., 2012, MATSUI et al., 2015, MUNOZ et al., 2015, WANG et al., 2017). Oliveira et al., 2017 evidenced this optimized performance using sclerotic dentin, which is enriched with greater amount of Ca. In this scenario, stable chemical salt formation was demonstrated and favored for bond strength values to dentin (OLIVEIRA et al., 2017). Other acid functional monomers is also present in other dentin bonding systems, even 10-MDP seems to be reliable and the most investigated one (YOSHIHARA et al., 2010).

It is also relevant to highlight that the presence of hydrophobic ingredients promote greater resistance (VAN LANDUYT et al., 2008, YOSHIHARA et al., 2011) and minimize the damages resulted from hydrolytic degradation (FEITOSA et al., 2014).

The enzymatic activity also plays a relevant role on degradation of adhesive longevity. Host matrix metalloproteinases (MMP) of dentin are present in dentin since its formation (TAY; PASHLEY; YOSHIYAMA, 2002, TJÄDERHANE et al., 2013, VIDAL et al., 2014, BUZALAF et al., 2015, SCAFFA et al., 2017). Overall, these enzymes constitute a group of proteolytic enzymes dependent on zinc and Ca (TJÄDERHANE et al., 1998), which are capable to degrade almost all the proteins of the extracellular organic matrix such as dentin collagen, mainly MMP-8 colagenases and MMP-2 and -9 gelatinases (HANNAS et al., 2007). It has been demonstrated that numerous MMP present in dentin-pulp complex are activated with low pH (VUOTILA et al., 2002) both in physiological and pathological (biochemistry of the caries process and periodontal disease) conditions (TJÄDERHANE et al., 1998, PASHLEY et al., 2004, LEE et al., 1995, SULKALA et al., 2002, VAN STRIJP et al., 2003; NASCIMENTO et al., 2011, VIDAL et al., 2014, SCAFFA et al., 2017).

In adhesive process, the MMPs are activated due to the exposure of the collagen fibrils in demineralized dentin and their non-protection through incomplete monomer infiltration (HEBLING et al., 2005, CARRILHO et al., 2007a, CARRILHO et al., 2007b, PASHLEY et al., 2004). This fact may explain the progressive degradation of the hybrid layer over time (CARRILHO et al., 2007a, CARRILHO et al., 2007b, HASHIMOTO et al., 2000).

Seeking to mediate the MMP action in the hybrid layer, strategies can be used (PASHLEY et al., 2004) such as the application of chlorhexidine (CHX), which reveal satisfactory anti-proteolytic potential (HANNAS et al., 2007) even in low concentrations (GENDRON et al., 1999). Often, the CHX has been used as cleaning antimicrobial agent (PASHLEY et al., 2004). Its anti-proteolytic mechanism of action occurs by Ca-chelation through the addition of sodium chloride that reverses or prevents the action of MMP, especially MMP-2 and -9 present at dentin substrate (GENDRON et al., 1999). Among the types of CHX, aqueous solution of 2% chlorhexidine digluconate solution is the most accessible due to its low cost can be used in the adhesive process after the etching and before the adhesive application (HEBLING et al., 2005, HANNAS et al., 2007, KOMORI et al., 2009, WANG et al., 2013, ARAÚJO et al., 2014, FRANCISCONI-DOS-RIOS et al., 2015a, FRANCISCONI-DOS-RIOS et al., 2015b, GIACOMINI et al., 2017). This solution present high substantivity remaining in the organic matrix for a while (CARRILHO et al., 2007b). However, some studies showed that CHX allows temporary effect, with 18-month substantivity (BRESCHI et al., 2018, SADEK et al., 2010, RICCI et al. 2010).

The association of MDP and CHX could improve the adhesion and increase the longevity of restorative treatments, minimizing degradation effects. However, curent studies showed a possible interaction between these two components observing precipitates formation near the adhesive interface, which may result in a negative interaction of monomers with dentin (GIACOMINI et al., 2017, DI HIPÓLITO et al., 2012, WANG et al., 2013, ARAÚJO et al., 2014).

Therefore, considering the clinical challenges and possible interaction between these two beneficial strategies separately (MDP and CHX), more studies are needed to clarify this question specially in altered dentin substrate to seek improvements in the quality and longevity of adhesive approaches.

The aim of this study was to investigate the possible interaction of MDP with CHX in the different clinical realities: sound, carious and eroded dentin substrates using self-etching mode of a universal dentin bonding system. The null hypotheses tested were: 1) there is no difference in bond strength to normal, carious, and eroded dentin substrate; 2) there is no difference in bond strength between pretreatment with water or CHX; and 3) there is no difference on bond strength overtime (24hours and 6 months) regardless of the substrate and pretreatment.

2 ARTICLE

2 ARTICLE

The article presented in this Dissertation was written according to the Operative Dentistry instructions and guidelines for article submission

How carious and eroded dentin determine the impact of a universal bond system to dentin when associate with clorhexidine

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Key words: dentin bonding agents; dental caries; dental erosion; enzymes inhibitors.

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SUMMARY

The purpose of this study was to explore the possible interaction between two calcium-dependent agentes: 10-methacryloyloxydecyl-dihydrogen phosphate (MDP) and 2% digluconate chlorhexidine (CHX) in a MDP-based universal adhesive system in self-etching mode and a solution of CHX on substrates artificially modified by caries and erosion, through microtensile bond strength (μTBS). Additional characterization of the adhesive interface was performed by SEM/EDS analyses. Flat dentin surfaces were obtained from 120 specimens (n=20/group) prepared from extracted sound human third molars and randomly divided into three groups according to the dentin substrate: sound-control (S), artificial carious (C) and artificial eroded (E). Half of these specimens were pre-treated with distilled water (W) and other half with 2% CHX, constituting 6 groups: S-W, S-CHX, C-W, C-CHX, E-W, E-CHX. After, all the specimens were restored with Apder Single Bond Universal (self-etching mode) and two increments of composite resin (Filtek Z-350), following manufacturer's instructions. Slices (0.8mm) were obtained to SEM analysis and beams (0.64mm²) were obtained and evaluated by EDS analysis and μTBS in universal testing machine (500N/ 0.5mm/min) after 24 hours and 6 months. Failure modes were classified using optical microscopy (40X). Data was statistically analyzed by two-way ANOVA and Tukey tests ($p<0.05$). Substrate type was a statistically significant factor ($p<0.0001$), whereas the pretreatment ($p=0.189$), time ($p=0.337$) and the interaction between three factors ($p=0.452$) were not significant. In conclusion, carious and eroded dentin substrates negatively interfered on the bond strength of an MDP- based universal adhesive systems, regardless its use with CHX. Likely, the reduction of available calcium from these substrates impaired the effectiveness of this system.

Key words: dentin bonding agents; dental caries; dental erosion; enzymes inhibitors.

INTRODUCTION

Since the introduction of bonding to dental structure, certainly the complexity of dentin represents the most challenging scenario in terms of longevity[1,2,3,4]. This is due to the characteristics of a biological and dynamic substrate, which result in limitations to create a long-term stable interaction of resin monomers to dentin[5,6].

In clinical practice routine, dentin substrates are frequently altered due to events as caries and erosion, which implies in a vulnerable substrate[1,3,4,7]. However, mostly the investigations regarding bonding are performed using sound dentin[7].

The dental caries still is the most common and challenging clinical dental disease[8], even robust scientific evidences and technological advances have driven for more conservative interventional procedures, as selective removal of the carious tissue[9,10,11]. Simultaneously, the change in lifestyle has caused premature aging of the teeth and favored an increase in the prevalence of non-carious lesions, such as erosion[12,13,14]. In this case, the dental surface is demineralized by acids (extrinsic or intrinsic), without the bacterial involvement, which provokes the softening of surface followed by its wear. It can result in a complete loss of tissue specially considering mechanical removal, for instance exuberated by abrasion[12,13,14].

Due to changes in these substrates, the residual demineralized tissue can still represent a fragile substrate in terms of bonding, which can impair for the sucess of the adhesion in long-term, regardless of adhesive system category[7,15].

Both demineralized substrates can affect their interaction with the different restorative systems[1,2,16,17,18]. Therefore, studies reported that the dental substrate, mainly dentin is the most challenging[1,2,3,4]. Besides the mineral composition devoided of mineral from altered substrates, one still has to take the biological dynamic that involves the organic matrix into account[4,7].

To restore this substrate, different types of dentin bonding systems are available. The last category of bonding system launched in the market was classified as universal systems[19,20]. They were introduced enabling professionals to choose any technical strategy: etch-and-rinse or self-etch mode. Its friendly use conciliated with promising bonding performance seems to be interesting[20,21,22]. Since one of the main ingredient is an acidic functional monomer, as 10-MDP (10-

methacryloyloxydecyl dihydrogen phosphate), these systems enable the advantages of chemical stable interaction with dentin and reduction of sensitivity[19,20].

