Aus den Zahnmedizinischen Kliniken der Universität Bern

Klinik für Zahnerhaltung, Präventiv- und Kinderzahnmedizin

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Modification of phosphoric acid with polyphenol-rich plant extracts: assessment of bond strength to non-eroded and eroded dentine

Inaugural-Dissertation zur Erlangung der Doktorwürde der Zahnmedizin

der Medizinischen Fakultät der Universität Bern

vorgelegt von

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Von der Medizinischen Fakultät der Universität Bern auf Antrag der Dissertationskommission als Dissertation genehmigt.

Promotionsdatum:

Der Dekan der Medizinischen Fakultät:

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1 ABSTRACT

This study aimed at developing phosphoric acids modified with different polyphenol-rich plant extracts and to verify their effect on the 24h shear bond strength (SBS) to non-eroded and eroded dentine. In this view, the groups contained experimental phosphoric acid (PA), either plain or modified with polyphenol-rich plant extracts, and a commercially available phosphoric acid was used as positive control. The groups were: 1) LabPA (37% phosphoric acid prepared in the laboratory); 2) PA-GSE (LabPA+2% Grape seed extract); 3) PA-Blueberry (LabPA+2% Blueberry extract); 4) PA-Cranberry (LabPA+2% Cranberry extract); 5) PA-GT (LabPA+2% Green tea extract); 6) CommPA (commercial PA, Kerr Gel Etchant, 37.5% phosphoric acid). For SBS, a total of 192 dentine specimens were prepared from sound extracted permanent human molars, by grounding the teeth until the mid-coronal dentine. All specimens underwent a standard smear layer, half of them were straight restored (non-eroded dentine, n=96) and the other half underwent erosion challenge (eroded dentine, n=96; 10 cycles of 1h exposure to human saliva followed by 5 min immersion in 1% citric acid). Consequently, 96 non-eroded and 96 eroded specimens were etched (15s) with the PAs, according to the experimental groups (n=16 specimens/group) and subjected to an etch-and-rinse adhesive system, followed by standardized restoration with a composite resin. After 24 h incubation in humid chamber, the specimens were subjected to SBS using a universal testing machine. The failure mode of each specimen was determined under a stereomicroscope and described qualitatively. Data was analysed with Q-Q plots, Shapiro-Wilk and Levene's tests for normality and equality of variances, and General Linear Model (GLM) with SBScalc as outcome variable, and "Acid Group" and "Presence of Erosion" as factors. Both factors individually caused an effect to the outcome ($p<0.001$), but there was no interaction between the factors ($p=0.818$). All LabPAs (modified or not with plant extracts) had higher SBS than CommPA, and the presence of extract did not influence the results. Non-eroded dentine had significantly higher SBS than eroded specimens. In conclusion, erosion decreases SBS, and the addition of plant extracts did not influence SBS after 24h of restoration.

2 INTRODUCTION

2.1 DENTIN BONDING

Dentin bonding is a chemical tissue repairing process, where the mineral content in the dentin matrix is substituted with resin monomers to form a biocomposite comprising dentin collagen and cured layer. This layer is expected to form a tight and long-lasting connection between dentin and composite resins. (Tjäderhane, 2015)

Etch-and-rinse (ER) or self-etch (SE) are the common techniques in the application of dentin bonding. The main aim of both techniques is to create a pathway for adhesive resin infiltration into collagenous matrix. In ER bonding technique, this pathway is achieved with the application of an acid, which dissolves the minerals 5-10 µm deep and leaves the highly porous dentin collagen network emersed in water. The collagen network is then infiltrated with resin monomer. (Pashley *et al.*, 2011)

In order to establish long lasting and durable bonding between the adhesive resin and dentin, the morphological and physical variations in human dentin must be well understood and demonstrated. The bonding mechanism relies mainly on the penetration of the primer and adhesive resin into the preconditioned dentin surface in order to create micromechanical interlocking with the dentin collagen. This layer with mechanical linkage between resin monomers and dentin components is paramount for a durable resin-dentin bond and has been known as hybrid layer or resin–dentin interdiffusion zone. (Perdigão, 2010)

Monitoring the hybrid layer is an important study subject since the contemporary hydrophilic dentin bonding systems deteriorate over time. The process of resin monomer diffusion within the hybrid layers occurs inappropriately and results in a lack of infiltration at the bottom of the hybrid layer. (Hass *et al.*, 2016)

