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THE ROLE OF COPPER NANOPARTICLES ON ANTI-MICROBIAL ACTIVITY OF TOTAL ETCH ADHESIVE AND SELF ETCH ADHESIVE SYSTEMS

[1]*Dr. Akshita Balivada, [2]Dr. Maitreyi J. Desai, [3]Dr. R. Vinay Chandra and [4]Dr. K. J. Nanda Kishore

[1,2]Post Graduate Student, [3]Professor and Head, [4]Professor, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College and Hospital, Bangalore.

***Corresponding Author: Dr. Akshita Balivada**

Post Graduate Student, Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College and Hospital, Bangalore.

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ABSTRACT

Several investigations have been conducted which revealed microgaps at the interface between dentin and adhesive restorations in vivo. This implies that even the latest adhesive systems are not capable of producing a complete seal in clinical situations. Once invaded ,bacteria proliferate at the bonding interface, permeate the unsealed dentinal tubules, and may cause pulpal responses. Therefore, the possibility of adhesive systems having antibacterial effects is attractive with respect to the prevention of infections. With this in mind, the objective of this study is to evaluate and compare the effect of addition of copper nanoparticles on anti-microbial activity of total etch adhesive and self etch adhesive systems. **Methodology:** Two systems were used. 3M ESPE Adper Single Bond 2 and Neofill Self Etch 7th Generation Bonding agent. Experimental adhesives were formulated by incorporating copper nanoparticles into the adhesives. Four Groups were made: G1: Total etch adhesive + 0 wt.% copper nanoparticles (Negative Control), G2: Total etch adhesive + 1 wt.% copper nanoparticles, G3: Self etch adhesive + 0 wt. % copper nanoparticles (Negative Control), G4: Self etch adhesive + 1 wt. % copper nanoparticles .The antimicrobial activity of adhesives against Streptococcus after 12 hours and 24 hours was measured and compared. **Results**: There is a statistically significant difference on antimicrobial activity once the copper nano particles are incorporated, but no statistically significant difference between antimicrobial activity of G2 and G4.There is a significant difference in the antimicrobial activity between 12 and 24 hours.

KEYWORDS: Adhesive system; copper nanoparticles; antimicrobial activity.

INTRODUCTION

In the oral environment, water, enzymes, occlusal stresses, temperature and microorganisms derived from plaque biofilms challenge the resin-dentine interface.

Streptococcus and other bacterial species can then bond to this suitable biofilm and, in the presence of gaps and weakened interface, may invade the inner area of teeth and allow for the development of caries adjacent to tooth-restorative margins.[18,19]

Although biofilm cannot be eliminated, one can reduce the pathogenicity of biofilm and make the restorative interface less prone to caries adjacent to restorations. This can be achieved by developing dental materials with antibacterial properties to reduce biofilm formation at the tooth-restoration margins.[8]

The antibacterial activity of copper and silver nanoparticles has been extensively investigated. Copper nanoparticles showed higher antibacterial activity compared with silver nanoparticles against Escherichia coli, Bacillus subtilis and Staphylococcus aureus.[8]

AIM

- To evaluate and compare the effect of addition of copper nanoparticles on anti-microbial activity of total etch adhesive and self etch adhesive systems.

METHODOLOGY

The type of adhesive system used for the experiments were.
Total Etch 5th generation bonding agent (3M ESPE Adper Single Bond 2) and Self Etch 7th Generation Bonding Agent (Neofill Self Etch 7th Generation Bonding agent)

Four groups were made as follows.
G1: Total etch adhesive + 0 wt.% copper nanoparticles (Negative Control)
G2: Total etch adhesive + 1 wt.% copper nanoparticles
G3: Self etch adhesive + 0 wt. % copper nanoparticles (Negative Control)
G4: Self etch adhesive + 1 wt. % copper nanoparticles

- Adhesives for the experimental groups were formulated by incorporating copper nanoparticles into the adhesives (20 nm size with 99.9% purity).
  Lawn Culture of Streptococcus species were done on chocolate agar plates.
- Two filters papers were placed to help test the samples.
- Triplicate values of the zone of inhibition was obtained for each group at 12 and 24 hrs, which was further subjected to statistical analysis and tabulation of results.
- The antimicrobial activity of adhesives against Streptococcus was measured in terms of inhibition zones[5] and were compared at 12 hours and 24 hours time points.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics

Descriptive analysis includes expression of Zone of Inhibition of Streptococcus in terms of Mean & SD for each group.

