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ORIGINAL ARTICLE

Effects of six different preventive treatments on the shear bond strength of orthodontic brackets: *in vitro* study

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Abstract

Objective: The aim of this study is to evaluate the effect of six different prophylactic agents on shear bond strength (SBS) of orthodontic brackets.

Materials and methods: One hundred twenty-six freshly extracted mandibular bovine incisors were used. Teeth were randomly divided into 7 equal groups (18 per group) as follows: group-1 served as control with no pre-treatment; group-2 enamel treated with fluoride varnish (Fluor Protector, Ivoclar Vivadent); group-3 containing casein-phosphopeptide–amorphous calcium-phosphate (CPP–ACP) paste (GC Tooth Mousse, RECALDENT™); group-4 with ozone (HealOzone, Kavo); group-5 with glycine powder (Perio Flow, EMS); group-6 with hydroxyapatite powder 99.5% (Coswell S.p.A.); group-7 with a toothpaste made of hydroxyapatite nanocrystals (BioRepair® Plus, Coswell S.p.A). Brackets were all bonded using the same technique with transbond XT (3 M Unitek, Monrovia, CA). All the bonded specimens were stored for 24 h in deionized water (37 °C) and subjected to thermal cycling for 1000 cycles. The SBS was measured with an Instron Universal Testing machine and the adhesive remnant was assessed with the adhesive remnant index (ARI) using a stereomicroscope at 10× magnification.

Results: Statistical differences (ANOVA) were found among the seven investigated groups ($F = 12.226$, $p < 0.001$). SBS of groups 2, 5 and 6 were significantly lower than the control group ($p < 0.05$). ARI scores (chi-square test) were correlated with the differences of SBS values.

Conclusion: CPP–ACP paste, ozone or BioRepair® did not compromise on bracket bond strength. Fluoride, glycine or hydroxyapatite significantly decreased the SBS; only the fluoride group showed significant clinically low (<6 MPa) SBS values.

Keywords

Biorepair, casein-phosphopeptide–amorphous calcium-phosphate, glycine, hydroxyapatite, orthodontic brackets, ozone, shear bond strength, topical fluoride

History

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Introduction

The fixed orthodontic treatment can lead to an accumulation and retention of plaque on the tooth surface. This is the main cause of decalcification and caries. Even if these undesired events could be controlled and reduced by maintaining good oral hygiene, the use of mineralizing agents before and during the orthodontic fixed treatments is an important and valid option to reduce these possible adverse lesions. It has been reported that almost half of the patients who received fixed orthodontic treatment develop enamel decalcification (white spot lesions).[1] In addition, the etching process can cause a permanent demineralization of the enamel with a loss of 5–10 µm, resulting in a further weakness of the enamel surface.[2]

In order to improve the mineralization of the tooth, many preventive treatments have been suggested. The most popularly known is the one that promotes the use of topical fluoride before, during or even after the enamel etching. Fluoride ions linked with hydroxyapatite form fluorhydroxyapatite and promote the re-mineralization of tooth's surface.[3,4] Another material used is the synthetic calcium hydroxyapatite: this is one of the few materials that are classed as bioactive. Thus, synthetic hydroxyapatite (HA) is considered a logical mineral compound to substitute the natural mineral constituent of tooth.[5,6]

Recently, a milk protein derivate, casein-phosphopeptide–amorphous calcium-phosphate (CPP–ACP), has been recommended for caries prevention and enamel remineralization. The use of CPP–ACP provides a saturation of the enamel structure by the localization of the ACP on the tooth surface. The presence of CPP can guarantee the availability of calcium and phosphate in a soluble and biological form for all oral cavities and even providing a large reserve in dental plaque.[7]

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Another agent used as a preventive technique is the ozone. Good evidence has been reported for prophylactic ozone applications in restorative dentistry prior to etching and positioning dental sealants and restorations.[8]

Before the placement of brackets, a widespread treatment, which consists air-polishing, is used to remove the plaque deposit. Glycine powder has been reported to be effective in the removal of biofilm, with the benefit of a low abrasive action on the tooth surface. Air-polishing with glycine powder is used to debride and treat tooth surfaces in operative dentistry as well as in orthodontics.[9,10]

The last material tested is BioRepair® Plus (Coswell S.p.A., Bologna, Italy): a fluoride-free toothpaste made of hydroxyapatite nanocrystals, which have been introduced because of its excellent biological properties, lack of toxicity, and inflammatory and immunological responses. The micro-particles activate due to their ability to bond to natural tissues, thus filling micro-gaps in the enamel.[11]

Tooth pre-treatments with those agents act on the enamel and can therefore interfere with the bonding mechanism and the bond strength of brackets. Different studies have already been done concerning the use of almost all these materials but always with different protocols, so the evaluation and the comparison are limited.[12–17]

The aim of this study is to use the same protocol, reducing the bias as much as possible with all six different techniques to make a direct and complete comparison between them. Thus, it is possible to obtain better and more specific results on the possible negative effect of these agents on the shear bond strength (SBS) of orthodontic brackets.