2.2 MATRIX METALLOPROTEINASES (MMP)

Matrix metalloproteinases (MMPs) are endogenous Zn^{2+}– and Ca^{2+}–dependent enzymes, which have a potent degrading capability of extracellular matrix components present in the dentine, and which are one of the responsible factors for the dentin-resin bonding failure. MMPs consist of mainly a prodomain and a catalytic domain. They are usually expressed as inactive zymogens, and the prodomain must be separated from the catalytic one for it to be activated. The activity of MMPs is regulated by cleavage of the propeptide, which may occur at multiple levels, including autolysis, serine protease

plasmin, or other MMPs. (Visse & Nagase, 2003)

The cysteine cathepsins were recently discovered as a member of proteases also located in the dentin. The cathepsins have shown to play an important role in the degradation of extracellular matrix. Dental erosion and failure of existing adhesive restorations were constantly related to high content of cysteine cathepsins in the oral cavity. (Mazzoni *et al.*, 2015)

MMPs have an important function in tissue remodelling and the regulation of cell-matrix interactions during the primary stages of tooth morphogenesis. MMP-20 (enamelysin) has unique structural and enzymatic properties, which is capable of degrading amelogenin, and may have an important role during enamel development. (Caterina *et al.*, 2002) MMP-20 is produced by mature odontoblasts, which secret it into the dentinal fluid and it is embedded into the dentin and may be released during caries progression. (Sulkala *et al.*, 2002)

Prior to activation, MMPs are secreted in an inactive form. Following activation, MMPs gain the potency to degrade extracellular matrix components. The MMPs -2, -8 and -9 are activated by low pH (4.5) value which also significantly contributes to the progression of dentin erosion. Surprisingly, the degradation process of dentin organic matrix takes places after neutralization of the pH value in the human saliva and not immediately when the pH value falls. Consequently, The MMPs are activated, pH value is neutralised and both dental caries and erosion processes occur. (Buzalaf *et al.*, 2012)

The MMPs and the cysteine cathepsins, which are activated by the acidic components of the adhesive system, subject the partially exposed collagen fibrils network to enzymatic degradation, and for this reason it can negatively impact the dentin bonding. Therefore, the collagenolytic activity, together with the hydrolysis of the resin component, temperature, bacterial acids, and mechanical stresses contributing factors to the long-term loss of interfacial integrity of the adhesive restoration. (Braga & Fronza, 2020)

2.3 MMP AND THE DEMINERALIZATION OF DENTIN

The effect of the MMPs during caries was reported in the demineralization of dentin matrix stage. Accompanied by the *Streptococcus mutans*, MMPs were associated with degradation of triple-helical collagen fibrils in mineralised tissues. It has been demonstrated that dentin matrix degrading activity is not performed by bacteria. *In vitro* experiments have shown that cariogenic bacteria could cause only demineralization of the dentin surface, but were not able to degrade the dentin matrix, which is necessary for cavity formation. (Hannas *et al.*, 2007) The degradation of the dentin matrix is therefore mainly driven by the MMPs.

For erosion, MMPs also have a main role in the progression of dentin demineralisation. The erosive cycles occur normally in the oral cavity and consist of demineralisation followed by remineralisation processes. This leads mainly to softening of the outermost dentin layer. Softening occurs irregularly leaving some sound dentin areas unaffected, hence, adhesive resins adhere to different surfaces than those for which they were originally created. Moreover, erosion can cause removal of the dentinal plugs and dissolve the organic intertubular dentin. The remnants are usually the exposed collagen fibrils which are easily attacked by the activated MMPs. Eroded dentin surfaces directly impair the longevity and the stability of the adhesive restorations. (Cruz *et al.*, 2015)

Exposed dentin areas have a higher vulnerability to erosion. Dissolution of the minerals in the dentin take place usually with low pH values. After minerals dissolution, the organic matrix is highly exposed to breakdown by the MMPs. The enzymatic degrading action of MMPs to the demineralized organic matrix significantly enhances the demineralization process. The demineralised organic matrix can be hardly removed through standard tooth brushing movements. The layer of the demineralised organic matrix protects partly the dentin against mechanical forces, such as abrasion and decreases the ionic diffusion into the demineralized layer. This has been shown to decrease progression of dentin erosion. However, the extent to which this protective effect is relevant is not determined yet, as the demineralised organic matrix can be degraded by activated MMPs. (Buzalaf *et al.*, 2012)

2.4 PLANT EXTRACTS AND DENTIN BONDING

It has been proven by previous studies that the use of natural plant-based cross linkers remarkably contributes to a stabile dentin collagen, and it increases significantly the collagen resistance to degradation. Additionally, improvement of the longevity to the composite restorations and the stabilisation of the hybrid layer took place. (Liu & Wang, 2013) (Liu *et al.*, 2013) (Hass *et al.*, 2016)

