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Original Research Article

Comparison of shear bond strength as affected by ozonated water, fluoride varnish & casein phosphopeptide – Amorphous calcium phosphate

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ABSTRACT

Background: A translatory research to validate antimicrobial and re-mineralization agents to reduce white spot lesions which would not unfavorably affect shear bond strength and focus on ozonated water being a valid and relatively inexpensive method for orthodontic patients to follow as a home follow up care.
Materials and Methods: Tooth surfaces of 60 Extracted Human Premolar Teeth were cleaned and divided into four groups of 20 each and then treated with fluoride varnish, CPP-ACP, Ozonated water and distilled water before bonding brackets and later subjected to SBS testing.
Result: SBS of teeth treated with Ozone water and CPP-ACP were close to ideal SBS.
Conclusion: Ozone water is a valid and relatively inexpensive method for orthodontic patients to follow as a home follow up care.

1. Introduction

After Buonocore[1] introduced bonding, the old technique of banding on individual teeth was replaced by bonding. Maintaining good oral hygiene is important for everyone and especially during orthodontic treatment as the brackets and other orthodontic auxiliaries act as deposition area for food particles resulting in plaque formation. Plaque or bio-film is a thin coating of microorganisms with high levels of acidogenic bacteria notably Streptococcus mutans and Lactobacilli along with organic debris. They lower the oral pH and often initiates demineralization and formation of caries. Brackets, archwires, ligatures and other orthodontic appliances complicate the use of conventional oral-hygiene measures resulting in drop in pH in orthodontic patients resulting in white spot lesions, frank caries, halitosis and periodontal disease. Demineralization of enamel around brackets can be an extremely rapid process. Orthodontic patients need constant motivation and counseling to maintain oral hygiene as this may slack due to poor compliance or sheer boredom.

Re-mineralizing agents can be prescribed for daily use to decrease the incidence of decay. Fluoride and its anti-cariogenic and re-mineralization properties have been well documented. Milk protein derivative, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) complex, has been advocated for caries prevention and enamel re-mineralization. Ozone is an important disinfecting agent due to its antibacterial action. Though ozone is often placed on a caution list but it is documented in literature that controlled ozone application has been found to be extremely safe and free from side effects. The ozone molecule is a powerful agent that can be used on microorganisms. It is lethal to bacteria, viruses, and fungi, yet the more highly evolved human cells are not damaged by ozone in lower concentrations. Ozone therapy is currently being widely practiced in the field of dentistry to treat periodontal and endodontic problems.

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2. Materials and Methods

This is a prospective, in-vitro study to assess the effect on Shear Bond Strength after treatment of tooth surface with various products to control plaque and possible white spot lesions. Materials used were, Sample-60 Extracted Human Premolar Teeth ,Storage -Distilled water,Brackets-Metal MBT Bracket (AO Master Series 0.022* 0.028), Bonding-37% Orthophosphoric Acid (3M ESPE), Bonding Agent (Ortho Solo), Composite (Ormco Enlight),Test Variables-Ozonated Water, Tooth Mousse (GC Tooth Mousse Plus)-CPP-ACP, Fluoride Varnish (Ivoclar Fluor Protector N Varnish 7700ppm), Lab Equipments- Universal Testing Machine (Instron Universal Testing *Machine).*

60 Human Maxillary and Mandibular premolars were selected for this study. The extracted teeth were washed under running water, followed by debridement with an ultrasonic scaler and stored in distilled water for ten days. The teeth are cleaned by scaling and polished with pumice using rubber cups at low speed and then rinsed under tap water for 15mins and stored in distilled water before test process being initiated. The teeth were randomly allocated to the 4 study groups. They were mounted in acrylic cube block of self-cure methyl methacrylate with dimensions of 1.8mm×2.1mm×2.6mm. Color coded in four different colors according to group they were allocated to. The teeth were mounted with its long axis perpendicular to the base of the block. They were stored in distilled water for a period of 10 days. Portable Ozone Generating Machine was used to create ozonized water for this study. The machine generates 400mg/hr of ozone was made to work for 30 mins in a beaker containing 500ml distilled water. Since the ozone in the water will divert back to oxygen in 20 minutes, the prepared water was used within 15 mins of preparation.

