Brazilian Dental Science

unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.4322/bds.2022.e3053

Sodium trimetaphosphate combined to calcium as a strategy to improve dentin-bonding interface

Trimetafosfato de sódio associado ao cálcio como uma estratégia para melhorar a interface adesiva da dentina

Rafael Simões GONÇALVES[1], Giovanna Speranza ZABEU[1,2], Wendy Saori HISSANO[1], Marina Ciccone GIACOMINI[1,3], Polliana Mendes Candia SCAFFA[4], Daniela RIOS[5], Heitor Marques HONÓRIO[5], Linda WANG[1]

1 - Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Odontológicos, Bauru, SP, Brazil.
2 - Centro Universitário Unisagrado, Curso de Odontologia, Bauru, SP, Brazil.
3 - Centro Universitário das Faculdades Integradas de Ourinhos, Curso de Odontologia, Ourinhos, SP, Brazil.
4- Oregon Health and Science University, Biomaterials and Biomechanics, Portland, Oregon, USA.
5 - Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Odontopediatria, Ortodontia e Saúde Coletiva, Bauru, SP, Brazil.

ABSTRACT

Objective: The aim of this study was to evaluate the effect of STMP as biomimetic analog of dentin matrix on the dentin bond strength submitted to artificial cariogenic challenge over time. **Material and Methods:** The total number of teeth used in the experiment was 60 teeth, which were divided into 6 groups (n = 10). Of these total amount, 10 teeth were not submitted to the artificial cariogenic challenge (ACC), serving as control group (Sound Dentin - SD) while the other 50 were submitted to an ACC (7d/37ºC), being treated with treatment solutions according to each group: SD- deionized water/sound dentin, CD- deionized water/ artificial caries dentin, GIII- STMP, GIV- STMP + $Ca(OH)_2$, GV- STMP + NaF, and GVI- NaF. After treatments (24h), the specimens were restored (Adper Single Bond Universal + Filtek Z250), to obtain resin–dentin sticks with a cross sectional area of 0.8mm^2, approximately. Two-third of these sticks were stored in artificial saliva (37°C) for analyzes after 6 and 12 months. The 1/3 remains were subjected to μTBS test (baseline). Data were analyzed by two-way ANOVA and Tukey tests ($p<0.05$). **Results:** In general, the highest μTBS values were obtained in sound condition (SD), while the artificial caries condition (CD) determined minimum values. Groups treated with NaF (with or without STMP- GV and GVI) were not able to improve adhesion over time. Only the use of STMP + $Ca(OH)_2$ (GIV) improved the μTBS compared to the others caries-challenged dentin after 1 year. The adhesive failure pattern was predominant in all time. **Conclusion:** The use of the STMP associated with $Ca(OH)_2$ seems to be a viable therapeutic strategy conciliating the biomimetizing capacity to the adhesive process satisfactorily even its performance is not superior to initial condition.

KEYWORDS

Dentin; Dental adhesive; Protease inhibitors.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar o efeito do STMP como análogo biomimético da matriz dentinária na resistência de união à dentina submetida a desafio cariogênico artificial ao longo do tempo. **Material e Métodos:** foram utilizados um total de 60 dentes neste experimento, os quais foram divididos em 6 grupos (n = 10). Desse total, 10 dentes não foram submetidos ao desafio cariogênico artificial (DCA), servindo como grupo controle (Dentina Hígida - DH) enquanto os outros 50 foram submetidos ao DCA (7d / 37ºC), sendo tratados com soluções de tratamento específicas para cada grupo: DH- água deionizada / dentina hígida, DC- água deionizada / dentina submetida ao DCA, GIII- STMP, GIV- STMP + $Ca(OH)_2$, GV- STMP + NaF e GVI- NaF. Após os tratamentos (24h), os corpos-de-prova foram restaurados (Adper Single Bond Universal + Filtek Z250), para obtenção de

palitos de resina-dentina com área transversal de aproximadamente 0,8mm^2. Dois terços desses palitos foram armazenados em saliva artificial (37°C) para análises após 6 e 12 meses. Os outros 1/3 foram submetidos ao teste μTBS (baseline). Os dados foram analisados por ANOVA a dois fatores e testes de Tukey ($p < 0,05$). **Resultados:** Em geral, os maiores valores de μTBS foram obtidos em condição hígidas (DH), enquanto a condição subtmetidas ao DCA determinou os menores valores. Os grupos tratados com NaF (com ou sem STMP associado -GV e GVI) não foram capazes de melhorar a resistência de união, ao longo do tempo. Somente o uso de STMP + Ca $(OH)_2$ (GIV) melhorou o μTBS em comparação com as outras condições desafiadas por cárie após 1 ano. O padrão de falha adesiva foi predominante em todos os tempos. **Conclusão:** O uso do STMP associado ao Ca $(OH)_2$ parece ser uma estratégia terapêutica viável conciliando a capacidade biomimetizante ao processo adesivo de forma satisfatória mesmo que seu desempenho não seja superior à condição inicial.

PALAVRAS-CHAVE

Dentina; Adesivo dentinário; Inibidores de proteases.