The polyphenolic natural component known as proanthocyanidin (PAC) is a plant-based substrate that has been shown to be an efficient collagen cross-linker. One of its main advantages is that it is a strong inhibitor of the MMPs, which leads to interruption of the matrix degrading process. (Ferreira & Slade, 2002) It has been established that grape seed extract and cranberry juice extract have high content of PAC. (Wang *et al.*, 2021)

The PAC-rich grape seed extract (GSE) has been shown to be one of the best cross-linkers regarding hybrid layer stabilisation. Less than 30 seconds application time is sufficient to demonstrate high cross-linking features of this extract. Previous studies have used GSE as a priming agent, however others added it as component to the phosphoric

acid-etchant. Promising results were stated since the degradation of the exposed collagen fibrils by MMPs was significantly reduced. (Liu *et al.*, 2013) These studies, however, have not included human saliva in their models. Saliva also contains MMPs, which can also impact dentin degradation, driving the demineralization forward.

Green tea extract (GTE), which contains polyphenolic compounds based on catechins, such as (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epigallocatechin gallate, has also proven to interrupt the matrix-degrading process by inhibiting the activity of MMPs. (Demeule *et al.*, 2000) One of the main characteristics of GTE is its light color. So, any staining of the dentin during the dentin-etching process is very minor setback, which makes GTE a clinically acceptable acid-etchant. (Liu *et al.*, 2013)

In recent publications, it has been stated that the potency of cranberry juice extract (CJE) as a cross-linker was less significant in comparison to the GSE and GTE. However, CJE containing 0.65% PAC was a contributing factor to a stabilised demineralized dentin collagen. The effectiveness of CJE as an MMPs inhibitor was also evidenced by the dramatically reduced matrix-bound enzymatic activity, which almost completely inactivated the gelatinolytic activity in the collagen films after 30 seconds of treatment. (Wang *et al.*, 2021)

2.5 MODIFICATION OF THE PHOSPHORIC ACID

The traditional concentrations of phosphoric acid used at 30-40% demineralises the dentin to a depth of 5–8 µm. This depth cannot be usually achieved by the infiltration of the bonding resin, therefore, this discrepancy between etching depth and adhesive resin infiltration leads to unprotected collagen, which is rapidly degraded by activated MMPs. (Pashley *et al.*, 2011) The oral metabolic bacterial activity induces also acidic pH, which activates the MMPs and stimulates the release of cathepsins, which accelerate collagen degradation near hybrid layer. Rapid resin-dentin breakdown and dissolution of unprotected collagen fibrils occur commonly with the usage of the traditional etch-and-rinse adhesives. (De-Paula *et al.*, 2020)

Previous study trials were attempting to modify the traditional acid etchant. The biomodification process took place by incorporating cross-linkers, such as PAC, into the 37% phosphoric acid-etchant. PAC was mainly obtained from grape-seed extract, which was compatible with the phosphoric acid concentration (37%). Phosphoric acid etchants incorporated with polyphenol cross-linkers provided stable dentin-resin interface with low nanoleakage after aging and optimal collagen cross-linking, highlighting feasible usage with all adhesive restorations. (De-Paula *et al.*, 2020)

The concentration of the polyphenol cross-linkers has an important role in the efficacy of the plant-based extracts. The commercial CJE, which was used in previous studies, contained less than 1% of PAC, and it did not achieve an exceptional effect. Therefore, this would be very important information for the manufacturing process of biomodified acid-etchants. This will have profound effects on the composition, potency and PAC content of the resulting extract. For example, raw cranberry, similar to grape and cocoa seeds, contains higher amounts of total phenols than other common fruits including blueberry, apples, red grapes and strawberries. But, it has two major classes of phenolics identified, and only 56% is PAC. Due to high content of PAC in GSE (95%), it has been shown to be the ideal plant-based extract incorporated into the acid-etchant. (Castellan *et al.*, 2011)

The polyphenol cross-linkers, epigallocatechin-3-gallate (EGCG) and epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG-3Me) modified etch-and-rinse adhesives (Single Bond 2, SB 2), have been also tested for their antibacterial effect and the stability to the hybrid layer. EGCG and EGCG-3Me modified adhesives showed remarkable antibacterial effect against *S. mutans* and increased bonding stability to dentin. In addition, the inhibitory effect of EGCG and EGCG-3Me on MMPs was demonstrated for the first time with direct evidence. Modification with EGCG-3Me had better antibacterial effect, protease inhibition, and less influence on the degree of conversion of the adhesive than with EGCG. Therefore, adhesive modification with EGCG-3Me at a concentration of 400 µg/mL may be a method to be considered to improve the long-term use of adhesive restorations. (Yu *et al.*, 2017)

2.6 AIM

Some studies have added plant-extracts to phosphoric acids, but they either have used lower concentrations of the acid (only 10%), or they have not included human saliva in their models for dental erosion formation. Considering that the saliva also contains MMPs, which can affect the demineralization process and the bond strength of the adhesive system, incorporating it into the model is of interest. In the present study, we include human saliva in our model, and we also add the plant extracts to phosphoric acid at clinically relevant concentration.