Inferential Statistics

One-way ANOVA Test followed by Tukey's post hoc test was used to compare the mean Zone of Inhibition (in mm) against Streptococcus between 4 groups at 12 and 24 hrs time intervals.

Student Paired t Test was used to compare the mean Zone of Inhibition (in mm) against Streptococcus between 12 and 24 hrs time intervals in each group.

The level of significance was set at $P<0.05$.

RESULTS

INHIBITION ZONES FORMED AFTER 12 HOURS

FIGURE 3a
G1: Total etch adhesive + 0 wt. % copper nanoparticles (Negative Control).

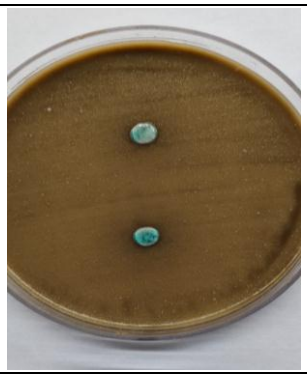


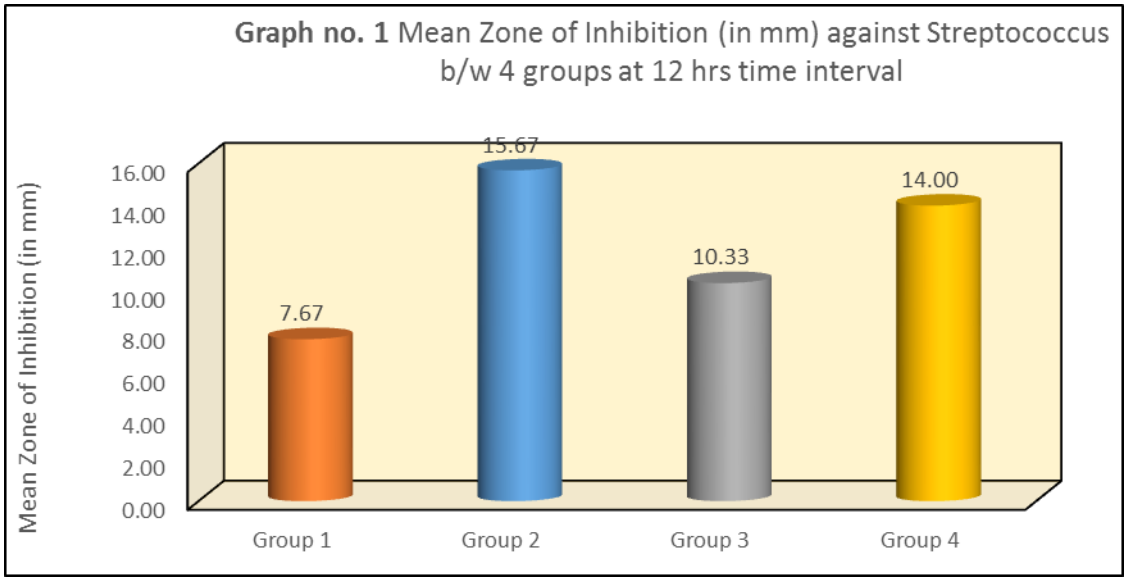
FIGURE 3b
G2: Total etch adhesive + 1 wt. % copper nanoparticles.

FIGURE 3c
G3: Self etch adhesive + 0 wt. % copper nanoparticles (Negative Control).

FIGURE 3d
G4: Self etch adhesive + 1 wt. % copper nanoparticles.

Table -1: Comparison of mean Zone of Inhibition (in mm) against Streptococcus b/w 4 groups at 12 hrs time interval using One-way ANOVA Test.

Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	7.67	0.58	7	8	<0.001*
Group 2	3	15.67	0.58	15	16	
Group 3	3	10.33	1.53	9	12	
Group 4	3	14.00	1.00	13	15	



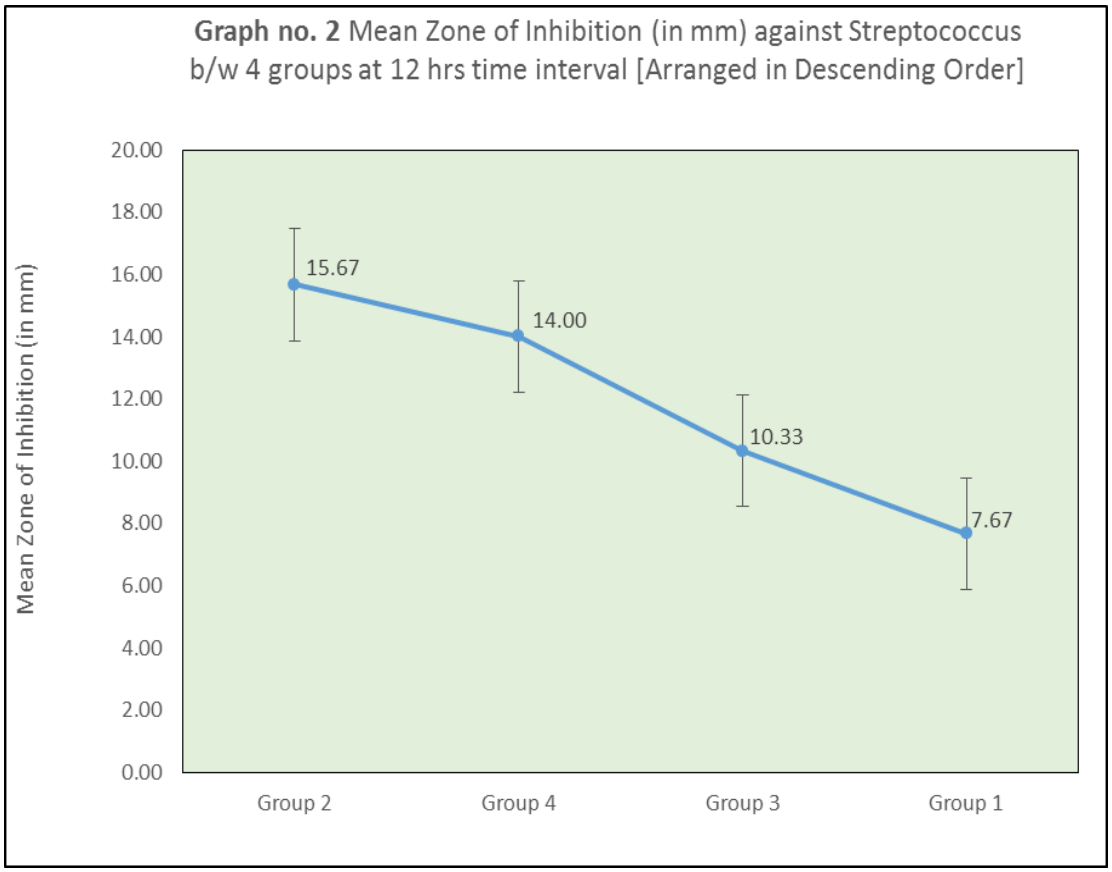


Table -2- Multiple comparison of mean diff. in mean ZOI against Streptococcus b/w 4 groups at 12 hrs time interval using Tukey's Post hoc Test.

(I) Groups	(J) Groups	Mean Diff.(I-J)	95% CI for the Diff		p-value
			Lower	Upper	
Group 1	Group 2	-8.00	-10.61	-5.39	<0.001*
	Group 3	-2.67	-5.28	-0.05	0.04*
	Group 4	-6.33	-8.95	-3.72	<0.001*
Group 2	Group 3	5.33	2.72	7.95	0.001*
	Group 4	1.67	-0.95	4.28	0.25
Group 3	Group 4	-3.67	-6.28	-1.05	0.009*

Table -3: Comparison of mean Zone of Inhibition (in mm) against Streptococcus b/w 4 groups at 24 hrs time interval using One-way ANOVA Test.