INTRODUCTION

Dental tissue is frequently challenged. Among all these events, dental caries represents the most frequent one, requiring for restorative therapeutic approaches Therefore, scientific bases have been raised to guide for materials and techniques to allow long term successful service [1-3]. As a biofilm and sugar dependent disease, dental caries determines lesions due to two simultaneously processes: acidic dissolution of dentin minerals promoted by bacterial agents and the degradation of dentinal matrix with continuous infiltration of bacteria into the intertubular dentin [4]. In this process, intrinsic proteolytic enzymes of dentin are activated, which intensify even more the degradation of extracellular matrix including collagen [4-6]. Matrix Metalloproteinases (MMPs), mainly the collagenolitic MMP-2 and -9, and the cysteine cathepsins (CCs) are the most investigated host-derived enzymes [5-8]. All these events promote significant impact on the substrate in distinct levels, impairing the adhesive restorative procedures [1,3,7-9].

Previous studies of Mazzoni et al. (2006) [10] and Nishitani et al. (2006) [11] indicated the increasing of the gelatinase activity from dentin demineralized with phosphoric acid or self-etch adhesives compared to not exposed substrate, which in turn, contribute to the adhesive interface degradation. Also, Giacomini et al. (2017) [3] evidenced the different susceptibility of degradation according to dentin substrate conditions, wherein caries affected dentin is more prone to degradation overtime in comparison to eroded or sound dentin. In this scenario, strategies that minimize this effect have been widely investigated, especially mostly regarding the association of chlorhexidine as proteolytic inhibitor [7,12]. Other synthetic agents with this purpose has also been tested [13,14]. Natural antibacterial agents have recently employed with the ability to increase collagen synthesis [15,16]. New proposals aim to preserve the collagen fibrils and favor for structured dentin remineralization, which characterizes the biomimetic strategy [14].

Sodium trimetaphosphate (STMP - $Na_3P_3O_9$) also presents the potential for collagen type I phosphorylation and has been extensively investigated as a remineralization agent [14,17,18]. It seems to induce the directional biomimetic growth of apatite resulting in a strengthen dentin structure [17,19]. This process is based on the phosphorylation of serine and threonine residues present in the non-collagenous proteins as the dentin matrix protein-1 (DMP-1). This protein regulates dentin mineralization, by controlling the size and orientation of the apatite nucleation in the organic matrix [20]. As a result, the phosphate group, which has high affinity for Ca^{2+} ions, can trap these ions to form inorganic crystals that are essentials for dentin remineralization [20,21]. Furthermore, the preservation and stability of collagen fibrils would be positive for the dental adhesive procedure, since it depends on the mechanical interaction of the hydrophilic monomer of adhesive systems to the collagen fibrils for forming the hybrid layer [22]. With the perspective of the use of STMP as a biomimetic agent previously evidenced by Liu et al. [14], its association with $Ca(OH)_2$ could lead to the development of calcium phosphate precursors, which would favor for conditions to the growth of mineral crystals needed for remineralization [23,24]. More recently, it was evidenced its successful potential,

allowing both remineralization of dentin and showing its ability as proteases inhibition [25,26].

Considering the beneficial, the aim of this study was to evaluate the effect of STMP once its considered a biomimetic analog of dentin matrix on the artificial carious dentin bond strength over time. The hypotheses tested were that there were no differences in the dentin bond strength when comparing sound and artificial carious dentin treated or not with STMP and, there were no differences when the STMP were associated or not with $Ca(OH)_2$ or NaF.

MATERIALS AND METHODS

Sixty fresh extracted, caries-free human molars were used in this study, after approval of the Committee of Ethics in Research from the Bauru School of Dentistry, University of São Paulo (FOB-USP) (protocol no. 48755115.0.0000.5417).

Experimental design

This *in vitro* study involved two factors: treatment (in 6 levels) and time (in 3 levels). The main response variable was the bond strength by means of microtensile bond strength (μTBS).

Specimen preparation and groups distribution

The occlusal enamel of sixty human sound third molars was removed horizontally (perpendicular to the long axis of the tooth) using a water-cooled diamond disc (Extec Corp, Enfield, CT, USA) coupled to a cut machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) to expose flat dentin surface. The dentin surface was standardized using #600-grit SiC paper under running water for 60 s (Politriz APL-4 Arotec, Cotia, SP, Brazil) to standardize the smear layer. Fifty specimens were varnished with acid-resistant varnish (Revlon International Corp, New York, NY, USA), except for the occlusal surface, and exposed in 30 mL of 50 mM acetate buffer solution containing 2.2 mM $CaCl_2$, 2.2 mMKH_2PO_4, at pH 5.0, for 7 days in order to produce a caries-like lesion in dentin [27,28]. The remain ten teeth were kept sound as control group. Therefore, they were randomly divided into six groups (n=10), resulting from the combination of the dentin demineralization and treatment solution. After, specimens were individually immersed in 40 mL of treatment solution for 24 h, at room temperature, according to their groups division as described below (Table 1 and Figure 1).