Therefore, the aim of this study was to develop phosphoric acids modified with polyphenol-rich plant extracts and to assess the 24h shear bond strength when using these acids, testing on non-eroded and eroded dentine.

3 MATERIALS UND METHODS

The present study was divided into 2 parts: 1) Development of the modified phosphoric acids (MPAs); and 2) Evaluation of shear bond strength (SBS) of composite restorations performed with the MPAs to non-eroded and eroded dentine.

3.1 DEVELOPMENT OF THE MODIFIED PHOSPHORIC ACIDS (MPAS)

Development of the MPAs

A solution of phosphoric acid (37%) was prepared in the laboratory and, from that, a total of 5 MPA groups (Table 1) were developed. One group, the negative control, contained no polyphenols, and the other 4 groups contained 2% of polyphenol-rich extract: Grape seed extract; Blueberry extract; Cranberry extract; Green tea. The 5 MPA groups were gelled by adding polyethylene glycol (Figure 1), as used in some commercialized acids. In addition, a commercially available phosphoric acid (OptiBond Gel Etchant, Kerr, 37.5% phosphoric acid) was also tested, as positive control (Figure 2).

Figure 1 : 5 MPA groups

Figure 2, Kerr commercial phosphoric acid

Table 1: Descripition of experimental groups

Group	Ingredients
Negative control group	- Phosphoric acid (37%) solution and polyethylene glycol
Grape seed extract	- Phosphoric acid (37%) solution , grape seed extract (2% of polyphenol) and polyethylene glycol
Blueberry extract	- Phosphoric acid (37%) solution , blueberry extract (2% of polyphenol) and polyethylene glycol
Cranberry extract	- Phosphoric acid (37%) solution , cranberry extract (2% of polyphenol) and polyethylene glycol
Green tea extract	- Phosphoric acid (37%) solution , green tea (2% of EGCG) and polyethylene glycol
Positive control group	- Phosphoric acid (37.5%, OptiBond Gel Etchant, Kerr)

3.2 SHEAR BOND STRENGTH (SBS) OF COMPOSITE RESTORATIONS PERFORMED WITH THE MPAS TO NON-ERODED AND ERODED DENTINE

Specimen preparation

A total of 192 dentine specimens were prepared from sound extracted permanent human molars, obtained from a pooled bio-bank. The local ethical committee considers pooled bio-banks as irreversibly anonymized and they waive the necessity of previous ethical approval. The occlusal surfaces of the teeth were ground until the mid-coronal dentine with 220-grit silicon carbide abrasive papers (LaboPol-21, Struers), under constant cooling. The specimens were further ground with 500-grit silicon carbide abrasive paper. Then, the molars were apically shortened with a water-cooled diamond saw (IsoMet Low Speed Saw, Buehler) and embedded in self-curing acrylic resin (Paladur, Heraeus Kulzer). All the specimens were kept in a humid chamber at 4˚C, until the beginning of the experiment. They were subjected to a standardized smear layer, which was formed by grinding the specimens for 5 s with 500-grit silicon carbide abrasive paper (LaboPol-21, Struers), under constant cooling. For the non-eroded dentin specimens (n=96), the smear layer was formed immediately prior to the experimental procedure, whereas for the eroded specimens (n=96), the smear layer was made prior to the erosive challenges.

For the erosive challenge, the 96 specimens were submitted to 10 erosive cycles, each consisting of 1h exposure to human saliva (37˚C), followed by 5 min immersion in citric acid with constant movement (1%, pH 3.6, 25˚C, 70 rpm, travel path 50 mm). The human saliva was used because it contains MMPs and might influence the degradation of the organic matrix. The whole mouth stimulated saliva was collected from fifteen volunteers and pooled. Again, the local ethical committee considers pooled saliva as irreversibly anonymized samples, and prior ethical approval was not necessary.