Materials and methods

One hundred twenty-six freshly extracted mandibular bovine incisors, obtained from the same farm in order to have equal level of fluoride concerning their feeding, were used. Teeth were cleaned of debris and then polished with non-fluoridate pumice and rubber prophylactic cups at low speed for 15 s. Tooth selection criteria included: integrity of buccal and lingual enamel surfaces under visible light at 4× magnification, absence of traumatic injuries, cavities, enamel erosions, smooth and flat buccal surface suitable for bonding. They were then stored in distilled water for no more than 2 weeks.

Teeth were randomly divided into 7 equal groups (18 teeth each) as follows:
group 1: served as *control* with no pre-treatment;
group 2: *fluoride* varnish (Fluor Protector, Ivoclar Vivadent, Schaan, Liechtenstein) according to manufacturer's instructions;
group 3: paste containing *CPP–ACP* (GC Tooth Mousse, RECALDENT™, Tokyo, Japan) and stored for 24 h in a solution with artificial saliva in order to promote the activation of the product;
group 4: *ozone* (HealOzone, Kavo Dental GmbH, Biberach, Germany) applied with a silicon cup for 60 s;
group 5: *glycine* powder (Perio Flow, EMS Electro Medical Systems S.A., Nyon Swiss) by air-flow for 60 s;
group 6: *hydroxyapatite 99.5% powder* (Coswell S.p.A., Bologna, Italy) by air-flow for 60 s;
group 7: toothpaste made of hydroxyapatite nanocrystals (*BioRepair Plus*®, Coswell S.p.A).

Brackets (Edgewise Standard, 4.4 mm × 3.2 mm, Leone S.p.A., Florence, Italy) were all bonded using the same standard technique: enamel surfaces were treated with 37% phosphoric acid (Etching gel 3M, Unitek, Monrovia, CA) for 60 s, rinsed with a water spray for 20 s and air dried. All brackets were bonded with transbond XT (3M Unitek, Monrovia, CA) and light-cured with the same LED lamp (Bluephase Polywave, Ivoclar Vivadent, Schaan, Liechtenstein) for 25 s. We used the same type of bracket throughout this work (Edgewise Standard, 4.4 mm × 3.2 mm, Leone S.p.A.). All the brackets were bonded by a unique operator. All the bonded specimens were then stored for 24 h in deionized water (37 °C) and subjected to thermal cycling for 1000 cycles.

For SBS tests, each tooth was mounted on self-cured acrylic resin blocks with a mounting jig used to align its buccal surface so that it was perpendicular to the bottom of the mold. Specimens were then mounted in the jig and the SBS was measured with an Instron Universal Testing machine (Model 3343, Instron Corp., Canton, MA). Continuous shear force was applied at a crosshead speed of 1 mm per minute until bracket failure.

The force required to detach the bracket was recorded in Newtons (N) and converted to megapascals (MPa) using the following formula: bond strength (MPa) = debonding force (N)/$[w \times l]$(mm^2), where w = width of the bracket base, l = height of the bracket base and 1 MPa = 1 N/mm^2.

After the detachment, each tooth surface was examined under a stereomicroscope at 10× magnification to assess the amount of adhesive remnant, using adhesive remnant index (ARI).[18]

The ARI index was ranked from 0 to 3 as follows:
0 = no adhesive on the enamel;
1 = less than 50% adhesive on the enamel;
2 = more than 50% adhesive on the enamel;
3 = 100% adhesive on the enamel.

Statistical analysis

Description and inferential statistical analyses were performed using MedCalc statistical software (MedCalc Software, Ostend, Belgium).[19] Differences among groups were evaluated using the analysis of variance (ANOVA, $p < 0.001$) and between the effects of all the preventive agents used on SBS (Student–Newman–Keuls test, $p < 0.05$). The chi-square test was used to examine whether there were differences among the groups in the ARI. p Value was considered statistically significant if $p < 0.05$.