Test for treatment were - CONTROL: BLACK (DW): teeth were cleaned and left untreated, OZONATED WATER: PINK (OW) : teeth were rinsed with ozonated water for or 10 secs twice a day for 30 days, FLOURIDE VARNISH: RED (FV): teeth were dried thoroughly and a thin layer of varnish is applied using an applicator as per the manufacturer's instructions and allowed to dry for 1 minute. The Varnish was brushed off after an hour, TOOTH MOUSSE: WHITE (TM): teeth were treated with CPP-ACP as per the manufacturer's instructions and later rinsed off after 5mins performed twice daily for 30 days.The teeth were etched with 37% orthophosphoric acid for 30 secs and rinsed thoroughly. A thin coat of primer – a bond enhancer Orthosolo (by Ormco) was applied with an applicator.A thin layer of adhesive (Enlight by Ormco) was applied on the bracket base and placed on the center of facial surface of teeth with the help of bracket holding tweezer. Flash is removed after gently pressing with uniform pressure the bracket and then cured using a LED Light. One single operator did all the bonding. An Instron Universal Testing Machine with a load range between 0 to 100 KN with a max cross head speed of: 1mm/min was used to measure the shear bond strength. The Universal Testing Machine consists of two cross heads, upper and lower. The upper crosshead is movable, while the lower crosshead is stationary. The crossheads of the Instron are attached on a hydraulic framework connected to a force recording unit. The Acrylic blocks were positioned in the lower crosshead with the crown portion of teeth facing upwards, with the long axis parallel to the direction of the load application. A stainless steel wire loop (0.5 mm diameter) was fixed to the upper cross head and adjusted to engage the bracket at the other end. The crosshead of the Instron moved at a uniform speed of 1mm/minute. The load was progressively increased till bracket debonded from the tooth surface. Progressive debonding force was applied to the bracket. The force required to debond the bracket from the enamel surface was recorded. The debonding force was measured in terms of Newtons and was seen in the reading device attached to the Instron machine. The bond strength values obtained in terms of Newton's were converted into Megapascals using surface area of the bracket. The surface area of bracket base was determined by measuring the height and widths of the base. Newton was converted to Megapascals by the formula, Stress = Load/Area where 'load' is the newton value obtained from Instron machine and 'area' is the surface area of the bracket. This was repeated for all the samples in the same order as they were bonded.

The groups were tested individually and the debonded brackets retrieved for ARI testing. The brackets were separated and kept in respective colour coded packets. The teeth and brackets were examined and classified according to the Adhesive Remnant Index (ARI) put forth by Artun and Bergland. The enamel surface was observed under a stereomicroscope with a magnification of 40 X to assess the quantum of residual adhesive on the debonded tooth surface.

ARI scores were recorded, and the results are tabulated.

3. Result

One-way ANOVA showed that SBS of different groups were significantly different and was impacted by different treatments used ($p<0.001$), presented inTable 3 .

Descriptive statistics (mean & standard deviation, 95% confidence interval) :Table 2

The mean differences between

DW and OW (17.00),

DW and TM (18.133),

DW and FV (29.467),

FV and OW (-12.467),

FV and TM (-11.333) statistically significant ($p<0.05$)

TM and OW (-1.133) statistically not significant, ($p>0.05$): Table 4.

Table 1: ARI Scale

Score	Description
0	No adhesive left on the tooth.
1	Less than half of the adhesive left on the tooth.
2	More than half of the adhesive left on the tooth.
3	All adhesive left on the tooth, with distinct impression of the bracket mesh.

The means ± s.d. of SBS in FV (33.13 ±13.876) was statistically significantly lowest than other groups, in DW (62.60 ±12.816) statistically significant highest then other groups while OW (45.60 ±14.040) and TM (44.47 ±13.010) have no statistical significant difference to each other.

The chi-square test (*$p < 0.05$) indicated statistical differences in ARI scores between DW with other ($p<0.01$) was statistically significant.Table 5

4. Discussion

Controlling dental plaque before and during fixed orthodontic treatment, without compromising the SBS of brackets, has always been an area of research in orthodontics. Nagayoshi et al[2] proved that ozonated water is a very potent antibacterial agent against mutans streptococci and other microorganisms. This in vitro study was conducted to find out a home care cost effective way to probably reduce WSL in orthodontic patient.

