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Evaluation of the effect of ozone water on the shear bond strength between resin cement and dentin: an in-vitro study

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Abstract:
Background:*Biodegradation of bonded interface between resin cements and dentin is known to be the chief cause for bonding failure. Degradation of collagen is initiated by bacterial activity which in turn stimulates MMP production. Previous studies reported that ozone efficiently counteracts micro-organisms found in the dentin. Thus, in this study the effect of ozone water on the shear bond strength between resin cement and dentin was evaluated.*
Aim: *To evaluate the effect of the treatment of dentin slabs with ozonized water on the shear bond strength between resin-based cements and dentin*
Materials and methods: *Freshly extracted human molar teeth were collected. Flat dentin slabs of 3 x2 x 1 mm size were prepared and mounted on acrylic resin slabs. Mounted samples were etched with 37% phosphoric acid for 20s. These samples were divided into two groups. Samples under group 1 were treated with distilled water for 120s. Samples under group 2 were treated with ozonized water for 120s. All the samples were allowed to dry and resin-modified GIC of thickness 5mm was applied over it. After 24 hours, shear bond strength was evaluated using the Universal testing machine.*
Statistical analysis: *The results were statistically analyzed using the t-test and Mann -Whitney test.*
Results: *Samples treated with ozonized water showed higher shear bond strength than samples treated with distilled water.*
Conclusion: *Within the limitations of this study, it can be concluded that shear bond strength of resin-modified GIC to dentin surface is better with ozone water treatment than distilled water.*
Keywords: *Ozonized water, adhesion, anti-microbial agent*

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I. Introduction

Despite improved understanding of the adhesion science involved in dentin bonding over the past two decades, the average replacement time of a tooth-colored resin restoration is only 5.7 years[1],[2] Biodegradation of the bonded interface is still the major factor responsible for the replacement of most bonded restorations[3] The durability of bonds between cement and dentin requires stable collagen fibrils at the interface. Degradation of collagen fibrils can cause result in the failure of bonds between dentin and the cement. Proteolytic enzymes responsible for this degradation may be derived from oral bacteria, yeast or host. Bacterial products such as acids and proteinases are involved in matrix degradation by host-derived proteinases. Categories of host-derived proteolytic enzymes are matrix metalloproteinases(MMPs). Members of the family of proteinases are well known for their capacity to degrade native and denatured collagen type 1. It has been shown that MMP-1, MMP-2, MMP-8 and MMP-9 are present in dental plaque, in gingival crevicular fluid (GCF) and saliva. A significant correlation was found between total collagenase activity in whole saliva and that in crevicular fluid. The majority of the MMPs in saliva originate from the GCF, but a fraction of these MMPs may be synthesized in the parotid gland and the submandibular gland. Recently, MMP-2, MMP-8, MMP-9 and MMP-20 have been identified in soft dentinal lesions. They showed that acid-activated MMPs degrade the dentin organic matrix in vitro[4] Inhibition of MMPs reduced the gelatinolytic activity of human saliva and retarded caries progression in rats[5] Effective antimicrobial agents are required to overcome this problem. Ozonized water is known as a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses.[6] The advantages of ozone in the

liquid phase are its efficacy, ease of usage, lack of health hazards, rapid detrimental effects against microbes, and aptness for use as a soaking solution for dental and medical instruments and equipments.[7] Recently, we found that it reduced the viability of oral microorganisms including Gram-positive oral microorganisms, Gram-negative oral microorganisms, and Candida albicans, suggesting that ozonized water might be useful to control oral infectious microorganisms.[8]In the previous studies antimicrobial effect of ozonized water on the bacteria invading dentinal tubules was described. However, no information exists regarding the antimicrobial effect of ozone water in improving the cement-dentin bond strength at the interface. Hence, this in-vitro study aimed to evaluate the effect of the treatment of dentin slabs with ozonized water on the shear bond strength between resin-based cements and dentin.

II. Material and Methods

A total of 10 recently extracted caries-free maxillary first molars of approximately the same size and anatomy were selected. Patients' informed consent was obtained following which teeth were collected and preserved.

Preparation of dentin slabs:

The teeth were then mounted in quadrangular molds with an auto polymerizing acrylic resin (DPI-RR COLD CURE DENTURE REPAIR MATERIAL). The enamel of a tooth was removed with a high-speed air turbine handpiece (NSK-PANA AIR) with 5,30,000 RPM used at a drive air pressure of 0.215MPa using diamond burs (API SERIES, FLAT CONE SHAPED-TR-62C,10 Mohs hardness) under cooling water to expose the dentin surface. The occlusal one-third of the crown was cut with a slow speed lab micro motor unit (MARATHON M4 LAB) under cooling water. To prepare the dentin slabs into 1-mm thick pieces, cuts were made perpendicular to the long axis of the tooth. Finally, three dentin slabs were obtained from each tooth.