The deposition of stable MDP-Ca salts with the remaining mineral content seems to be responsible for this stability, due to the formation of a nanolayer resistant to degradation[23]. Investigations show increased bond strength to dentin and aid explaining the improved longevity in terms of bonding to dentin[19,21,22,24,25]. Oliveira et al. (2017)[3] evidenced this optimized performance using sclerotic dentin, which is enriched with greater amount of Ca. In this scenario, stable chemical salt formation was demonstrated and favored for bond strength values to dentin. Other acid functional monomers is also present in other dentin bonding systems, even 10-MDP seems to be reliable and the most investigated one[27].

As the enzymatic activity also plays a relevant role on degradation of adhesive longevity, host matrix metalloproteinases (MMP) may be inhibited once activated[28,29,30,31,32,33]. In adhesive process, the MMPs are activated by mineral loss and consequent exposure of the collagen fibrils and their non-protection through incomplete monomer infiltration[34,35,36,37,38]. This fact may explain the progressive degradation of the hybrid layer over time[35,36,39].

As MMPs action depends on zinc and Ca[40], strategies that deprive these ions are interesting[37], such as the application of chlorhexidine (CHX), which reveal satisfactory anti-proteolytic potential[41] even in low concentrations[42]. Often, the CHX has been used as cleaning antimicrobial agent[37]. Its anti-proteolytic mechanism of action occurs by Ca-chelation through the addition of sodium chloride that reverses or prevents the action of MMP, especially MMP-2 and -9 present at dentin substrate[42]. Among the types of CHX, aqueous solution of 2% chlorhexidine digluconate solution is the most accessible due to its low cost can be used in the adhesive process after the etching and before the adhesive application[1,17,18,34,41,38,43]. This solution present high substantivity remaining in the organic matrix for a while[36]. However, some studies showed that CHX allows temporary effect, with 18-month substantivity[44,45,46].

The association of MDP and CHX could improve the adhesion and increase the longevity of restorative treatments, minimizing degradation effects. However, current studies showed a possible interaction between these two components observing precipitates formation near the adhesive interface, which may result in a negative interaction of monomers with dentin[2,38,43,47].

Therefore, considering the clinical challenges and possible interaction between these two beneficial strategies separately (MDP and CHX), more studies are needed to clarify this question specially in altered dentin substrate to seek improvements in the quality and longevity of adhesive approaches.

The aim of this study was to investigate the possible interaction of MDP with CHX in the different clinical realities: sound, carious and eroded dentin substrates using self-etching mode of a universal dentin bonding system. The null hypotheses tested were: 1) there is no difference in bond strength to normal, carious, and eroded dentin substrate; 2) there is no difference in bond strength between pretreatment with water or CHX; and 3) there is no difference on bond strength overtime (24hours and 6 months) regardless of the substrate and pretreatment.

MATERIAL AND METHODS

Experimental design

This in vitro study involved the analysis of three factors: substrate condition (in three levels – sound (control), artificial carious and artificial eroded dentin), pretreatment of the dentin (in two levels - distilled water and 2% digluconate chlorhexidine solution (CHX)) and time (two levels - 24 hours and 6 months). The main response variable was the bond strength measured through a microtensile bond strength test (μTBS). Additionally, failure mode was assessed using optical microscopy (40X) and Scanning Electron Microscopy (SEM) and Dispersive Energy Spectroscopy (EDS) were used for additional qualitative analyses.

Specimen preparation and challenges protocols

One-hundred twenty specimens with a flat dentin surface (n=20/group) were randomized and prepared from extracted sound human third molars obtained under approval of Local Institutional Ethics Committee (protocol CAAE 79124217.0.0000) and stored in 0.1% salt solution of thymol at nearly 8°C. The occlusal enamel and roots were removed (perpendicular to the long axis of the tooth) using a water-cooled diamond disc (Isomet, Buelher Ltd. Lake Bluff, IL, USA). A 600-grit SiC abrasive paper was used under running water for 30 seconds (Politriz APL-4 AROTEC, Cotia, SP, Brazil) to standardize smear layer. The specimens were divided according to dentin substrate (sound - control [S], artificial carious [C] or artificial erosion [E]) and the pretreatment (water [W] or chlorhexidine [CHX]) to constitute the groups: S-W, S-CHX, C-W, C-CHX, E-W and E-CHX.

The control group (S) were maintained in artificial saliva (1.5 mM $Ca[NO_3]_2.4H_2O$, 0.9 mM $NaH_2PO_4.2H_2O$, 150 mM KCL, 0.1 mol/L Tris, 0.05 ppm F, pH 7.0). Artificial carious dentin lesions (C) were produced by cycles of 6 hours of demineralization solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50.0 mM lactic acid buffer, pH 5.0)[48], followed by 18 hours of remineralization solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130.0 mM KCl, 20 mM HEPES buffer, 5.0 mM NaN_3, pH 7.0)[49]. Each specimen was immersed in 30 mL of solution for each cycle. Daily, the solutions were renewed during the five days, followed by 48 hours of incubation in remineralizing solution, also daily renewed, totaling 7 days. For the creation of artificial erosion dentin lesions (E), the specimens were immesed in industrialized orange juice at pH 4.0 (Suco Del Valle do Brasil, Leão Alimentos e Bebibas Ltda, Linhares, ES, Brazil), composed of reconstituted orange juice, dietary fiber (acacia gum), vitamin C and natural aroma. The specimens were immersed for 5 minutes, three times a day, for 5 days and stored in artificial saliva in others periods. Orange juice was selected to this step since it is of high consumption by population and is of friendly use[2]. Both altered substrates were assessed by transverse microradiography (TMR) after complete challenge, to validate the formation of carious and erosion in dentin. In the artificial carious (Figure 1) a thin demineralization subsuperficial layer was evidenced with the preservation of the outer surface while eroded dentin revealed a superficial loss (Figure 2).

For restorative treatment, enamel selective acid-etching with 37% phosphoric acid gel (Dentscare LTDA, Joinville, SC, Brazil) was performed in all specimens for 30 seconds, followed by abundant washing with water and drying with absorbent paper (wet technique). Dentin was not etched since self-etching mode was selected for this study. The specimens from each dentin substrate were subdivided into two pretreatment groups (n=20), including application of distilled water (W) and 2% digluconate chlorhexidine (CHX) aqueous solution at pH 5.8 (Sigma-Aldrich, Saint Louis, MN, USA). After passive application for 30 seconds, excess was removed with absorbent paper. In sequence, the universal adhesive system (Apder Single Bond Universal, 3M ESPE, St Paul, MN, USA) was applied according to the manufacturer's instructions following self-etching protocol. It was light cured using a 1,000-mW/cm²

LED unit (Radii-Call, SDI, Bayswater, VIC, Australia). The composite resin was inserted in two increments of 2 mm layers (Filtek Z350 Universal Restorative, 3M ESPE) and light cured for 20 seconds. The specimens were stored immersed in artificial saliva for 24 hours at 37°C. The same unique operator performed all the procedures.

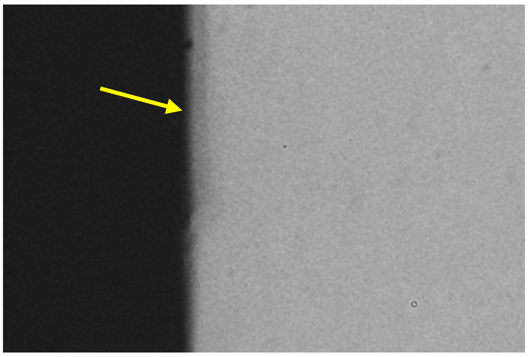

Figure 1: TMR image of artificially carious dentin substrate. A subsuface lesion is observed with a intact surface.

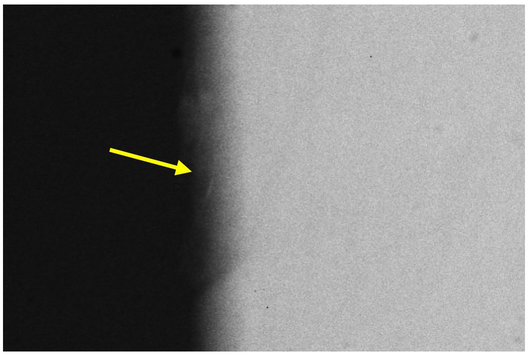

Figure 2: TMR image of artificially eroded dentin substrate. Absence of intact surface is showed.