Individuals receiving orthodontic treatment may be prescribed various forms of fluoride treatment. Topically applied sodium fluoride solution causes re-mineralization, mainly by reducing apatite dissolution by forming less soluble fluorapatite. The effects of fluoride on the prevention of tooth decay and re-mineralization of decalcified enamel have been elaborately described and documented by Bradshaw et al[3] and Jeevarathan et al.[4]

A milk protein derivate, casein-phosphopeptide amorphous calcium-phosphate (CPP–ACP), has been recommended for caries prevention and enamel re-mineralization. Reynolds et al.[5,6] Pliska et al[7] and Nongonierma et al[8] also proved it to be effective on reducing WSL. When topical fluoride is used, the availability of calcium and phosphate ions is a limiting factor for re-mineralization. A calcium-phosphate transporting system along with fluoride treatment can treat early tooth decay. CPP-ACP attaches to soft tissue plaque pellicle and hydroxyapatite, transfers calcium and amorphous phosphate to saliva and plaque liquid and acts as a resource for calcium phosphate ions. Calcium phosphate amorphous is biologically active and can release calcium phosphate ions to maintain super saturation level. Therefore, it reduces demineralization and increases re-mineralization. Nongonierma et al[8] explained the bio-functional properties of CPP-ACP and demonstrated that 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.

Ozone was discovered by CF Schonbein in 1839. It was first suggested as a disinfectant for drinking water in view of its powerful ability to inactivate microorganisms (against bacteria, fungi, protozoa, and viruses). The aqueous form is easy to handle and is a potent microbicidal solution. Nagayoshi et al[2] concluded in his study that ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl, when used as an irrigant especially when combined with sonication, and showed a low level of toxicity against cultured cells. Ozone is a different kind of agent that does not act on the tooth structure, thus it does not affect the mineral properties but its versatility and amplitude of beneficial effect of local applications have become evident in orthopedics, cutaneous, and mucosal infections as well as in dentistry and is well documented by Bocci et al[9] Kshitish et al[10] and Talukdar et al.[11] Nagayoshi et al.[2,12] tested ozonated water in different concentrations and different time on various species of oral microorganisms and found 4mg/L concentration for 10 seconds was enough to kill 99.9% of bacteria including S.mutans. In his study he found that ozonated water reduced the viability of oral microorganisms including Gram-positive oral microorganisms, Gram-negative oral microorganisms, and Candida albicans, suggesting that ozonated water might be useful to control oral infectious microorganisms.

The harmful effects of Ozone on humans as gas and water form has been researched in abundance by Bocci et al,[13] Cross et al[14] and Uppu et al[15] and found that the gaseous form is harmful only in large quantities. Bocci[16] checked to ascertain if ozone is always toxic and concluded that depending upon its concentration and cumulative dose, ozone can be either therapeutic or toxic. Much of the ozone toxicity is neutralized by the powerful antioxidant system of blood as studied by Bocci et al.[9] Shinriki et al[17] described how blood plasma and cells are endowed with a powerful antioxidant system so that a fairly wide range of ozone concentrations between 40 and 80 micro g/ml per gram of blood (approximately 0.83-1.66 mM) are effective but not deleterious.

A standardized bonding protocol was followed in a dry field, and all testing of bond strengths was carried 24 hours after storage in distilled water at 37°C to permit attainment of maximum bond strength in a simulated oral environment without risk of desiccation as discussed by

Table 2: Distribution Means ± S.d. of SBS (N) in four groups

		Mean ±Std. Deviation Newton (N)	Mean ±Std. Mega Pascal (MPa)	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
DW	15	62.60 ±12.816	10.43±2.13	3.309	55.50	69.70	43	82
OW	15	45.60 ±14.040	7.6±2.33	3.625	37.83	53.37	28	75
FV	15	33.13 ±13.876	5.52±2.31	3.583	25.45	40.82	20	73
TM	15	44.47 ±13.010	7.41±2.16	3.359	37.26	51.67	19	64

Table 3: Comparison of means of SBS in four groups by one-way ANOVA

	ANOVA				
	Sum of Squares (N)	Df	Mean Square (N)	F	p value
Between Groups	6642.183	3	2214.061	12.246	.000**
Within Groups	10124.667	56	180.798		
Total	16766.850	59			