Table 1 - Groups division (n=10) according to the treatment proposed

Groups	Dentin Demineralization	Treatment Solution
SD	No	Deionized water (H_2Od)
CD	Yes	Deionized water (H_2Od)
GIII	Yes	1.5% STMP
GIV	Yes	1.5% STMP + $Ca(OH)_2$
GV	Yes	1.5% STMP + NaF 1,100ppm
GVI	Yes	NaF 1,100ppm

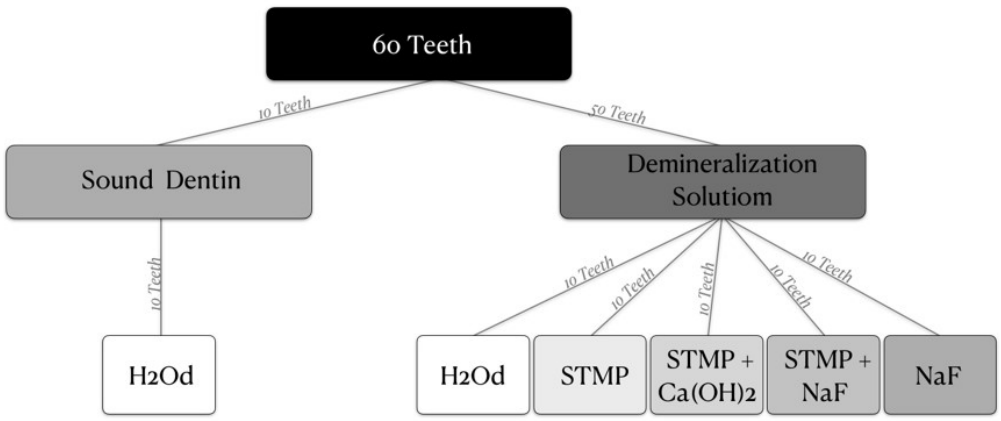

Figure 1 - Schematic illustration of the groups tested.

SD- Deionized water/Sound dentin: no demineralization and further treatment was performed on dentin surface, being considered the positive control group.

CD- Deionized water/artificial caries dentin: specimens were submitted to an artificial cariogenic challenge and then immersed in deionized water, serving as negative control group.

GIII-1.5% STMP solution: specimens were immersed in STMP solution only.

GIV-1.5% STMP solution + saturated solution of $Ca(OH)_2$: after the exposure of the dentin sticksin the 1.5% STMP solution, the specimens were treated with saturated solution of $Ca(OH)_2$ in the same way and time treated with STMP solely.

GV- 1.5% STMP solution + sodium fluoride (NaF): the specimens were immersed in 1.5% STMP solution, however sodium fluoride was added in its composition.

GVI-NaF solution: specimens were immersed in NaF solution only..

Treatment Solution Preparation

STMP solution

Protein phosphorylation with STMP requires alkaline hydrolysis into linear form. Thus, STMP was hydrolyzed at pH 12 for 5 h followed by neutralization to pH 7.4 with minimal reduction in its phosphorylation potential [29,30]. The STMP (Sigma-Aldrich Co., St. Louis, MO, USA) solution was prepared at a concentration of 1.5%.

Sodium Fluoride solution

The fluoride solution was made in a 280 μg F/mL concentration, which was selected to simulate the dilution (1:3 weight/weight) that occurs in the oral cavity when 1,100 ppm dentifrices are used [31]. Fluoride solution was made from the salts NaF.

Restorative procedure and sticks obtainment

The "multi-mode" adhesive system (Adper Scotchbond Universal - 3M ESPE, St. Paul, MN, USA) was applied as self-etching system in wet-bonding technique in accordance with the manufacturer's instructions described in Table 2. After the bonding procedures, all teeth were restored with a microhybrid composite restoration Filtek Z250 (3M ESPE - St. Paul, MN, USA- A2 shade) in three increments of 2 mm. Each increment was light polymerized for 40 s using a LED light curing unit set at 1,200 mW/cm^2 (Radii-cal, SDI Limited, Bayswater, Victoria, Australia). After, the restored teeth were stored in artificial saliva (1.5 mmol/L CaCl2, 0.9 mmol/L KH2PO4, 20 mmol/L Hepes, 150 mmol/L NaCl, pH 7.0) at 37 ºC for 24 h.

The specimens were sectioned longitudinally in the mesio-distal and buccal-lingual directions across the bonded interface, using a slow-speed diamond saw to obtain resin–dentin sticks with a cross sectional area of approximately 0.8 mm width (±0.2 mm) measured with a digital caliper (Digimatic Calliper, Mitutoyo, Tokyo, Japan).

After sticks random selections, 2/3 of these were stored in artificial saliva at 37 °C for analysis after 6 months (1/3) and 12 months (1/3), this solution was replaced once a week during all experiment. The 1/3 remain specimens was subjected to baseline mechanical test through microtensile bond strength test (24 h after the restorative procedure). A mean of 20-24 sticks were obtained per tooth and distributed in thirds for each of the tested times.

Microtensile bond strength test (μTBS) and failure mode

The specimens were fixed with cyanoacrylate glue (Super Bonder Flex Gel, Henkel Loctite,

Table 2 - Information of manufacturer, lot number and composition of the adhesive system investigated in the study

Material	Composition*	Manufacturer's Instructions
Adper Scotchbond Universal	10-MDP methacryloyloxydecyl dihydrogen phosphate, dimethacrylate resins, HEMA 2-hydroxyethyl methacrylate, methacrylate modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	Self-etch strategy
3M ESPE		1. Scrub adhesive for 20 s on
(Lot:579967)		dentin
		2. Air thin for 5 s
		3. Light cure for 10 s

*Information provided by the manufacturer.