Scanning Electron Microscopy (SEM) – Protocol 1

After restorative procedures, the specimens were longitudinally sectioned, perpendicular to the bonding interface, using an Isomet 1000 digital saw (Buehler, Lake Bluff, IL, USA) to obtain slices of approximately 0.8 mm thickness. One slice from each subgroup was randomly selected for initial analysis (24 hours) and 6 months in SEM. The slices were stored in artificial saliva until the analysis protocol. Then, they were immersed in 18% hydrochloric acid solution for 30 seconds to remove superficial smear layer, washed for 30 seconds in distilled water, followed by immersion in 5% sodium hypochlorite solution for 15 minutes to remove all non-infiltrating collagen by adhesive system and subsequent washing for 30 seconds. The specimens were kept drying for 12 hours in room temperature and then mounted in aluminum stubs to be metallized with palladium gold in metallizer (DentronVaccum, Desk IV Moorestonn, NJ, USA). After, all specimens were analysed in adhesive interface in SEM (JSM – T22OA, JOEL LTDA, Tokyo, Japão) with magnification of X1,500[50,51].

Microtensile bond strength test (μTBS)

The remain slices of the restored specimens were again longitudinally sectioned for making resin-dentin sticks of approximately 0.64 mm² area (0.8x0.8 mm). The thickness was measured using a digital caliper (Mitutoyo Americana LTDA, Aurora, IL, USA). Following, the sticks were fixed in device (JIG 1 – Plus, Odeme Dental Research, Luzerna, SC, Brazil) with cyanoacrylate resin (Super Bonder Power Flex Gel – Loctite, Henckel LTDA, Itapevi, SP, Brazil) and tested oin tension at universal testing machine (Instron 3342, Instron Co., Canton, MA, USA) at a 0.5 mm/min speed and with a 500 N load cell. The μTBS was expressed in MPa by division of the maximum load (kgf) by the specimen cross-sectional area (mm²). For this test, operator was blinded regarding the stick group.

Each fractured surface was analyzed with a handheld digital microscope (Dino-Liteplus digital microscope, AnMo Electronics Corp, Hsinchu, China) at approximately X40 magnification and classified in the adhesive (failure in the adhesive layer), cohesive in dentin, cohesive in composite resin, or mixed. The experimental unit considered was the tooth, so the sticks of each tooth were divided in two times for initial evaluation and of 6 months. During aging, the sticks were stored in weekly renewed artificial saliva at 37°C.

For the statistics analysis, data was collected. As they satisfied the assumptions of a normal distribution, the equality of variance was tested for all the variables using Statistica software (Statsoft, Tulsa, OK, USA). Finally, three-way ANOVA and multiple comparisons tests (Tukey) for individual comparisons were performed, with $p \leq 0.05$.

Dispersive Energy Spectroscopy (EDS) – Protocol 2

A stick of each group was randomly selected for initial analysis (24 hours) and 6 months in EDS. The specimes were polished in 600-grit SiC abrasive paper and etched with 37% phosphoric acid gel for 10 seconds. Following they are cleaned using an ultrasonic apparatus (Merse, Campinas, SP, Brazil) for 10 min. For biological tissues preservation, it was used a 2.5% glutaraldehyde solution buffered solution with 0.1 M sodium cacodylate solution (Merck KGaA, Darmstadt, Germany), for 12 h at approximately 8 ºC, followed by washing for 3 min and immersion in distilled water for 1 h, with renewal in every 20 min. The specimes were then dehydrated in ethanol solutions (Merck KGaA, Darmstadt, Germany) as follows: 25%

for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min and 100% for 60 min. At the end, they were immersed in a hexamethyldisilazane (HMDS) solution (Merck KGaA, Frankfurter Str. 250, D-64293 Darmstadt, Germany) for 10 min in an exhaust system[52](SOUZA-GABRIEL et al., 2016).

The sticks were fixed on stubs with a double-sided adhesive carbon tape and were sputter-coated with gold in a vacuum metallizing machine and examined with a scanning electron microscope (FEI Inspect S50, FEI Company, Hillsboro, OR, USA) at magnification of X5,000 at an accelerating voltage of 25 kV, a working distance of 10m. The images were observed and quantified chemical elements by EDS (EDAX PhiZAF Quantification - Standardless).

Table 1. Composition of adhesive system – Adper Single Bond Universal

Adhesive system	Composition
Scothbond Universal (3M ESPE, St. Paul, MN, EUA)	Methacryloiloxydecyl dihydrogen phosphate, dimethacrylates, 2-Hydroxyethylmethacrylate, methacrylate modified polyalcenic acid copolymer, filler, ethanol, water, initiators, silane.

RESULTS

Bond strength values of mean and standard deviation are shown in the table 1. The type of substrate was the only significant factor ($p<0.0001$). Pretreatment ($p=0.189$), time ($p=0.337$) and the interaction between the factors ($p=0.452$) were not statistically significant.

Table 2. Mean (MPa) and standard deviation values of bond strength of a universal bonding system to dentin substrates treated or not with chlorhexidine.

Groups	Initial	6 months
S-W	39.27 (10.16) Aa+	39.23 (9.88) Aa+
S-CHX	40.55 (15.75) Aa+	33.39 (13.64) Aa+
C-W	27.67 (13.09) Ba+	26.17 (10.69) Ba+
C-CHX	24.09 (7.21) Ba+	24.44 (7.70) Ba+
E-W	25.73 (12.64) Ba+	26.63 (12.75) Ba+
E-CHX	25.83 (10.71) Ba+	24.87 (8.94) Ba+

N=20. Different capital letters mean statistical significance between substrates (S x C x E) ($p<0.05$). Equal lowercase letter means no statistical significance between pretreatments (W x CHX) ($p<0.05$). Equal symbol means no statistical significance between time (initial x 6 months) ($p<0.05$).

Overall, the results suggest that sound dentin substrate consistently demonstrated the highest bond strength values, being statistically different from the carious and eroded dentin substrates. The bond strength was compromised related to altered dentin substrates, presenting lower values in artificial carious and eroded conditions, regardless of pretreatment and initial ou 6 months of aging *in vitro* evaluation. Between the demineralized substrates, they presented similar performance, with no statistical differences between them.

Regarding time, no difference was noted for any condition, even BS values decreased after 6 month-aging. The same performance was attributed to CHX, which did not determine any differences regarding substrate or time.

Figure 3 shows the distribution of failure mode analysis, revealing that mixed failure was observed mostly in all groups, except for demineralized dentin substrates pretreated with CHX in which adhesive failure was most prevalent. The cohesive failures were not absent, even in small percentage

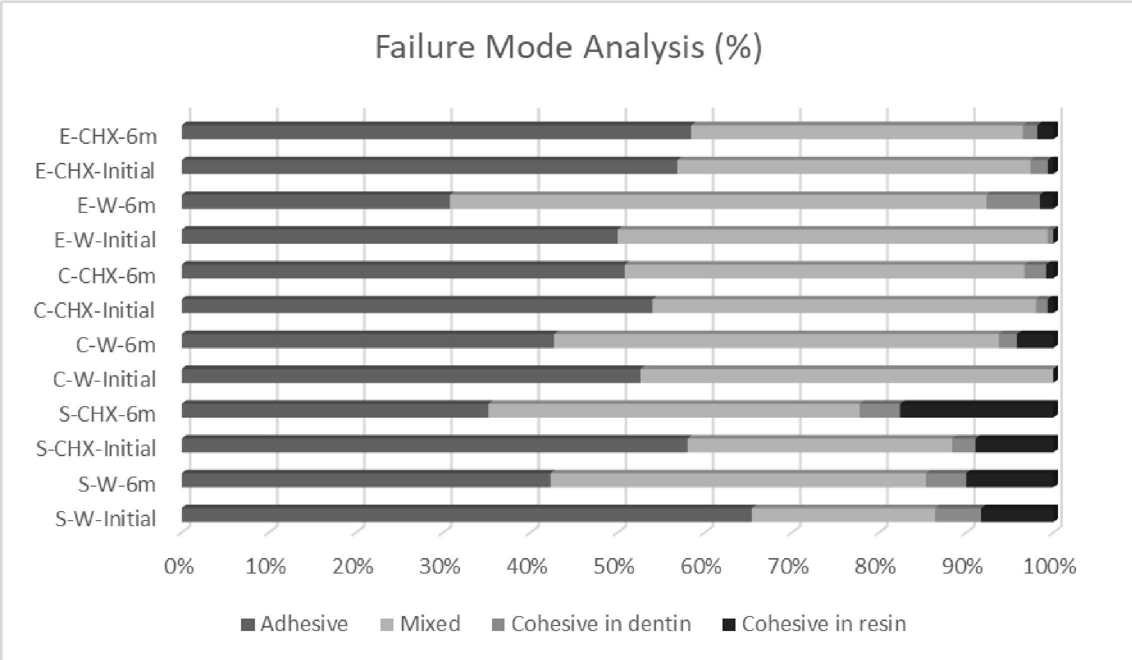

Figure 3 - Classification of the failure mode distribution (%) for all substrates, pretreatment and time evaluation (initial and 6m). Predominance of adhesive and mixed failure pattern in all groups.