*Significant p<0.05, ** highly significant p<0.01

Table 4: Student– Newman–Keuls test (Comparison between the effects of all the variables used on SBS)

Group	Group	Mean Difference (N)	p value
TM	OW	-1.133	>0.05
DW	OW	17.000	<0.05*
	TM	18.133	<0.05*
	FV	29.467	<0.05*
FV	OW	-12.467	<0.05*
	TM	-11.333	<0.05*

Table 5: Frequency of distribution of ARI score and chi-square comparison between each single group and the control one

	ARI Scores			Chi-Square (d.f)	p value
	0	1	2		
DW	1	12	2	14.80 (2)	0.001**
OW	1	13	1	19.20 (2)	0.000**
FV	13	2		8.067 (1)	0.005**
TM	3	12		5.40 (1)	0.020*

*Significant p<0.05, ** highly significant p<0.01(Chi-Square, d.f for Black colour with other 33.949, 6 p<0.01)

Titley et al,[18] and Dunn.[19] The variables tested in the research consisted of a control group left untreated and stored in distilled water against a group treated with a standard Fluoride varnish (Ivoclar Flor Protector) applied as per the standard instructions of the product. The other test group consisted of the common prescription of CPP-ACP (Tooth Mousse) advocated by clinicians to patients to combat WSL during and after orthodontic treatment. The other important variable tested was ozonated water. An important objective was to ascertain if any difference existed between CCP-ACP and ozonated water in SBS. This would provide translatory value in clinical practice as India is a developing nation with many orthodontic patients barely able to meet the financial demands of orthodontic treatment. CCP-ACP when prescribed to a patient entails an additional financial burden every other month for a period of six months to one year, while an ozone generator would be a small onetime investments permitting multiple uses for multiple users without excessive maintenance or recurring costs. Ozonated water was prepared in the current research study in a cost effective way by using an ozone generating machine (400mg/Hr) commonly used for household disinfecting processes. It was installed according to the manufacturer instruction and the bubbling stone of the device was immersed into the bottom of 500 ml beaker filled with cooled distilled water for 30 minutes similar to previous study by Anumula et al[20] which was proved effective against streptococcus mutans.

The results of the current research showed that the control group (where no treatment was given and the teeth were stored in distilled water) had highest SBS followed by Ozonated water group and very closely by the group treated with CCP-ACP. The group treated with Fluoride varnish exhibited the lowest.

The results were encouraging as the SBS values of teeth treated with Ozonated water and Tooth Mousse were not statistically significantly different from each other and in good standing when compared to the ideal bond strength range of 5.9–7.8 MPa given and benchmarked in his study by Reynolds.[21] This definitely indicates that substituting tooth mousse with ozonated water mouth wash is a viable economical option for orthodontic patients without compromising on bond strength. Hence, an important objective of this research has been served.

Wang et al[22] showed that fluoride treated teeth cause detachment after bonding. Similarly in the current research, the results indicated that the SBS was statistically significantly decreased by the application of the fluoride varnish in the FV group when compared to control DW, the TM and OW groups. The benchmarked minimal value recommended as optimum for orthodontic purposes is 6 MPa according to Reynolds[21] and Whitlock et al[23] and the value in the current research was found to be lower than the minimal acceptable.

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. The worst score to be considered is an ARI of 3 .Considering the results of the current research it can be concluded 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. This should go to prove that that the bond between bracket and resin was stronger than that between the resin and enamel. However Hildebrand et al[24] demonstrated, ARI scores do not always correlate with SBS values, and in vitro studies do not represent clinical situations precisely.

5. Conclusion

As the present research is an in-vitro study there is a strong possibility of it not simulating the oral conditions completely. The test teeth were devoid of the actual oral conditions of plaque/food debris/ saliva etc. hence is may be considered inadequate to simulate in vivo conditions. However, this research has been able to meet an objective of providing validation on ozonated water versus CCP-ACP as an effective economic home care hygiene follow up. Larger samples size studies with inclusion of various dimensions could be planned for the future.

6. Source of Funding

None.

7. Conflict of Interest

None.

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