Experimental procedure

All specimens were placed in tap water and kept in room temperature for one hour prior to the experiment, so that they reached room temperature. Then, the etching, adhesive system and restoration were applied (set up can be observed in Figure 3).

Initially, the dentine specimens were briefly water sprayed and gently air-dried. A self-adhesive tape with a perforation in the center (~2 mm ø) was placed on the dentine surfaces, framing the area to be treated. This is made to guarantee a defined, isolated and

standardized bonding area. For the restoration, the dentine specimens were etched for 15s with the phosphoric acid according to each experimental group (Table 1), then they were rinsed with water spray for 15 s and slightly airdried for 5 s. Subsequently, the adhesive system (OptiBond FL, Kerr) was applied according to the manufacturer's instructions. Firstly, the OptiBond FL Prime was applied with a microbrush, lightly scrubbing for 15 s followed by gently air-dried for 5 s. Next, the OptiBond FL Adhesive was applied also with a microbrush, creating a thin coating and light cured for 10 s (LED curing unit; Demi, Kerr; power density of 1500 mW/cm^2). For the restorative procedure, a split Teflon mold was fixed on the dentine surfaces (inner diameter 1.5 mm, bonding area 2 mm^2, height 2 mm) and filled with composite resin (Filtek Z250, 3M ESPE, shade A4). The composite was light cured for 20 s (LED curing unit; Demi, Kerr). The specimens were straight placed in black light-tight boxes (Figure 4) to avoid any further influence of ambient light, and then kept in an incubator (UM 500, Memmert) at 37°C and 100% humidity. After 24 h, the specimens were subjected to SBS testing.

Figure 3: Set up for the experimental procedure

Figure 4: Isolation through black box

SBS analysis

SBS was analysed with a universal testing machine (Zwick Z1.0 TN, Zwick), using a stainless-steel wire (diameter 0.6 mm) at a crosshead speed of 1 mm/min. The maximum force (N) was recorded and the SBS (in MPa) was calculated: maximum force (N) dividing by the bonding area (mm^2).

Statistical analysis

Data was analysed with Q-Q plots, Shapiro-Wilk and Levene's tests for normality and equality of variances. We used a General Linear Model (GLM) with SBS as outcome variable, and "Acid Group" and "Presence of Erosion" as factors. Significance level was set at 5%. Failure modes were described qualitatively.

4 RESULTS

The initial test of Between-Subject effect is presented in the table below, showing that each factor ("Acid Group" and "Presence of Erosion") individually caused an effect to the outcome ($p<0.001$), but there was no interaction between both factors ($p=0.818$). This means that the Acid Group caused an effect on the SBS values, with some acids performing better than others. Also, the Presence of Erosion caused an effect, where non-eroded specimens had significantly higher SBS values. There was no interaction between the variables, where generally non-eroded dentine presented higher values than eroded dentine, independent of the acids. And all acids performed similarly, independent of the presence of erosion (in other words, if an acid had higher SBS values for non-eroded dentine in comparison to another acid, this pattern continued for eroded dentine). While using the same acid-etchant at eroded and non-eroded specimens, significantly higher bonding strength of the restoration was stated at the non-eroded specimens than the eroded ones ($p<0.008$).

Below are the comparisons between the acid groups:

Table 2: Tests of Inter-subject effect

Dependent variable: SBS

Source	**Type III Sum of Squares**	**df**	**Mean of squares**	**F**	**Sig**
Corrected model	3067.357[a]	11	278.851	12.651	<.001
Constant term	41177.200	1	41177.200	1868.126	<.001
Acid group	1030.455	5	206.091	9.350	<.001
Presence Erosion	1988.125	1	1988.125	90.197	<.001
Acid group	48.777	5	9.755	.443	.818
Mistakes	3967.558	180	22.042		
Overall	48212.115	192			

Corrected overall variation	7034.915	191			

R-Quadrat =.436 (corrected R-Square =.402)

Reference category = 1

Table 3: Test results

Dependent variable: SBS

Source	**Sum of Squares**	**df**	**Medium of square**	**F**	**Sig.**
Contrast	1030.455	5	206.091	9.350	<.001
Mistakes	3967.558	180	22.042		