Representative SEM images (x1500 and x5000) of sound, carious and eroded dentin are presented respectively in figures 4, 5 and 6 combined with their subgroups (water and CHX, initial and 6 months).

The imagens showed homogeneous distribution of adhesive agent constituting a shallow hybrid layer with the presence of some resinous tags, which are notable on the groups treated with CHX. When altered substrates are observed, a discontinuous structure is visible even for carious (figure 5) and eroded dentin (figure 6).

EDS analysis (figure 7) indicated constant values of chemical elements in bonding interface (B), compatible with the percentage components of the adhesive system used (table 1) and dentin substrate, as carbon (53-86%), oxygen (5-27%), calcium (1-10%), silicon (1-10%), phosphor (1-6%) and clorine (0-1%).

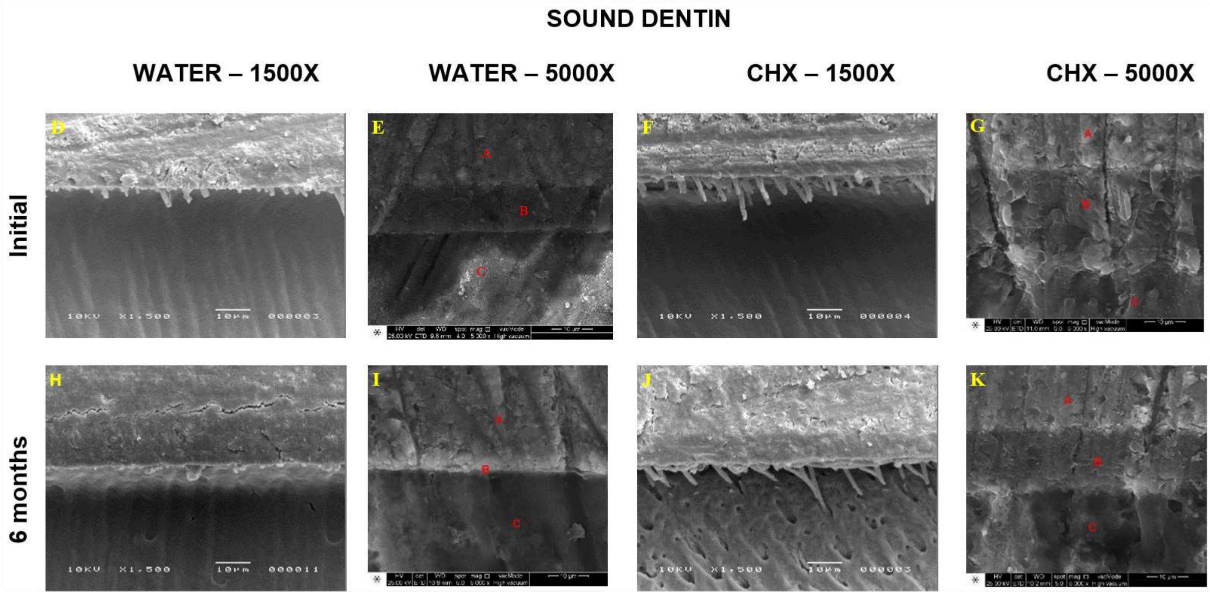

Figure 4: Representative SEM images (x1500 and x5000) of the **sound groups** (W and CHX) at initial and 6m evaluation. Area legend: A – resin composite, B – adhesive interface and C – sound dentin substrate. A specific and particular pattern of self-etching adhesive system were observed with eventual formation of short resin tags of sparsely and homogeneous distribution.

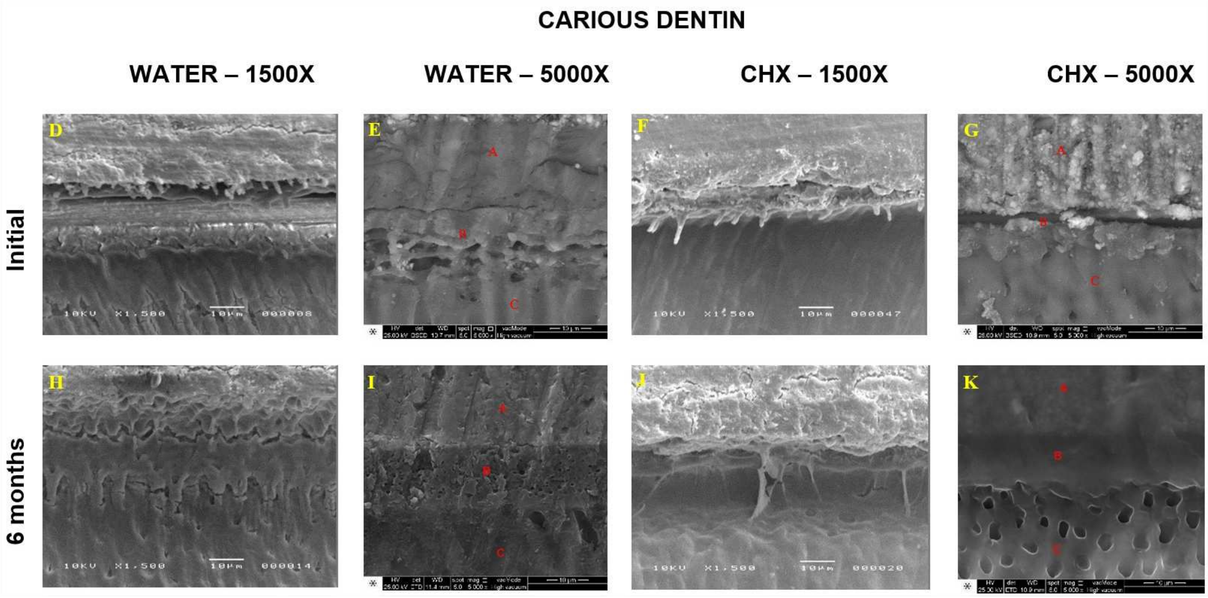

Figure 5: Representative SEM images (x1500 and x5000) of the **carious groups** (W and CHX) at initial and 6m evaluation. Area legend: A – resin composite, B – adhesive interface and C – carious dentin substrate. A pattern similar to that found in the sound group was observed, but with lower lower homogeneity.

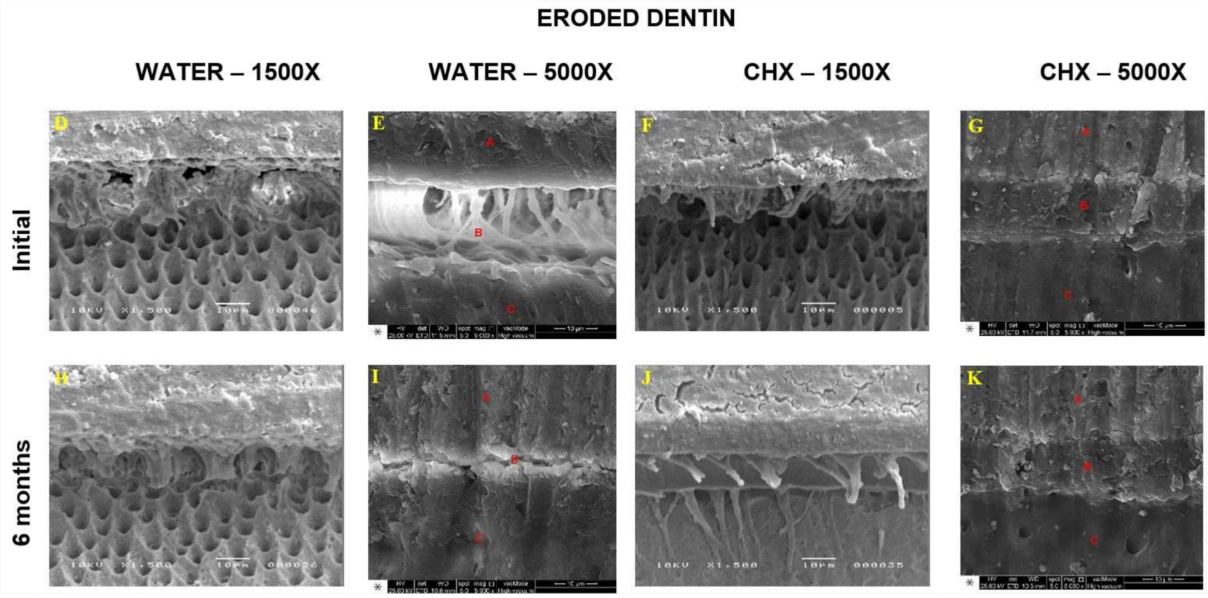

Figure 6: Representative SEM images (x1500 and x5000) of the **eroded groups** (W and CHX) at initial and 6m evaluation. Area legend: A – resin composite, B – adhesive interface and C – eroded dentin substrate. Standard feature of eroded dentin substrate was observed with exposure of the dentin tubules.