Results

One-way ANOVA showed that SBS of different groups were significantly different and was impacted by different treatments used ($p < 0.001$). Descriptive statistics (including mean and standard deviation) and the differences between each group are presented in Table 1. The main differences were between the control group (17.38 ± 5.38) and the fluoride (6.62 ± 5.71), glycine (11.02 ± 4.74) and hydroxyapatite (10.86 ± 4.09) groups. There were no statistical differences

Table 1. Results from one-way ANOVA test ($p < 0.001$) and between the effects of all the preventive agents used on SBS (Student–Newman–Keuls test, $p < 0.05$).

Factor	*n*	Mean (MPa)	SD	Different from factor no.*
(1) Control	18	17.38	5.38	(2)(5)(6)
(2) Fluoride	18	6.62	5.71	(1)(3)(4)(5)(6)(7)
(3) CPP–ACP	18	14.87	4.07	(2)(5)(6)
(4) Ozone	18	17.10	5.61	(2)(5)(6)
(5) Glycine	18	11.02	4.74	(1)(2)(3)(4)(7)
(6) Hydroxyapatite	18	10.86	4.09	(1)(2)(3)(4)(7)
(7) Biorepair	18	16.01	4.11	(2)(5)(6)

Continuous variables are reported as means ± standard deviation.
*$p < 0.05$.

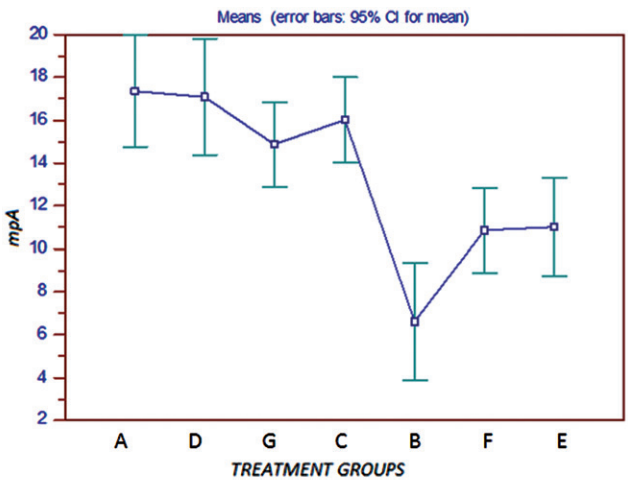

Figure 1. Average SBS of brackets compared by the different agents used. A–G = groups 1–7.

between the control group and other groups (CPP–ACP, ozone, BioRepair®). Fluoride group showed the lowest values and was statistically different from all the other groups (Figure 1).

Table 2 presents ARI index values. The chi-square test (*$p < 0.05$) indicated statistical differences in ARI scores between control group and fluoride (<0.0001), glycine (0.03) and hydroxyapatite (<0.03) according to the results of ANOVA on SBS values.

This was characterized by a shift from ARI scores of 0 and 1: groups with a higher SBS are set mainly in ARI 1 while those with lower SBS are set on score 0.

Table 2. Frequency of distribution of ARI score and chi-square comparison between each single group and the control one.

	ARI scores				
	0	1	2	3	*p* Values*
Control	1	11	4	2	
Fluoride varnish	16	2	0	0	<0.0001
CPP–ACP	4	12	2	0	0.2114
Ozone	4	12	2	0	0.2114
Glycine	12	4	2	0	0.0311
Hydroxyapatite	9	6	2	1	0.0311
Biorepair	4	12	1	1	0.2640

*$p<0.05$.

Discussion

The enamel demineralization adjacent to orthodontic brackets is still a severe problem. In order to reduce these adverse outcomes, different protocols with different agents have been proposed. Even though all these prophylactic techniques have been proved to act positively on the caries and WSL incidence, it is still not clear if their proprieties may play a positive, neutral or negative role on SBS of brackets.

In this *in vitro* study we have considered six different prophylactic agents. Fluorides are known as the most favored remineralizing agent. Topically applied sodium fluoride solution causes remineralization, mainly by reducing apatite dissolution by forming less soluble fluorapatite. This material acts on the tooth surface by delivering freely available ions which enter in the enamel and reform stronger crystals. Moreover, the remineralization of enamel using synthetic apatite or hydroxyapatite has been proved to be beneficial. HA crystals exhibit high levels of biomimetic properties due to their composition, structure, morphology, bulk and surface physical–chemical properties.

In previous studies, the effect of fluoride application before acid etching on SBS has been reported with different and controversial results. Generally, fluoride pre-treatment may induce lower SBS values [13, 21] or values similar to the control groups.[20,21] In our results the SBS of orthodontic bracket was significantly and highly decreased by the application of the fluoride varnish.