Table 4: Post-Hoc-Tests

Acid groups

Dependent variable: SBS

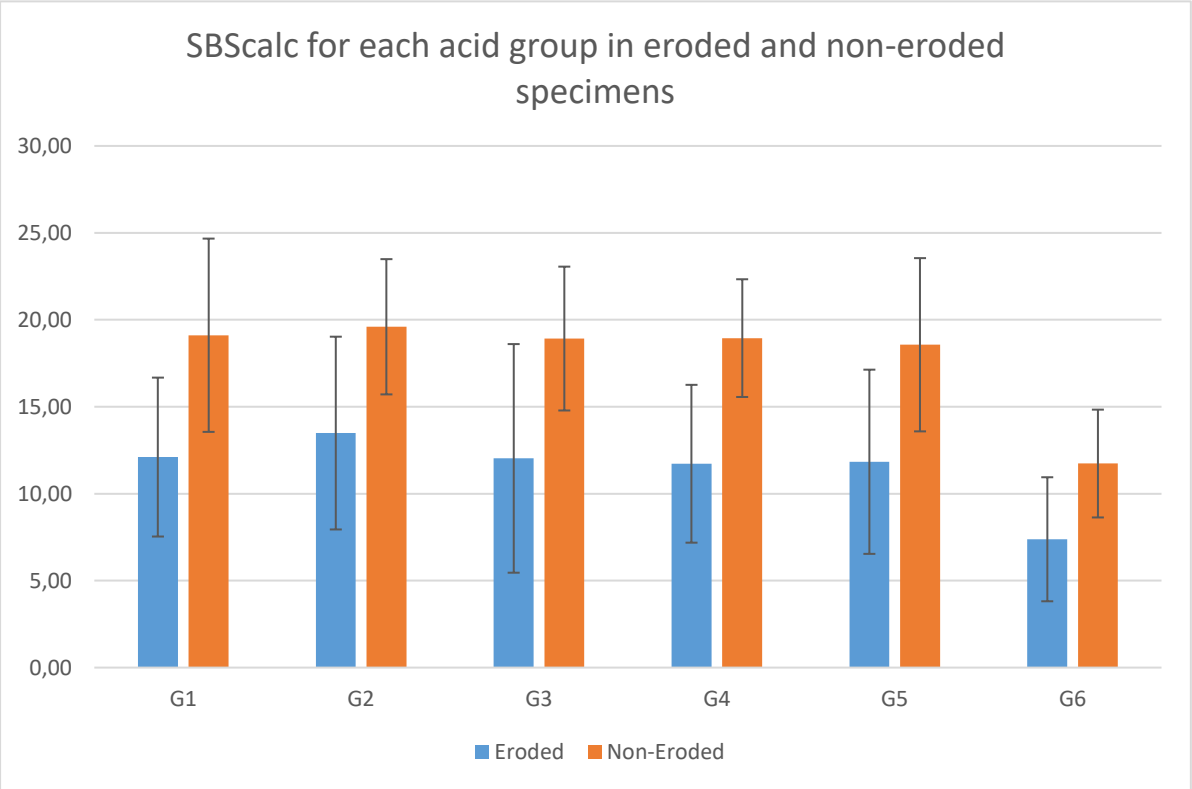
Tukey-HSD

(I)Acid group	**(J) Acid group**	**Average difference (I-J)**	**Std.-mistakes**	**Sig.**
Phosphoric acid Commercial (KERR)	Negative control	-6.19937*	1.173722	<.001
	Phosphhoric acid with OPC	-6.98625*	1.173722	<.001
	Phosphoric acid with Blueberry	-591906*	1.173722	<.001
	Phosphoric acid with Cranberry	-.577750*	1.173722	<.001
	Phosphoric acid with Green Tea	-5.64422*	1.173722	<.001

Table 5: SBScalc

Tukey-HSD[a,b]

Acid group	N	Subset 1	Subset 2
Phosphoric acid commercial (KERR)	32	9.55688	
Phosphoric acid with Green Tea	32		15.20109
Phosphoric acid with Cranberry	32		15.33438
Phosphoric acid with Blueberry	32		15.47594
Negative control	32		15.75625
Phosphoric acid with OPC	32		16.54313
Sig.		1.000	.863



5 DISCUSSION

5.1 DIFFERENCE IN BOND STRENGTH BETWEEN ERODED AND NON-ERODED SPECIMENS

The present study modified phosphoric acid with different polyphenol-rich plant extracts and verified the effect of these modified phosphoric acids on the 24 h shear bond strength (SBS) of an etch-and-rinse adhesive to non-eroded and eroded dentine. Since the above-mentioned graph demonstrated the significant difference in adhesion strength of the standardized adhesive restorations between the eroded specimens and the non-eroded specimens, it proves the negative immediate effect of eroded dentin surfaces on the bonding stability.