SP, Brazil) to a jig, which was mounted on a universal testing machine (Instron, Norwood, MA, USA) and subjected to tensile forces at a crosshead speed of 0.5 mm/min until debonding. μTBS (MPa) was calculated by dividing the peak force (N) by the cross-sectional area of the failed interface (mm^2), measured by a digital caliper.

The failure mode of the sticks was classified as cohesive (failure exclusive within dentin or resin composite), adhesive (failure at resin/dentin interface), or mixed (failure at resin/dentin interface). The classification was performed under a stereomicroscope at 200x magnification (Stemi SV11, Carl Zeiss, Jena, Germany). Specimens with premature failures were included in the tooth mean calculation.

Statistic

Data of μTBS mean and standard deviation were collected for each group and analyzed by two-way ANOVA and Tukey tests. All statistical analyzes were carried out using Statistica software (Statsoft®, Tulsa, OK, USA). Statistical significance was established at $\alpha = 0.05$.

RESULTS

The two-way ANOVA test revealed that the factors treatment ($p<0.0000$) and time ($p<0.0000$) were statistically significant as were their interaction ($p<0.0000$) (Table 3)

Table 3 - Means values μTBS in MPa (SD) according to substrate condition and time of analyzes

Substrate Condition	μTBS (MPa)		
	Initial	6-month	12-month
Sound	45.28 (1.35)	45.96 (3.65)	39.06 (4.73)
(Positive Control)	**Aa**	**Aa**	**Ba**
Demineralized	8.72 (0.54)	5.19 (2.87)	3.37 (0.93)
(Negative Control)	**Ad**	**ABc**	**Bc**
Demineralized +	8.35 (1.52)	5.64 (2.18)	4.11 (2.81)
1.5% STMP	**Ad**	**ABc**	**Bc**
Demineralized +	16.01 (1.50)	12.78 (1.16)	9.11 (2.50)
1.5% STMP + Ca(OH)2	**Ab**	**Ab**	**Bb**
Demineralized +	13.25 (2.67)	6.16 (2.96)	2.82 (3.38)
1.5% STMP + NaF	**Abc**	**Bc**	**Bc**
Demineralized +	10.14 (1.82)	5.95 (1.56)	4.73 (2.50)
NaF	**Acd**	**Bc**	**Bc**

For each line, different capitals letters indicate significant differences of μTBS values between the times of analyzis. For each column, different lowercase letters indicate differences between the dentin condition for each time (Dunn's test, n = 10, p < 0.05).

Regarding to the dentin treatment, in initial time (24 h storage), it can be observed that sound condition (positive control group) reached the highest dentin bond strength. On the other hand, the artificial carious dentin showed the lowest results (negative control group), which did not differ when compared to the artificial caries dentin treated with NaF or 1.5% STMP solutions only. Among the groups submitted to artificial cariogenic challenge, only 1.5% STMP supplemented with $Ca(OH)_2$ was statistically higher compared to the all other treatments, except for the group treated with 1.5% STMP associated with NaF, even the μTBS showed lower values compared to the sound condition ($p> 0.05$).

After 6-month storage, 1.5% STMP associated with $Ca(OH)_2$ on artificial caries dentin performed similarly to immediate time, being effective overtime. After 12-month storage, the sound condition and the specimens submitted to an artificial caries challenge that were treated with 1.5% STMP + $Ca(OH)_2$ were not able to stabilize the bond strength overtime. Interesting to point that in particular, this group kept the highest μTBS values despite of sound condition after 12 month.

Overall, on the others conditions, the μTBS showed lower values compared to their respective immediate time assessments. Despite this performance, the sound condition maintained the highest μTBS values followed by the specimens treated with 1.5% STMP + $Ca(OH)_2$. The groups treated or not with 1.5% STMP supplemented or not with NaF or only NaF showed the lowest results, with no statistical significant differences between them.

In general, the majority of the specimens (88.3%) showed adhesive failures. Cohesive failures were observed in 5.2% of the specimens and mixed failures in 1.4%. A small number of premature failures (5.2%) were observed in the present study (Figure 2).

DISCUSSION

Evidences has robustely proven the role of dentin intrinsic enzymes on the degradation process of dentin on carious process revealing their presence and/or activity [6]. These enzymes are bound to the organic matrix or prevenient from the saliva and may lead to the degradation of both the collagen and other organic components of the dentin matrix. In this way, all dentin proteins are targets of the action of these proteases [6].