Preparation of groups:

The prepared slabs were mounted on auto polymerizing acrylic resin block (DPI-RR COLD CURE DENTURE REPAIR MATERIAL). All the samples were etched with 35% phosphoric acid (AQUA ETCH) for 20s followed by rinsing with water for 20S. The samples were allowed to dry. The samples were divided into two groups. Samples under group 1were treated with distilled water for 120s. Samples under group 2 were treated with ozonized water (Five mL of distilled water was sparged with ozone gas from an ozone-generating device Eltech-DOZ200, Eltech Engineers, Mumbai, India with a range of 300s) for 120s. Samples were allowed to dry and resin-modified GIC (FUJI PLUS) of thickness 5mm was applied over it.

Shear bond strength testing:

The shear bond strength was evaluated with a universal testing machine (INSTRON 5960) with a 0.5mm crosshead blade loaded at a crosshead speed of 0.5mm/min. The load necessary to break the bond between specimen and crown was measured and represented in MPa and the descriptive statistics were determined.

Statistical analysis:

Data obtained were tabulated in an excel sheet. It was then analyzed using SPSS software (version 17). Data were assessed for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test. Independent sample t-test was performed to evaluate the statistical difference between groups.

III. Result

Data assessed for normality and homogeneity of variance using the Shapiro -Wilk test and Levene's test were found to be normally distributed. Independent sample t-test revealed that group 1 and group 2 were extremely significant from each other($p<0.001$) in all the parameters.

	Group	N	Mean	Std.Deviation	Std.Error Mean
Time (Sec)	Group 1 Group 2	2678 2333	27.55 24.30	16.896 15.405	.326 .319
Extension(mm)	Group 1 Group 2	2678 2333	-.4584560 -.4048658	.28232525 .25906190	.00545562 .00536347
Load (N)	Group 1 Group 2	2678 2333	-19.8334 -33.6887	11.96073123 17.95095553	.23112794 .37164663

[Table.1]:*t-TEST- Group statistic*

		Levene's Test For Equality Of Variances not assumed		t-test for equality of means						
		F	Sig.	T	df	Sig(2-tailed)	Mean Difference	StdErrorDifference	95%Confidence interval of the difference	
									Lower	Upper
Time(sec)	Equal variances assumed	37.485	.000	-7.090	5009	.000	-3.256	.459	-4.157	-2.356
	Equal variances not assumed			-7.135	4998.613	.000	-3.256	.456	-4.157	-2.362
Extension(mm)	Equal variances assumed	33.812	.000	6.964	5009	.000	0.5359015	0.00769586	.03850289	.06867741
	Equal variances not assumed			7.005	4995.510	.000	0.5359015	0.00765054	.03859174	
Load (N)	Equal variances assumed	421.521	.000	-32.509	5009	.000	-13.85529	.42619589859	-14.6908	-13.0198
	Equal variances not assumed			-31.658	3967.676	.000	-13.85529	.43765436	-14.7133	-12.9972

[Table.2] : *Independent sample test*

	Group	N	Mean Rank	Sum Of Ranks
Time (sec)	Group 1 Group 2 Total	2678 2333 5011	2631.73 2361.68	7047765.00 5509801.00
Extension (mm)	Group 1 Group 2 Total	2678 2333 5011	2381.44 2648.98	6377494.00 6180072.00
Load (N)	Group 1 Group 2 Total	2678 2333 5011	3045.77 1886.41	8156568.00 4400998.00

[Table.3] :*NPar Tests*
Mann-Whitney test

	Time (sec)	Extension (mm)	Load (N)
Mann-Whitney U	2787190.0	2790313.0	1678387
Wilcoxon W	5509801.0	6377494.0	4400998
Z	-6.591	-6.530	-28.297
Asymp.sig (2 –tailed)	.000	.000	.000

[Table.4] : *Test statistics* [a]
a – Grouping variable: Group

IV. Discussion

The significant loss of collagen from the dentin specimens after the in situ periodfollowstheprevious work[9]Potential sources of proteolytic enzymes responsible for this degradation are the oral microflora, host cells like polymorphonuclear granulocytes, macrophages, and fibroblasts or endogenous proteinases present in

dentin. Oral bacteria may release proteolytic enzymes that degrade the collagenous dentine matrix. Microorganisms could contribute to collagen degradation by pathogens such as toxins, acids and enzymes.[10],[11] These bacterial products could be responsible for the upregulation of interleukin 1. Interleukin 1 present in GCF and saliva is responsible for the stimulation of polymorphonuclear leukocytes and macrophages to release MMPs.[12]It has been reported that ozone, in the gaseous or liquid phase, has a strong ability to oxidize with a reliable detrimental effect against microbes.[7],[13],[14] and it is generally noted that oxidation due to ozone damages the cell walls and cytoplasmic membranes of fungi and bacteria.[15] After the membrane is damaged by oxidation, its permeability increases and ozone molecules can readily enter the cellscausing the microorganism to die. The results of the present study showed that dentin surfaces treated with ozonized water beforetheapplication of resin-modified GIC showed better bond strength than those which were not treated with it.

V. Conclusion

Within the limits of the study it may be concluded that treatment of dentin surface with ozonized water before application of resin-modified GIC showed improved shear strength.

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