This feature can be explained by the erosion process that takes place. Teeth that are subjected to several cycles of erosion obtain several structural defects such as mineral loss, fibril collagen exposure, and opening of dentinal tubules. (Zimmerli *et al.*, 2012)

Deeper demineralized layer becomes highly involved in the low bonding stability due to high degree of demineralisation in the eroded dentin. During the application of the adhesive, the hybrid layer formed in the eroded dentin is usually significantly thicker than that in healthy dentin. This inhomogeneous thickness of the hybrid layer contains usually several porosities, which can initiate crack propagation in the hybrid layer leading to early failure of the adhesive restoration (Wang & Spencer, 2004). This undesired heterogeneous layer, which contains hydrophilic areas and demineralized zones, is usually not fully infiltrated by the resin. This may explain the lower SBS values for eroded dentin because resin monomers may not penetrate as deeply as acid-etchant (Cruz *et al.*, 2015).

Therefore, the reduced 24 h bond strength to the eroded dentin observed in this study can be attributed to the inefficient and inferior formation of the hybrid layer due to the collapse of the demineralised collagen fibrils and the excessively high water content, which caused low adhesive infiltration in deep layers and also generally an improper polymerisation process (Flury *et al.*, 2017).

In a previous study with Energy Dispersive X-ray Spectroscopy (EDX), a significant reduction was observed in the content of calcium and phosphorus in the eroded dentin specimens as a result of demineralization caused by several erosion cycles. Chemical bonding to dentin of the acidic phosphate monomer (10-methacryloyloxydecyl dihydrogen phosphate (MDP)) is obtained mainly via calcium. Thus, the reduced bond strength to eroded dentin in comparison to non-eroded dentin may have been caused not only by

inferior hybridization of the eroded dentin, but also by the lower content of calcium (Zumstein *et al.*, 2018).

5.2 HIGH BOND STRENGTH VALUES BETWEEN THE ACID GROUPS ON NON-ERODED DENTIN

In this present study, it was stated that all the groups showed higher bond strength (SBS) values on the non-eroded dentin specimens than on the eroded ones. This confirms the concept that the use of a sound-dentin substrate results in ideal bond strength. The main requirement in achieving an ideal hybridization in the adhesive-resin interface is usually fulfilled by non-eroded dentin. The minimal reported differences between the acid-etchant groups are discussed in the next paragraphs.

In sound, non-eroded dentin substrate resin monomers displace water within and around the collagen fibrils without reducing the size of the already small porosities created by acid etching and completely replace that water uniformly within the infiltrating resin. When infiltration is complete, light activation is applied to cure the resin and result in an ideal hybrid layer. The morphological and physiological homogeneity have always been described as the major success to achieve uniform, reproducible and reliable bonding (Carvalho *et al.*, 2009).

It was proven in a previous study, that eroded dentin surfaces require generally certain preparation with diamond bur in order to enhance the bonding properties of the substrate by removing the non-uniform demineralized layer and showing off a fresh non- eroded dentin substrate. Short term and long-term bonding to eroded dentin was clearly enhanced by the way the eroded dentin surface was prepared (Zimmerli *et al.*, 2012).

5.3 SIGNIFICANT DIFFERENCE IN BOND STRENGTH BETWEEN KERR PHOSPHORIC ACID AND THE OTHER 5 PHOSPHORIC ACID GELS ON NON-ERODED DENTIN

Table 4 and the graph have stated a significant difference in bond strength between the commercial Kerr phosphoric acid and the other five manufactured phosphoric acid gels on non-eroded dentin after 24 hours, including the polyphenols contained gels and the negative control gel which does not contain polyphenols. Firstly, this confirms that the laboratory preparations were good on the immediate bond strength on the dentin substrate.

Furthermore, previous studies which applied the use of natural plant-based cross linkers on the manufactured gels and that led to a stabile dentin collagen interface, and it

enhanced significantly the collagen resistance to degradation. Moreover, a highly stable hybrid layer was monitored and contributed to the general immediate stabilization of the adhesive restoration. (Liu & Wang, 2013) (Liu *et al.*, 2013) (Hass *et al.*, 2016)

The PAC-rich grape seed extract (GSE) which has been demonstrated in our study as oligomer-proanthocyanidin (OPC) proved to be a very potent cross-linkers and incorporating this component into the manufactured acid-etchant contributed to the hybrid layer stabilisation. Several studies applied the usage of GSE in different methods, e.g., incorporating the GSE into the priming agent. the degradation of the collagen fibrils by the MMPs was significantly reduced after the application of GSE. (Liu *et al.*, 2013)

Green tea extract has unique practical characteristics. Its light color hindered the discoloration of dentin during dentin-etching. Its optimal viscosity was very helpful during application and rinsing procedures. It has also proven to interrupt the matrix-degrading process by inhibiting the activity of MMPs. It has been classified as clinically acceptable acid-etch (Demeule *et al.*, 2000) (Liu *et al.*, 2013)