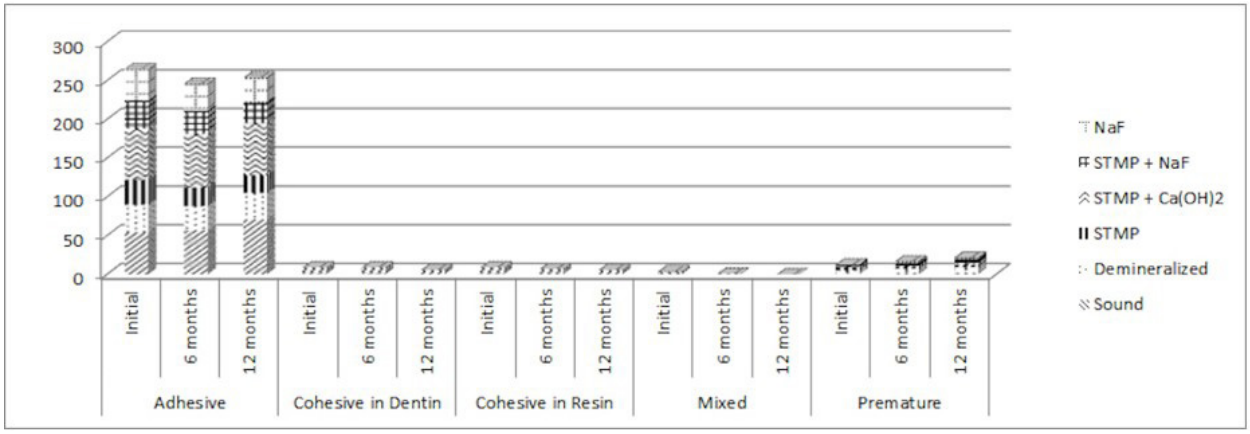

Figure 2 - Failure mode distribution according to treatment and time.

MMP-2 and -9 have been identified as the major responsible for the destruction of the organic matrix of the dentin tissue during the carious process [32]. These proteases are capable of degrading almost all components of the extracellular matrix, especially type I collagen fibrils [5,6].

In the present in vitro study, dentin from freshly extracted third human molars were used to evaluate the bonding performance on artificial caries dentin challenge over time. As carious substrate is more fragile due to demineralization and exposed organic matrix susceptibility for degradation, it might be more representative to analysis the pretreatment effect of STMP [3,33]. Moreover, Gower [34] (2008) stated that when remineralized, such promoted hybrid layers with bonding process would not contain enough free water to allow endogenous proteases to hydrolyze collagen easily.

Therefore, the focus of this study relied on STMP as this agent aid to reverse demineralization, induce remineralization, inhibit proteolytic enzymes and also exert potential for dentin biomimetization [14,17,18,25,26]. To explore its potential with different combinations, it was associated or not with NaF or $Ca(OH)_2$ solution. A group with NaF was included in this study precisely because of its well-known remineralizing potential, in addition to its potential to inhibit MMPs. Based on this evidence, we would like to investigate if its association with the STMP could enhance this remineralizing potential, since the STMP is considered a phosphorylating agent and a potential remineralizing agent. The 1.5% concentration was chosen based on previous studies carried out in our laboratory [26]. It showed to be the best concentration to act both as a remineralizing agent and as an inhibitor of the gelatinolytic activity of MMPs [26]. In general, the data showed higher μTBS values for the group treated with STMP associated with saturated solution of $Ca(OH)_2$. Therefore, this scenario validated the stablished scenario of artificial caries substrates to be more prone to the assess the effect of STMP.

The addition of saturated $Ca(OH)_2$ solution increased the potential of STMP by approximately 16% compared to STMP itself or with the other treated artificial carious groups. The more plausible explanation is associated with the role of this solution on the phosphorylation of the type I collagen fibrils. The treatment of the dentin surface with calcium hydroxide likely contributed to the formation of inorganic calcium phosphate crystals necessary for dentin remineralization [20,21]. Thus, this ability to stabilize calcium and phosphate, guiding mineral deposition into gaps of the collagen matrix certainly contributes to improve the mechanical properties of the demineralized dentin [17]. Dentin remineralization by STMP associated with a calcium solution was previously reported [26] and is also supported by our results. The combination of STMP with calcium hydroxide (Ca(OH)2) might also favor dentin remineralization by the formation of a calcium phosphate precursor that promotes conditions for mineral crystal growth required for remineralization [23,24]. The above mentioned performance indicates that the use of STMP supplemented with $Ca(OH)_2$ may be more effective in promoting the remineralization of dentin. Its use is based on the specific phosphorylation of serine and threonine residues present in DMP-1 proteins that the phosphate group can obtain Ca^{2+} ions to promote the formation of inorganic crystals necessary

for the remineralization of dentin [20,21]. The phosphorylation of proteins present on the surface of an organic template by STMP could trap calcium ions by electrostatic force resulting in prenucleation clusters [20]. Such clusters may guide mineral deposition to the desired gap region areas within the collagen matrix [35-37]. In event that involves calcium and phosphate as in this investigation, STMP acts as a stabilizer for Ca^{2+} and induces hierarchical remineralization of hybrid layer and may increase the durability of resin-dentin bond [38].

As reported by Kawasaki et al. (1999) [24], the remineralization of dentin occurs neither by mineral nucleation in the organic matrix nor by its spontaneous precipitation, but by the growth of residual inorganic crystals in the body of the lesion. In consequence, this remineralization should be responsible for the improvement on its mechanical properties related to the observed results.

According to the μTBS values, it has been shown that the use of the STMP solution by itself was not sufficient to improve the bond strength of a universal adhesive system to the dentin. In addition, when this solution was associated with NaF or when only the NaF solution was used, no difference was also observed in the values of μTBS compared to the artificial carious group and between these groups.