Considering hydroxyapatite, few studies have suggested that this material should be used as direct adjunct in the adhesive. With these materials the SBS values are even higher than the untreated control specimens, but the authors have found that – if the percentage of HA particles is higher (10%), values collapse.[22]

In the present study, the application of HA powder with air-flow resulted in lower SBS compared with the control group and similar values compared with the fluoride group. Therefore, we can assume that the treatment with fluoride and hydroxyapatite may increase the enamel resistance to acid, resulting in a lower bonding strength.

The glycine group showed low values of SBS too. This material can erase biofilms and remove dental plaque reducing negative effects of other powder, and it is used during routine visits and before orthodontic brackets bonding. It is a soluble material that does not has any direct action on the enamel, and therefore should not affect the SBS as it has previously been reported. We believe that some particles of the powder may have been left on the enamel surface, thus altering the SBS. Thus, it is important to wash carefully the widely spaced teeth after the use of this kind of powder.[14]

On the other hand, the SBS was not affected significantly by the use of the other three techniques: CPPACP, ozone and BioRepair® Previously studies have reported that, the use of CPP–ACP may reduce the SBS values.[15,23]

According with other recent studies, we have not found such a difference between the CPP–ACP group and the control one: there is no statistical difference even if the treated tooth has a lower mean of SBS. We may speculate that with more applications of CPP–ACP and so with a higher availability of remineralizing ions fulfilling the enamel surface, the SBS would be lower.[24,25]

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Ozone is a different kind of agent that does not act on the tooth structure, thus it does not affect the mineral properties. Therefore, we know that it has a strong oxidizing effect that might have negative consequences on adhesion, since oxygen is a well-known polymerization inhibitor.

In agreement with other studies, we have not noticed any differences after the use of this technique on SBS.[16,26]

This could be explained considering that we used an etch-rinse technique that can promote the removal of both superficial mineralized components and residual oxidants. Further studies should be done in order to compare a group treated with self-etch adhesive systems (no rinsing): residual oxygen may be incorporated within the smear layer, making this adhesion more susceptible to oxygen.

The sixth agent used is a commercial toothpaste: BioRepair® Plus (Coswell S.p.A., Bologna, Italy): a fluoride-free toothpaste made of hydroxyapatite nanocrystals.[27,28]

The use of the toothpaste seems to be a safe prophylactic agent in terms of bracket bond strength. However, we suppose that protocol should be modified and improved for testing the SBS after more applications.

The ARI results indicate that more than 85% samples were included between scores 0 and 1. The most desired clinical condition is a low ARI score with less composite remaining on the tooth surface in order to reduce enamel damage during debonding procedures. Considering our results, we can confirm that all the test groups showed a good ARI index (<1) and the distribution of the values was in accordance with the SBS group values: the group with higher SBS manifested a greater part of composite remnants left on the enamel tooth surface.

A limit of this study, owing to the difficulties in obtaining human incisors, should be considered the use of bovine tooth. However, bovine enamel has already been used in several other studies as a substitute model without statistically significant differences in SBS comparing bovine and human enamel [29–31].

The results should be carefully compared with other findings due to the differences in methodology, such as tooth selection, different types of preventive agents, application time, type/concentration of material, etching and bonding system.

The advantage of this research is that, most of the commonly used prophylactic techniques and even a new material (BioRepair® toothpaste) have been considered and therefore can be compared. Considering the limitation of this *in vitro* study, these results indicated that SBS is negatively affected when fluoride varnish and hydrossyapatite are used before acid etching and bonding, but no relevant differences have been noticed in tooth treated with BioRepair®, ozone and CPP–ACP. SBS values ranged from 1 to 26 MPa: only the group treated with fluoride agent has several values lower than 6 MPa, that is the minimal one recommended as adequate for orthodontic purposes.[32,33]

Conclusions

- CPP–ACP, ozone and BioRepair® application did not affect significantly the SBS. Further studies should be done with more than just one single application of these preventive agents.
- Fluoride and hydroxyapatite reduced SBS values when used just before the bonding of the bracket.
- However, based on these results, we conclude fluoride varnish is the only agent that caused a significant negative effect of SBS value lower than 6 MPa and cannot be recommended.
- Human clinical trials should be done in order to confirm these results.

Declaration of interest

Authors have no relevant financial or non-financial relationship to disclose. All authors have made substantive contribution to this manuscript, and all have reviewed the final paper prior to its submission.

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