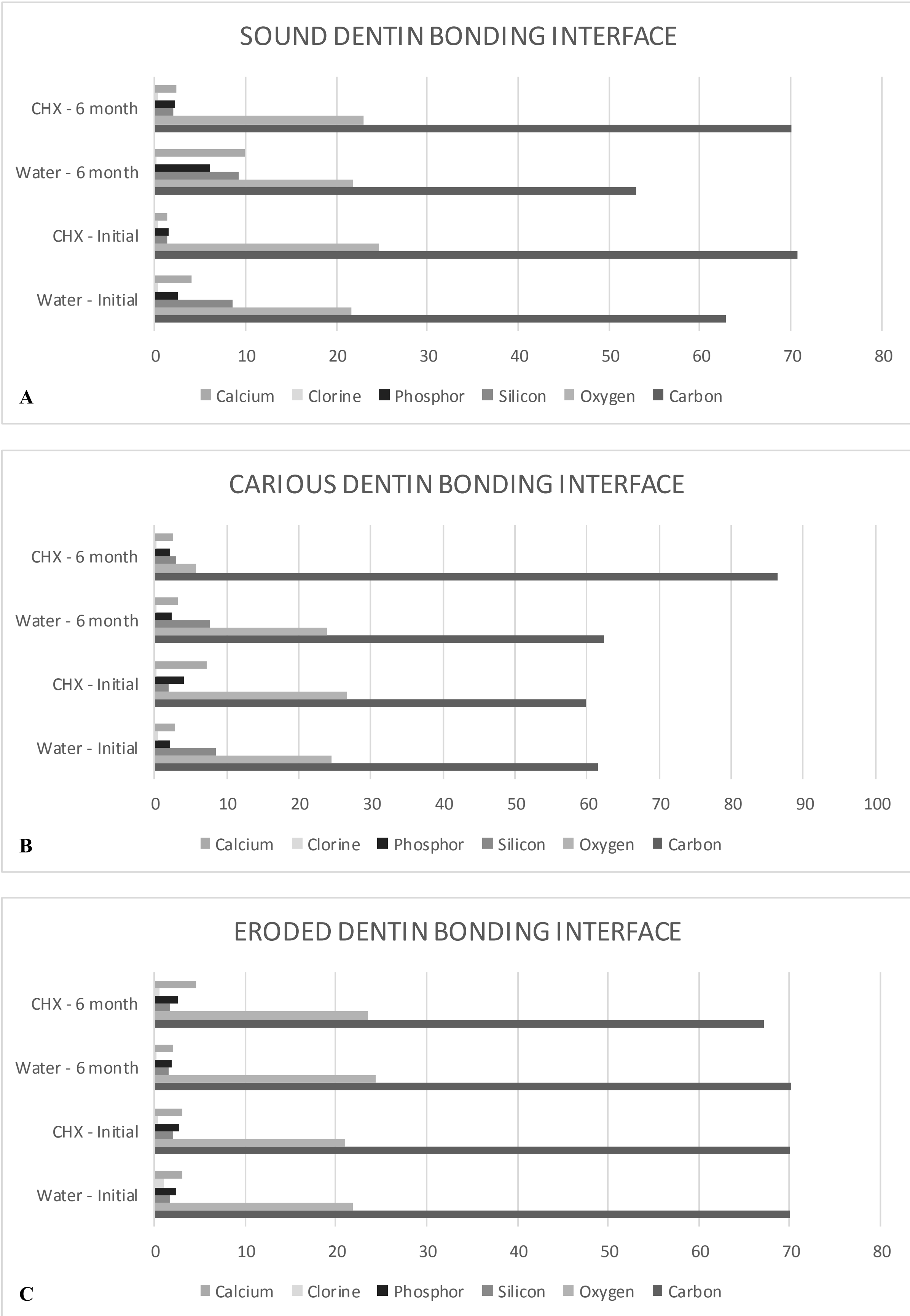

Figure 7: Percentage of components present in the resin-dentin bonding interface. Carbon was de most frequent element in all the groups in analysis and clorine more variable.

DISCUSSION

In clinical practice the most common substrates are morphological and structurally modified, as carious and eroded dentin, which frequently reach dentin and calls for restorative treatment. As most studies often use sound dentin, this study purpose to evaluate the influence of demineralized substrate in different interactions. Both artificially carious and eroded impaired bond strength of the self-ecthing mode universal adhesive to dentin (table 2), which rejects the first null hypothesis tested. This performance may suggest that a lower mineral content do affect negatively the chemical interaction of these minerals with MDP-based bonding agents, even immediately. Therefore, deprived content of calcium in dentin likely reduce the formation of stable Ca-based salts.

This poor performance regarding BS for the carious are supported by Isolan et al. (2018)[7]. In this systematic rewiew, significant higher bond strength to sound dentin compared to carious substrates, regardless of the cycling protocols is observed. Also, the lower values found for eroded dentin are in accordance to literature, which indicates impairment of adhesive quality to these substrates[1,2,16,17,18].

Controversally, Giacomini et al. (2017)[2] did not present differences between sound and eroded substrates. Only artificially carious dentin hampered BS to dentin. This difference may be attributed probably due to structural and chemical changes of carious dentin[53,54,55] as the denuded collagen fibrils of organic matrix are degraded[55,56]. In eroded dentin, the main modification relies on the mineral loss, without affecting suitable organic matrix. For artificial carious substrate the greater commitment of adhesive quality is likely due to the degradation of collagen[16,55].

Another relevant difference relies on the application mode of the universal adhesive system, when compared to Giacomini et al. 2017[2], who used the similar conditions, but combined with etch-and-rinse system. Even Muñoz et al. (2015)[21] did not observe any difference between application modes (etching-and-rinse or self-etching) in the use of the Apder Single Bond Universal, it may highlighted that the it was performing using sound dentin. Based in the present study, this substrate overestimate the bonding performance and probably is not realistic enough to simulate clinical conditions.

In Giacomini et al. (2017)[2] study, etching-and-rinse strategy used on artificial carious and eroded substrates underwent exacerbated mineral lack. This scenario may impaired the ability of bonding of the acidic functional monomer MDP to dentin, with reduced formation of Ca-MDP salt.

The 10-MDP monomer is mostly present in composition of universal adhesive systems and allows a chemical bonding with dental structure by Ca-dependent action mechanism, forming a stable nanolayer with various MDP-Ca salts in adhesive interface[27,57]. Substrates with lower Ca ions concentration associated with MDP-based bonding systems could result in a greater adhesion commitment, especially on modified substrates if they undergo further demineralization by etching in adhesive process. Therefore, self-etching mode could allow more interaction of MDP with present Ca in substrate by greater availability of this ion, which would less harmful for adhesion.

In terms of pretreatment with CHX in initial and 6 month-evaluations, no statistical difference was observed between the grupos tested, regardless of substrate type and time, which accepts the second hypothesis tested. This association was preconized as CHX is robustly supported as an antiproteolytic agent[1,38]. However, as no influence was observed, it is supposed that available Ca concentration even in demineralized dentin was enough to allow all the mechanism of action for both agents (MDP and CHX) when self-ecthing mode was employed, as no adverse additional demineralization was provoked by phosphoric acid[27,42,57]. In Giacomini et al. (2017)[2] the difference between treatments (water and CHX) in all substrates (sound, carious and eroded) in etching-and-rinse mode would state this observation. Over time this perspective may change as substantivity of CHX achieve 18 months, according to others studies[44,45,46].

Considering the time of evaluation, no statistical difference was observed among initial and 6 month-groups, and then the third hypothesis was rejected. The sound dentin presented the highest values, regardless of time and pretreatment.

Failure mode analysis (figure 3) was compatible with literature, validating the bond strength test with predominance of adhesive failure patterns fallowing mixed in all the groups regardless of substrate, pre-treatment or time. The increase in percentage of cohesive fractures in the sound groups confirmed the fact that the adhesive resistance in sound substrate is higher than observed for carious and eroded demineralized substrates.

The representative SEM images analyses are shown in figure 4-6 supporting the quantitative data and in accordance with the literature[58]. Overall performance shows classical particular pattern of self-etching adhesive systems: Hybrid layer was not distinct from the SEM images and eventual formation of short resin tags of sparsely and homogeneous distribution were detected.