In this study, the use of fluoride does not seem to be effective to improve dentin bond strength. The effective role of F^- ions depends on the amount of CaF_2 deposited on tooth surface, which did not occur in this study perhaps due to the lack of association of calcium ions with the NaF solution.

Based on the methodology and results of this study, it is suggested that the use of STMP may have altered the nanostructure of the collagen fibrils, since the introduction of the phosphate group on the surface of type I collagen (DMP-1) could serve as nucleation sites to trigger the mineralization of the dentin [20]. Thus, with the additional use of $Ca(OH)_2$ solution, the phosphate group could have stabilized calcium ions resulting in pre-nucleation clusters [23,24]. Consequently, guiding the deposition of minerals to the desired areas within the collagen matrix, increasing the mechanical properties of the extracellular matrix of the dentin [35-37] as it could be seen in the improvement of the μTBS values of the group treated with STMP + $Ca(OH)_2$ of this study compared to others groups submitted to the cariogenic challenge. However, long term success of this material is limited by the influence of oral dynamic conditions. The 6-month performance highlighted the potential of 1.5% STMP + $Ca(OH)_2$ on comparison to the other tested treatment, even none yielded the performance showed by the control sound group. After 12 months, all groups showed a decrease in μTBS values compared to initial condition. The presence of SMTP supplemented with $Ca(OH)_2$ was not able to maintain the μTBS values over time, but it was more effective in comparison to other tested treatments.

All this scenario addresses to the reports that conciliation of innovative "smart" remineralization strategies could restore the modulus of elasticity of mineral-depleted dental collagen structures within bonding interfaces to reach values similar or superior to those of sound dentin [34,38]. When associated to self-etching approaches, both proteolytic and polymer degradation impact could be reduced [38].

The supplementation of calcium was made regarding the potential to remineralize dentin properly as stated by previous investigations. Even the use of 1.5% STMP + $Ca(OH)_2$ addressed to improve bonding strength to dentin, it is far from the expected potential as described during the analyzes of previous studies regarding their remineralization and anti-enzymatic roles [25,26]. Therefore, once applied in dentin, it is relevant to consider its interaction to the substrate and to the tested bonding agent. Dentin substrate itself can serve as a mechanical barrier and dilute this impact. However, the impact of its consuming by STMP or MMP is not clear during their mechanism of action. Also, studies point out that in some cases, agglomerates can be formed and also impair the bonding to dentin by mechanical interference. Therefore, it was considered a relevant point to be investigated. Also, 10-MDP is the main ingredient of this multimode adhesive system, which mechanism of action depends on the consumption of calcium to bond to dentin. When 1.5% STMP + $Ca(OH)_2$ are used, Ca ions are available from this solution and probably MDP and STMP can compete for this ion and so, a negative interaction can be established. Chemical analyzes of this prepared substrate could be tested to clarify this scenario.

The adhesive/dentin interface degradation is still a challenge in Dentistry [2,39]. The durability of bonding to dentin depends on the interaction

of monomers with the network of collagen fibrils [39,40]. Probably, insufficient adhesive penetration results in exposed fibers, where degradation of the adhesive interface may begin [39]. Degradation may also start breaking the covalent bonds between polymers by water addition, leaving exposed collagen fibrils [39]. Consequently, collagen network degradation reduces the adhesive bond strength to dentin, compromising the durability of adhesive procedures, over time [39].

Therefore, the proposed research aimed to evaluate the remineralizing potential of STMP. As it known that the STMP presents the potential for collagen type I phosphorylation, it has been extensively investigated as a remineralization agent. Furthermore, the phosphate group, which has high affinity for Ca2+ ions, can trap these ions to form inorganic crystals that are essentials for dentin remineralization. So, the intention was to investigate if this association could enhance the potencial of the STMP in remineralizing dentin submitted to a carious challenge. Furthermore, when the group tested with STMP only, the bond strength results was not satisfactory when this association was done. Thus, it might have suggested that the association of both brought an improved remineralizing potential but not better than initial condition. The association with $Ca(OH)_2$ is able to optimize STMP benefits and might be continuously investigated to enhance its performance to reach long-term action.

Author Contributions

RSG: conceived, designed and performed the analysis, wrote the paper. GSZ: performed the analysis and wrote the paper. WSH: collected the data and performed the tests. MCG: performed the tests and wrote the paper. PMCS, DR, HMH: contributed data and analysis tools. LW: conceived and designed the analysis, wrote the paper.

Conflict of Interest

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

Funding

This investigation was supported by FAPESP (São Paulo Research Foundation) #2015/02559-0. Also, it was partially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001" and PET/MEC/SESu.

Regulatory Statement

Not applicable.