The phosphoric acid with cranberry extract showed relatively high values. Cranberry extract gels were applied in previous studies, and it was a contributing factor to a stabilised demineralized dentin collagen. The effectiveness of cranberry extract as an MMPs inhibitor was also stated by the significantly reduced degradation enzymatic activity by the MMPs by almost completely inactivating the gelatinolytic activity in the collagen films after 30 seconds of application time. (Wang *et al.*, 2021)

The phosphoric acid with blueberry extract showed similarly high SBS results. High bonding strength of the dentin-resin interface was reported in table 3 and in the graph after 24 hours. Perhaps this extract has been used exclusively introduced and used in this study, as most of the previous studies directed their focus towards the Prothocyanidin-based extract such as cranberry juice, grape seed and green tea extracts as the most potent polyphenols or cross-linkers.

Other studies applied chlorhexidine as a modifier to the acid-etchant. (Loguercio *et al.*, 2016). The gel showed also very good handling characteristics due to its pleasant colour, ideal consistency, and less discoloration tendency after rinsing procedure. Regarding the results and the practical application of the gel, the introduction of the blueberry extract in the dental market could be recommended.

5.4 HYPOTHESIS OVER THE STATED HIGH BONDING STRENGTH VALUES OF THE NEGATIVE CONTROL GROUP GEL

Our statistics reported surprisingly very high bonding strength on the non-eroded dentin substrate after 24 hours in comparison to the eroded dentin.

The follow up measurements and statistics of our study will include in 1 year the shear bond strength measurements of the same 6 phosphoric acids with the same number of specimens and on both eroded and non-eroded dentin surfaces. The negative control group will be of great focus, to verify whether this acid-etchant, which contains no polyphenols and was fabricated in the laboratory, will have high stabilization of the hybrid layer after 1 year.

Moreover, the values of the five phosphoric acids, which were fabricated in our labs, showed very similar bonding strength values with no significant differences ($p>0.05$). This confirms that the addition of the polyphenols in our modified phosphoric acid did not impact negatively on efficacy of the etching properties of the gels.

The high values of the five phosphoric acid groups directed us to a conclusion, that there is certainly a common factor between these acids. We assume that this factor is the common manufacturing criteria of the phosphoric acids regarding the consistency, concentration (37%) and shelf life.

5.5 HYPOTHESIS OVER THE LOW BONDING STRENGTH OF KERR PHOSPHORIC ACID

The Kerr phosphoric acid showed significantly lower values in comparison with the other five phosphoric acids after 24 hours. The concluded hypothesis was, that this significance is strongly related to the manufacturing properties of the Kerr phosphoric acid regarding the consistency, concentration (less than 37%) and shelf life.

During our study we realised, that the consistency of the Kerr phosphoric acid was more viscous that the consistency of the other five phosphoric acid. The high viscosity would have attributed to the low values of its bonding strength. This feature must be further analysed in future studies.

As previously mentioned, a follow-up study including the same kinds of phosphoric acids, on eroded and non-eroded dentin, will include results after 1 year, and this might bring further light to the issue.

6 CONCLUSION

In conclusion, this study was performed to investigate the development of modified phosphoric acids with polyphenol-rich plant extracts, to assess the bond strength when using these acids, testing on non-eroded and eroded dentine.

There was a significant difference in adhesion strength of the standardized adhesive restorations between the eroded specimens and the non-eroded specimens, it proves the negative immediate effect of eroded- dentin surfaces on the bonding stability.

There was also a significant difference in bond strength between the commercial Kerr phosphoric acid and the other five manufactured phosphoric acid gels on non-eroded dentin after 24 hours.

The high values of the five phosphoric acid groups suggest that there is certainly a common factor between these acids. We assume that this factor is the common manufacturing criteria of the phosphoric acids regarding the consistency and concentration (37%).

The Kerr phosphoric acid showed significantly lower values compared with the other five phosphoric acids.

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8 CONFLICT OF INTEREST STATEMENT

It is hereby confirmed that the author of this study has no conflict of interest and was not paid for doing the work.

9 THANK YOU

I would like to thank the clinic Restorative, Preventive and Pediatric dentistry, University of Bern, including PD Dr. med dent. Thiago Saads Carvalho, Dr. med. dent. Samira Niemeyer, Dr. phil. nat. Tommy Baumann, Michael Stiebritz and finally Prof. Dr. Hendrik Meyer-Lückel.