The characteristics presented in the images also support the stable values of bond strength detected for the different groups in the comparison of time, regardless of the preatreatment used. However, poorly homogeneous images are observed in the demineralized groups, which suggest the greater complexity of the substrates when affected by caries or erosion.

For the eroded group, it is possible to note characteristic pattern of eroded substrates with great exposure of dentinal tubules throughout the dentin surface[4].

Possible ruptures can be seen in area corresponding to the composite resin, especially in images regarding 6 month-aged the sound groups, showing a probable degradation of the resin, which may correspond to the increase of cohesive fractures in resin overtime.

The difference between protocols used to obtain the SEM imagens resulted in different patterns of images. Thus, the higher magnification images (x5000) do not provide additional information compared to x1500.

EDS analysis served to detect the main chemical elements present in the adhesive interface of the x5000 images. Carbon was always present in greater quantity, followed by oxygen, calcium, silicon, phosphorus and chlorine, in a smaller quantity. All these elements are present in adhesive system, composite resin and chlorhexidine used, or even in the dentin, as Ca. Constancy between components is noted, following approximately the percentage of each element, regardless of substrate type, pretreatment and time. The variations observed between the components may be due to different positions of the analysis inside the interface, with prevalence of dentin or resin, which varied trouhgh the observation of Ca levels, for instance, although the size of the evaluation area is approximately the same in all assessments ($60\mu m^2$). These findings are in accordance with the literature that shows these same elements at the resin-dentin bonding interface, varying their concentrations if they are closer to the dentin or resin, as the carbon decrease when approaching the hybrid layer[59].

No difference was observed between SEM images regarding pretreatments, water and CHX, regardless the substrates and time. It was expected for a difference in the detection of chlorine, since it would be found only in CHX. However, it appears sporadically in the different groups, with a varied percentage. This non-correspondence only in the groups pre-treated with CHX may be due to the fact that the artificial saliva used for the aging of the specimens contains chlorine in potassium chloride (KCl) and a probable contamination may have occurred in the groups without pretreatment with CHX.

Therefore, the supposed competition for this study between MDP and CHX depending on Ca concentration present in substrate would not cause a interference in bonding effectiveness by bond strength in initial and 6 months in sound, carious and eroded substrates with MDP-based bonding systems.

CONCLUSION

Carious and eroded dentin substrates negatively interfered on the bond strength of an MDP-based universal adhesive systems on self-etchig mode, regardless its use with CHX. Likely, the reduction of available calcium from these substrates impaired the interaction and effectiveness of this system.

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3 DISCUSSION

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Giacomini et al. 2017 highlighted that the demineralized dentin determined the interaction effectiveness of an universal bond system. In clinical practice both carious and eroded lesions frequently involve dentin and calls for restorative treatment. However, these authors considered the use of etch-and-rinse mode to provoke the activation of proteolytic enzymes on purpose.

In the present study, controversially, we purposed the use of the same system but using the self-etching mode in order to evidence if there would be differences in this performance.

Both artificially carious and eroded impaired bond strength of the self-ecthing mode universal adhesive to dentin (table 2). Thus, the first null hypothesis tested was rejected. This performance may suggest that a lower mineral content do affect negatively the chemical interaction of these minerals with MDP-based bonding agents, even immediately. Therefore, deprived content of calcium in dentin likely reduce the formation of stable Ca-based salts.

This poor performance regarding BS for the carious are supported by Isolan et al. (2018). In this systematic rewiew, significant higher bond strength to sound dentin compared to carious substrates, regardless of the cycling protocols is observed. Also, the lower values found for eroded dentin are in accordance to literature, which indicates impairment of adhesive quality to these substrates (CRUZ et al., 2015, GIACOMINI et al., 2017, KOMORI et al., 2009, FRANCISCONI et al., 2015a, FRANCISCONI et al., 2015b).

Controversally, Giacomini et al. (2017) did not present differences between sound and eroded substrates. Only artificially carious dentin hampered BS to dentin. This difference may be attributed probably due to structural and chemical changes of carious dentin (MOHAMED et al., 2015, NAKAJIMA et al., 1999, WANG; SPENCER; WALKER, 2007) as the denuded collagen fibrils of organic matrix are degraded (PUGACH et al., 2009, WANG; SPENCER; WALKER, 2007). In eroded dentin, the main modification relies on the mineral loss, without affecting suitable organic matrix. For artificial carious substrate the greater commitment of adhesive quality is likely due to the degradation of collagen (CRUZ et al., 2015, WANG; SPENCER; WALKER, 2007).

Another relevant difference relies on the application mode of the universal adhesive system, when compared to Giacomini et al. 2017, who used the similar conditions, but combined with etch-and-rinse system. Even Muñoz et al. (2015) did not observe any difference between application modes (etching-and-rinse or self-etching) in the use of the Apder Single Bond Universal, it may highlighted that the it was performing using sound dentin. Based in the present study, this substrate overestimate the bonding performance and probably is not realistic enough to simulate clinical conditions.

In Giacomini et al. (2017) study, etching-and-rinse strategy used on artificial carious and eroded substrates underwent exacerbated mineral lack. This scenario may impaired the ability of bonding of the acidic functional monomer MDP to dentin, with reduced formation of Ca-MDP salt.

The 10-MDP monomer is mostly present in composition of universal adhesive systems and allows a chemical bonding with dental structure by Ca-dependent action mechanism, forming a stable nanolayer with various MDP-Ca salts in adhesive interface (FEITOSA et al., 2014, YOSHIHARA et al., 2011). Substrates with lower Ca ions concentration associated with MDP-based bonding systems could result in a greater adhesion commitment, especially on modified substrates if they undergo further demineralization by etching in adhesive process. Therefore, self-etching mode could allow more interaction of MDP with present Ca in substrate by greater availability of this ion, which would less harmful for adhesion.

In terms of pretreatment with CHX in initial and 6 months evaluation, no statistical difference was observed between the grupos tested, regardless of substrate type and time, which accepts the second hypothesis tested. This association was preconized as CHX is robustly supported as an antiproteolytic agent (KOMORI et al., 2009, WANG et al., 2013). However, as no influence was observed, it is supposed that available Ca concentration even in demineralized dentin was enough to allow all the mechanism of action for both agents (MDP and CHX) when self-ecthing mode was employed, as no adverse additional demineralization was provoked by phosphoric acid (GENDRON et al., 1999, FEITOSA et al., 2014, YOSHIHARA et al., 2011). In Giacomini et al. (2017) the difference between treatments (water and CHX) in all substrates (sound, carious and eroded) in etching-and-rinse mode would state this observation. Over time this perspective may change as substantivity of CHX achieve 18 months, according to others studies (BRESCHI et al., 2018, SADEK et al., 2010, RICCI et al. 2010).

Considering the time of evaluation, no statistical difference was observed among initial and 6 months groups, and then the third hypothesis was rejected. The sound dentin presented the highest values, regardless of time and pretreatment.

Failure mode analysis (figure 3) was compatible with literature, validating the bond strength test with predominance of adhesive failure patterns fallowing mixed in all the groups regardless of substrate, pre-treatment or time. The increase in percentage of cohesive fractures in the sound groups confirmed the fact that the adhesive resistance in sound substrate is higher than observed for carious and eroded demineralized substrates.

The representative SEM images analyses are shown in figure 4-6 supporting the quantitative data and in accordance with the literature (SAIKAEW et al., 2016). Overall performance shows classical particular pattern of self-etching adhesive systems: Hybrid layer was not distinct from the SEM images and eventual formation of short resin tags of sparsely and homogeneous distribution were detected.

The characteristics presented in the images also support the stable values of bond strength detected for the different groups in the comparison of time, regardless of the preatreatment used. However, poorly homogeneous images are observed in the demineralized groups, which suggest the greater complexity of the substrates when affected by caries or erosion.

For the eroded group, it is possible to note characteristic pattern of eroded substrates with great exposure of dentinal tubules throughout the dentin surface (SIQUEIRA et al., 2018).

Possible ruptures can be seen in area corresponding to the composite resin, especially in images regarding 6 month-aged the sound groups, showing a probable degradation of the resin, which may correspond to the increase of cohesive fractures in resin overtime.

The difference between protocols used to obtain the SEM imagens resulted in different patterns of images. Thus, the higher magnification images (x5000) do not provide additional information compared to x1500.