REFERENCES

1. Chaves LP, Ciantelli TL, Araújo DFG, Giacomini MC, Tjäderhane L, Scaffa PMC, et al. How proteolytic inhibitors interact with dentin on glass-fiber post luting over 6 months. J Mech Behav Biomed Mater. 2018;79:348-53. http://dx.doi.org/10.1016/j.jmbbm.2018.01.011. PMid:29358152.
2. Meereis CTW, Münchow EA, Rosa WLO, da Silva AF, Piva E. Polymerization shrinkage stress of resin-based dental materials: a systematic review and meta-analyses of composition strategies. J Mech Behav Biomed Mater. 2018;82:268-81. http://dx.doi.org/10.1016/j.jmbbm.2018.03.019. PMid:29627738.
3. Giacomini MC, Scaffa PMC, Chaves LP, Vidal CMP, Machado TN, Honorio HM, et al. Role of proteolytic enzyme inhibitors on carious and eroded dentin associated with a universal bonding system. Oper Dent. 2017;42(6):E188-96. http://dx.doi.org/10.2341/16-178-L. PMid:29144877.
4. Tjaderhane L, Buzalaf MA, Carrilho M, Chaussain C. Matrix metalloproteinases and other matrix proteinases in relation to cariology: the era of 'dentin degradomics'. Caries Res. 2015;49(3):193-208. http://dx.doi.org/10.1159/000363582. PMid:25661522.
5. Toledano M, Nieto-Aguilar R, Osorio R, Campos A, Osorio E, Tay FR, et al. Differential expression of matrix metalloproteinase-2 in human coronal and radicular sound and carious dentine. J Dent. 2010;38(8):635-40. http://dx.doi.org/10.1016/j.jdent.2010.05.001. PMid:20452393.
6. Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res. 1998;77(8):1622-9. http://dx.doi.org/10.1177/00220345980770081001. PMid:9719036.
7. Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res. 2005;84(8):741-6. http://dx.doi.org/10.1177/154405910508400811. PMid:16040733.
8. Scaffa PM, Breschi L, Mazzoni A, Vidal CM, Curci R, Apolonio F, et al. Co-distribution of cysteine cathepsins and matrix metalloproteases in human dentin. Arch Oral Biol. 2017;74:101-7. http://dx.doi.org/10.1016/j.archoralbio.2016.11.011. PMid:27923176.
9. Araújo DF, Chaves LP, Bim Júnior O, Garcia FCP, Ishikiriama SK, Honório HM, et al. Influence of 2% chlorhexidine digluconate on bond strength of a glass-fibre post luted with resin or glass-ionomer based cement. J Dent. 2014;42(6):735-41. http://dx.doi.org/10.1016/j.jdent.2014.02.019. PMid:24613604.
10. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjaderhane L, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials. 2006;27(25):4470-6. http://dx.doi.org/10.1016/j.biomaterials.2006.01.040. PMid:16687171.
11. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, et al. Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives. Eur J Oral Sci. 2006;114(2):160-6. http://dx.doi.org/10.1111/j.1600-0722.2006.00342.x. PMid:16630309.

12. Magalhães AC, Wiegand A, Rios D, Hannas A, Attin T, Buzalaf MA. Chlorhexidine and green tea extract reduce dentin erosion and abrasion in situ. J Dent. 2009;37(12):994-8. http://dx.doi.org/10.1016/j.jdent.2009.08.007. PMid:19733206.

13. Tjäderhane L, Mehtala P, Scaffa P, Vidal C, Paakkonen V, Breschi L, et al. The effect of dimethyl sulfoxide (DMSO) on dentin bonding and nanoleakage of etch-and-rinse adhesives. Dent Mater. 2013;29(10):1055-62. http://dx.doi.org/10.1016/j.dental.2013.07.014. PMid:23942144.

14. Liu Y, Li N, Qi Y, Niu LN, Elshafiy S, Mao J, et al. The use of sodium trimetaphosphate as a biomimetic analog of matrix phosphoproteins for remineralization of artificial caries-like dentin. Dent Mater. 2011;27(5):465-77. http://dx.doi.org/10.1016/j.dental.2011.01.008. PMid:21354608.

15. Ooshima T, Osaka Y, Sasaki H, Osawa K, Yasuda H, Matsumura M, et al. Caries inhibitory activity of cacao bean husk extract in in-vitro and animal experiments. Arch Oral Biol. 2000;45(8):639-45. http://dx.doi.org/10.1016/S0003-9969(00)00042-X. PMid:10869475.

16. Bedran-Russo AK, Castellan CS, Shinohara MS, Hassan L, Antunes A. Characterization of biomodified dentin matrices for potential preventive and reparative therapies. Acta Biomater. 2011;7(4):1735-41. http://dx.doi.org/10.1016/j.actbio.2010.12.013. PMid:21167964.

17. Zhang X, Neoh KG, Lin CC, Kishen A. Remineralization of partially demineralized dentine substrate based on a biomimetic strategy. J Mater Sci Mater Med. 2012;23(3):733-42. http://dx.doi.org/10.1007/s10856-012-4550-5. PMid:22271278.

18. Gu L, Kim YK, Liu Y, Ryou H, Wimmer CE, Dai L, et al. Biomimetic analogs for collagen biomineralization. J Dent Res. 2011;90(1):82-7. http://dx.doi.org/10.1177/0022034510385241. PMid:20940362.

19. Li X, Chang J. Preparation of bone-like apatite-collagen nanocomposites by a biomimetic process with phosphorylated collagen. J Biomed Mater Res A. 2008;85(2):293-300. http://dx.doi.org/10.1002/jbm.a.31397. PMid:17688292.

20. George A, Veis A. Phosphorylated proteins and control over apatite nucleation, crystal growth, and inhibition. Chem Rev. 2008;108(11):4670-93. http://dx.doi.org/10.1021/cr0782729. PMid:18831570.