EDS analysis served to detect the main chemical elements present in the adhesive interface of the x5000 images. Carbon was always present in greater quantity, followed by oxygen, calcium, silicon, phosphorus and chlorine, in a smaller quantity. All these elements are present in adhesive system, composite resin and chlorhexidine used, or even in the dentin, as Ca. Constancy between components is noted, following approximately the percentage of each element, regardless of substrate type, pretreatment and time. The variations observed between the components may be due to different positions of the analysis inside the interface, with prevalence of dentin or resin, which varied trouhgh the observation of Ca levels, for instance, although the size of the evaluation area is approximately the same in all assessments (60μm^2). These findings are in accordance with the literature that shows these same elements at the resin-dentin bonding interface, varying their concentrations if they are closer to the dentin or resin, as the carbon decrease when approaching the hybrid layer (TONAMI et al., 2015).

No difference was observed between SEM images regarding pretreatments, water and CHX, regardless the substrates and time. It was expected for a difference in the detection of chlorine, since it would be found only in CHX. However, it appears sporadically in the different groups, with a varied percentage. This non-correspondence only in the groups pre-treated with CHX may be due to the fact that the artificial saliva used for the aging of the specimens contains chlorine in potassium chloride (KCl) and a probable contamination may have occurred in the groups without pretreatment with CHX.

Finally, for the use of self-etching mode of a MDP based system, the competition of MDP with CHX for calcium seems not to be proven to affect the bonding performance to dentin in terms of strength. Likely, it may be related to the available content of calcium enough for both processes.

4 Final Considerations

4 FINAL CONSIDERATIONS

The supposed competition between MDP and CHX in adhesion to dentin was not observed in this study with self-etching mode of a universal adhesive system. Therefore, depending on Ca concentration present in substrate, there is no interference in bonding effectiveness assessed trough microtensile bond strength in initial and 6 months evaluation in sound, carious and eroded substrates. Carious and eroded dentin substrates negatively interfered on the microtensile bond strength of an MDP-based universal adhesive systems, regardless its use with CHX. Likely, the reduction of available calcium from these substrates impaired the interaction and effectiveness of this system Ca-dependent.

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APPENDIXES

APPENDIXES

APÊNCIDE A - DECLARAÇÃO DE USO EXCLUSIVO DE ARTIGO EM DISSERTAÇÃO/TESE

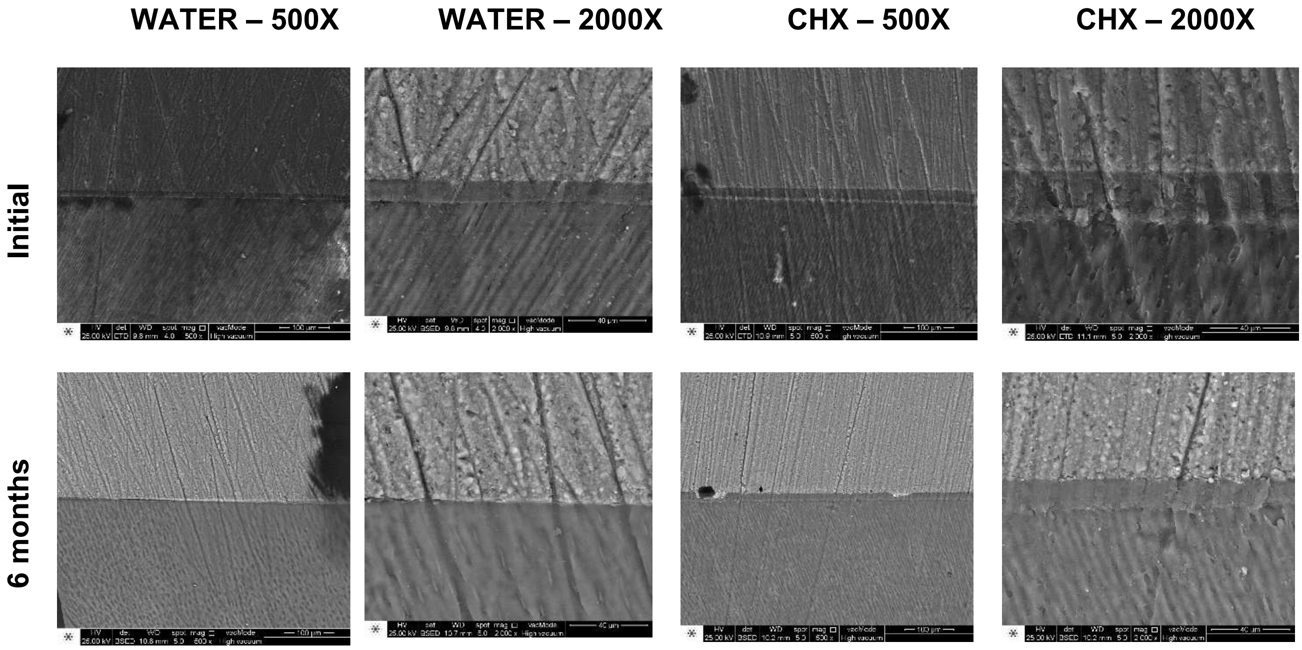
DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN DISSERTATION/THESIS

We hereby declare that we are aware of the article (How carious and eroded dentin determine the impact of a universal bond system to dentin when associate with clorhexidine) will be included in (Dissertation/Thesis) of the student (Juliana Carvalho Jacomine) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, january 15th, 2019.

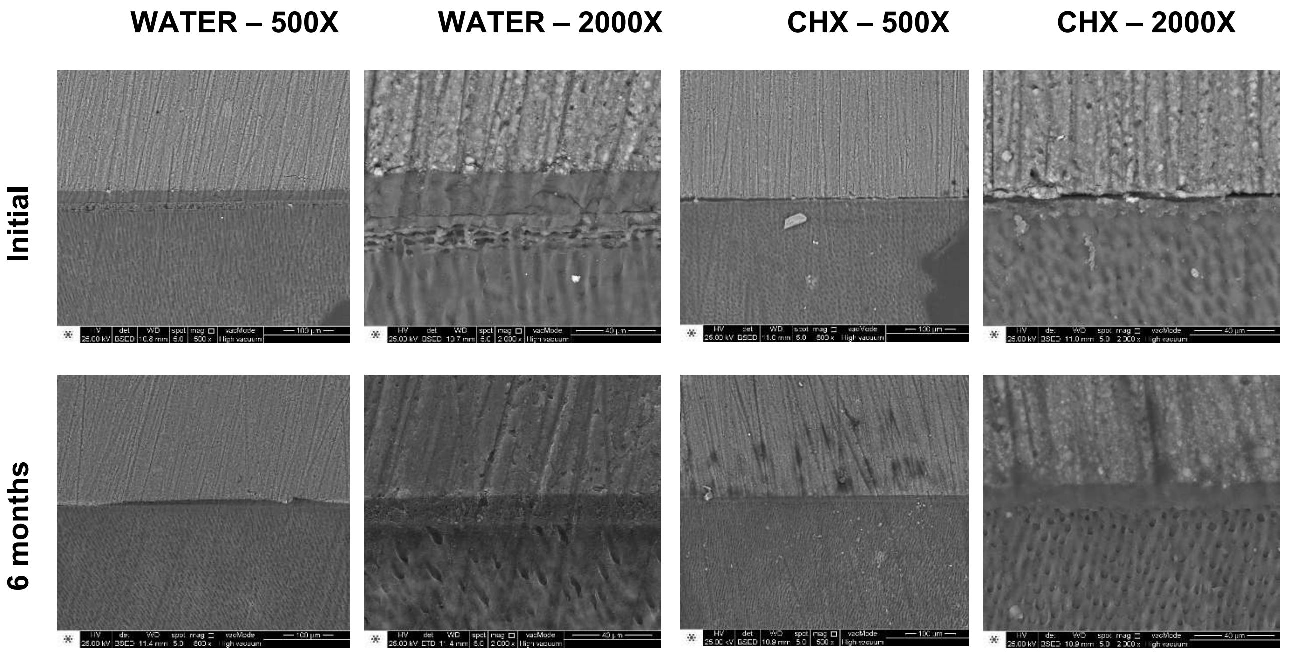
	Juliana Jacomine
Juliana Carvalho Jacomine	______________________
Author	Signature
	Linda Wang
Linda Wang	______________________
Author	Signature

SOUND DENTIN



Representative SEM images (x500 and x2000) of the **sound groups** (W and CHX) at initial and 6m evaluation with protocol 2.

CARIOUS DENTIN



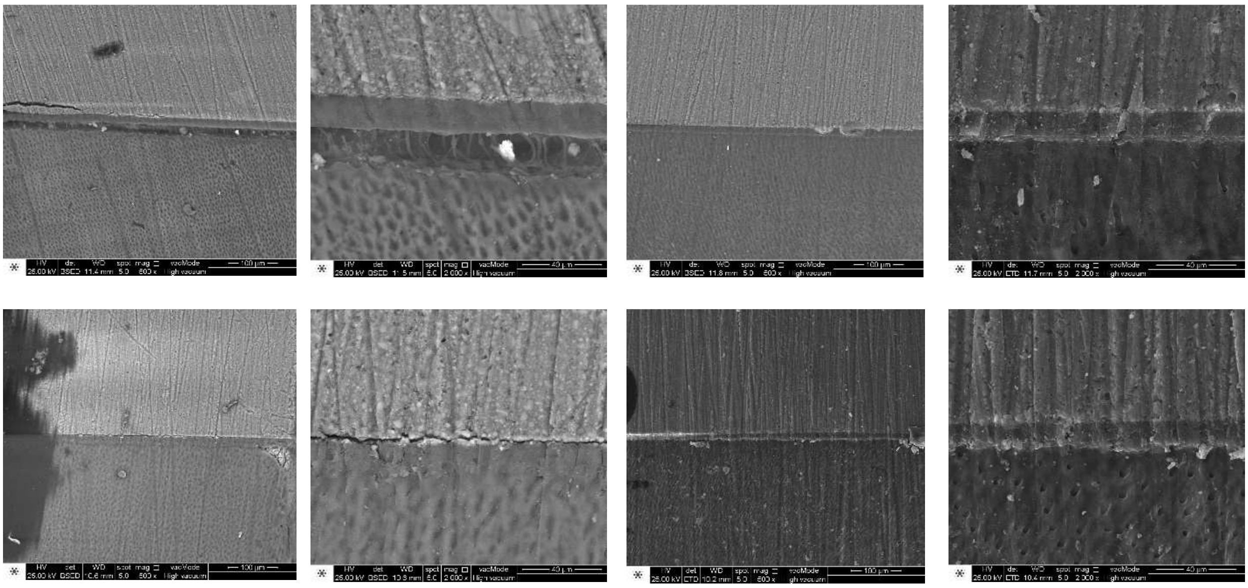
Representative SEM images (x500 and x2000) of the **carious groups** (W and CHX) at initial and 6m evaluation with protocol 2.

ERODED DENTIN

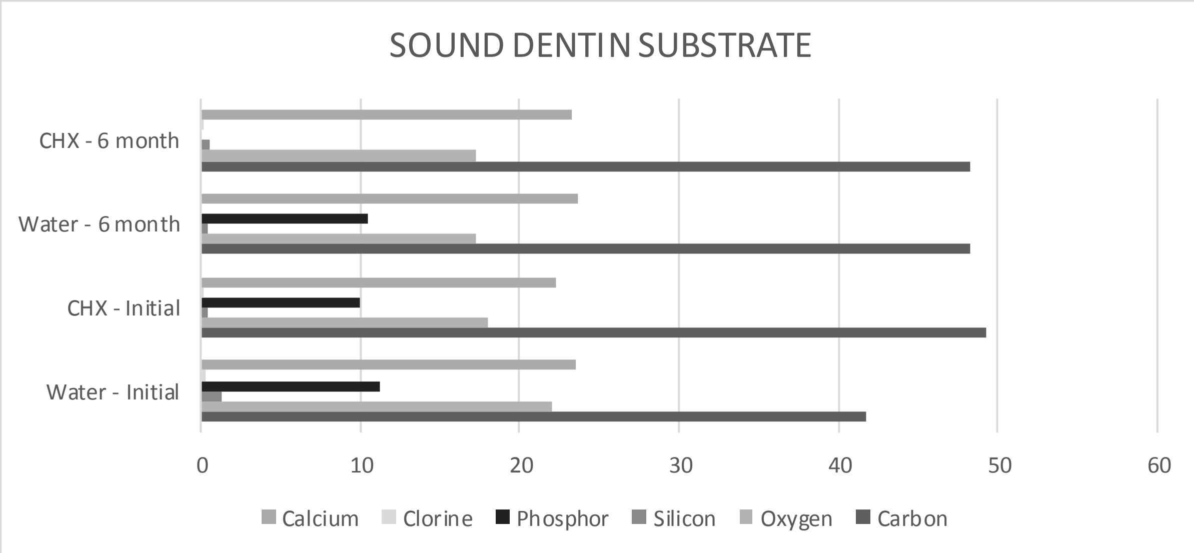
WATER – 500X	WATER – 5000X	CHX – 1500X	CHX – 5000X

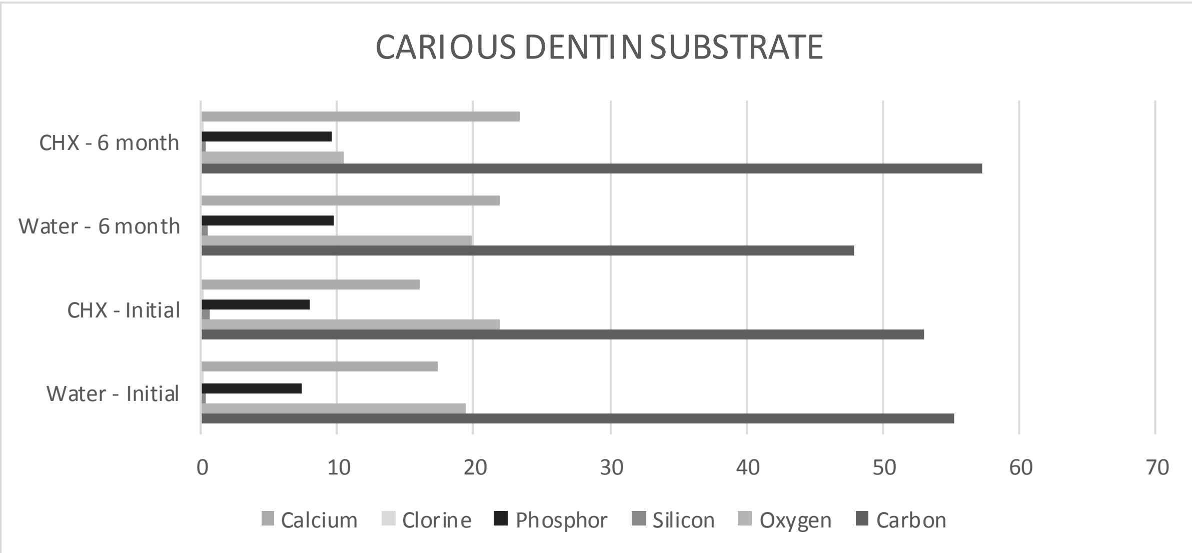
Initial

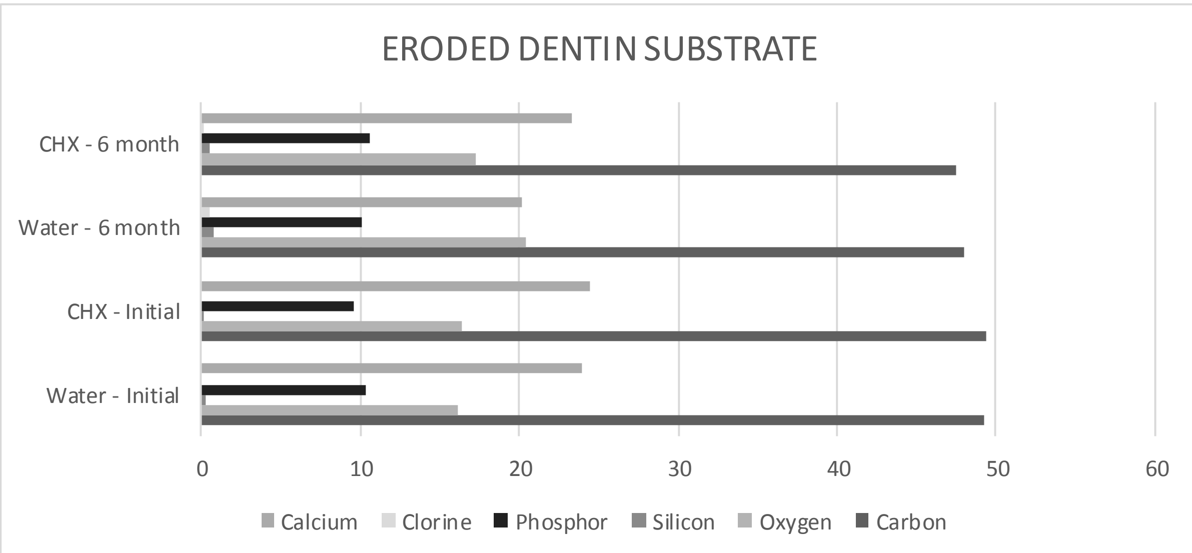
6 months



Representative SEM images (x500 and x2000) of the **eroded groups** (W and CHX) at initial and 6m evaluation with protocol 2.







Percentage of components present in the dentin substrate in different groups (sound, carious and eroded) at initial and 6m.