21. Wang X, Ma JB, Wang YN, He BL. Structural characterization of phosphorylated chitosan and their applications as effective additives of calcium phosphate cements. Biomaterials. 2001;22(16):2247-55. http://dx.doi.org/10.1016/S0142-9612(00)00413-0. PMid:11456064.

22. Pashley DH, Tay FR, Imazato S. How to increase the durability of resin-dentin bonds. Compend Contin Educ Dent. 2011;32(7):60-4, 66. PMid:21910364.

23. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc. 2000;131(7):887-99. http://dx.doi.org/10.14219/jada.archive.2000.0307. PMid:10916327.

24. Kawasaki K, Ruben J, Stokroos I, Takagi O, Arends J. The remineralization of EDTA-treated human dentine. Caries Res. 1999;33(4):275-80. http://dx.doi.org/10.1159/000016529. PMid:10343090.

25. Gonçalves RS, Scaffa PMC, Giacomini MC, Buzalaf MAR, Honório HM, Wang L. Use of sodium trimetaphosphate in the inhibition of dentin matrix metalloproteinases and as a remineralizing agent. J Dent. 2018;68:34-40. http://dx.doi.org/10.1016/j.jdent.2017.10.009. PMid:29079452.

26. Gonçalves RS, Candia Scaffa PM, Giacomini MC, Vidal CMP, Honório HM, Wang L. Sodium trimetaphosphate as a novel strategy for matrix metalloproteinase inhibition and dentin remineralization. Caries Res. 2018;52(3):189-98. http://dx.doi.org/10.1159/000484486. PMid:29342454.

27. ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res. 1982;16(3):201-10. http://dx.doi.org/10.1159/000260599. PMid:6953998.

28. Moron BM, Comar LP, Wiegand A, Buchalla W, Yu H, Buzalaf MA, et al. Different protocols to produce artificial dentine carious lesions in vitro and in situ: hardness and mineral content correlation. Caries Res. 2013;47(2):162-70. http://dx.doi.org/10.1159/000345362. PMid:23235318.

29. Shen CY. Alkaline hydrolysis of sodium trimetaphosphate in concentrated solutions and its role in built detergents. Ind Eng Chem Prod Res Dev. 1966;5:660-72.

30. Zhang K, Li Y, Ren Y. Research on the phosphorylation of soy protein isolate with sodium tripolyphosphate. J Food Eng. 2007;79(4):1233-7. http://dx.doi.org/10.1016/j.jfoodeng.2006.04.009.

31. Duke SA, Forward GC. The conditions occurring in vivo when brushing with toothpastes. Br Dent J. 1982;152(2):52-4. http://dx.doi.org/10.1038/sj.bdj.4804743. PMid:6950739.

32. Mazzoni A, Tjaderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res. 2015;94(2):241-51. http://dx.doi.org/10.1177/0022034514562833. PMid:25535202.

33. Komori PC, Pashley DH, Tjäderhane L, Breschi L, Mazzoni A, Goes MF, et al. Effect of 2% chlorhexidine digluconate on the bond strength to normal versus caries-affected dentin. Oper Dent. 2009;34(2):157-65. http://dx.doi.org/10.2341/08-55. PMid:19363971.

34. Gower LB. Biomimetic model systems for investigating the amorphous precursor pathway and its role in biomineralization. Chem Rev. 2008;108(11):4551-627. http://dx.doi.org/10.1021/cr800443h. PMid:19006398.

35. He G, Dahl T, Veis A, George A. Dentin matrix protein 1 initiates hydroxyapatite formation in vitro. Connect Tissue Res. 2003;44(Suppl 1):240-5. http://dx.doi.org/10.1080/03008200390181726. PMid:12952204.

36. Nijhuis AW, Nejadnik MR, Nudelman F, Walboomers XF, te Riet J, Habibovic P, et al. Enzymatic pH control for biomimetic deposition of calcium phosphate coatings. Acta Biomater. 2014;10(2):931-9. http://dx.doi.org/10.1016/j.actbio.2013.09.036. PMid:24095783.

37. Demichelis R, Raiteri P, Gale JD, Quigley D, Gebauer D. Stable prenucleation mineral clusters are liquid-like ionic polymers. Nat Commun. 2011;2(1):590. http://dx.doi.org/10.1038/ncomms1604. PMid:22186886.

38. Abuna G, Feitosa VP, Correr AB, Cama G, Giannini M, Sinhoreti MA, et al. Bonding performance of experimental bioactive/biomimetic self-etch adhesives doped with calcium-phosphate fillers and biomimetic analogs of phosphoproteins. J Dent. 2016;52:79-86. http://dx.doi.org/10.1016/j.jdent.2016.07.016. PMid:27472956.

39. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24(1):90-101. http://dx.doi.org/10.1016/j.dental.2007.02.009. PMid:17442386.

40. Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: a systematic review and meta-analysis. J Dent. 2015;43(7):765-76. http://dx.doi.org/10.1016/j.jdent.2015.04.003. PMid:25882585.

Linda Wang
(Corresponding address)
Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Odontológicos, Bauru, SP, Brazil.
Email: wang.linda@uol.com.br

Date submitted: 2021 May 23
Accept submission: 2021 Aug 03