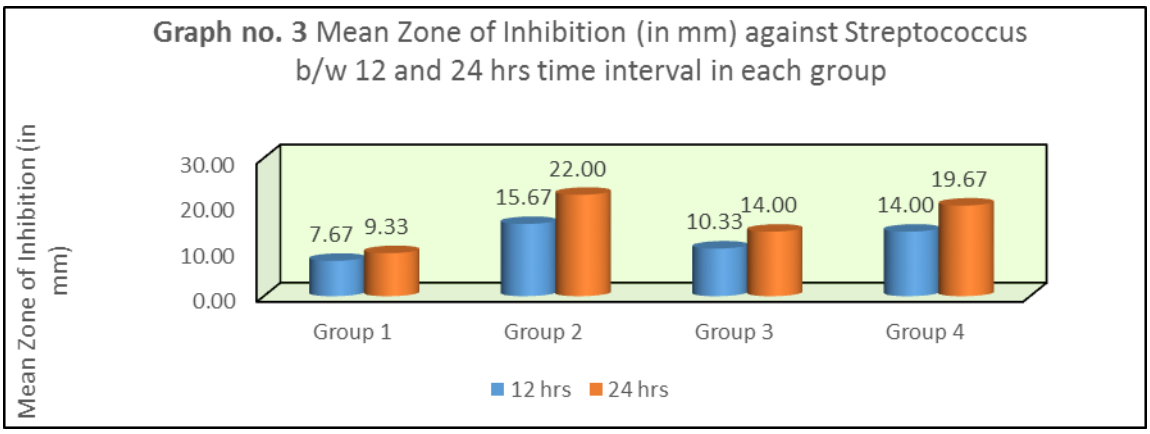
Groups	N	Mean	SD	Min	Max	p-value
Group 1	3	9.33	0.58	9	10	<0.001*
Group 2	3	22.00	2.00	20	24	
Group 3	3	14.00	2.00	12	16	
Group 4	3	19.67	1.53	18	21	

Table -4-Multiple comparison of mean diff. in mean ZOI against Streptococcus b/w 4 groups at 24 hrs time interval using Tukey's Post hoc Test.

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff		p-value
			Lower	Upper	
Group 1	Group 2	-12.67	-16.94	-8.40	<0.001*
	Group 3	-4.67	-8.94	-0.40	0.03*
	Group 4	-10.33	-14.60	-6.06	<0.001*
Group 2	Group 3	8.00	3.73	12.27	0.001*
	Group 4	2.33	-1.94	6.60	0.36
Group 3	Group 4	-5.67	-9.94	-1.40	0.01*

Table -5: Comparison of mean Zone of Inhibition (in mm) against Streptococcus b/w 12 and 24 hrs time interval in each group using Student Paired t Test.

Groups	Time	N	Mean	SD	Mean Diff	p-value
Group 1	12 hrs	3	7.67	0.58	-1.66	0.04*
	24 hrs	3	9.33	0.58		
Group 2	12 hrs	3	15.67	0.58	-6.33	0.002*
	24 hrs	3	22.00	2.00		
Group 3	12 hrs	3	10.33	1.53	-3.67	0.008*
	24 hrs	3	14.00	2.00		
Group 4	12 hrs	3	14.00	1.00	-5.67	0.003*
	24 hrs	3	19.67	1.53		



DISCUSSION

The idea behind the incorporation of copper into adhesive formulations was to keep its antimicrobial properties for longer periods of times.

Studies have shown copper concentration equal to or higher than 0.06% was capable to produce higher inhibition halo against S.mutans higher than the control adhesive, one may speculate that this slow release over time may supplement antimicrobial activity of the adhesive apart from the expected sealing and retention.[8]

In addition to superior antimicrobial activity copper is cheaper than other popular nanoparticles like silver nanoparticles; are easily available and the synthesis of copper nanoparticles is cost effective.

An additional advantage of copper nanoparticles is that they are easily oxidizable in air or aqueous media producing copper oxide nanoparticles.[9-12]

Copper oxide has an effective bactericidal action and can be easily blended with polymers or macromolecules producing stable polymers in regards to chemical and physical properties.

In the present study**,** the mean Zone of Inhibition against Streptococcus for Group 1 was 7.67 ± 0.58 mm, Group 2 was 15.67 ± 0.58 mm, Group 3 was 10.33 ± 1.53 mm and for Group 4 was 14.00 ± 1.00 mm. This difference in the mean Zone of Inhibition against Streptococcus between 4 groups at 12 hrs time interval was statistically significant at p<0.001.

Multiple comparison of mean difference between groups revealed that the Group 2 showed significantly higher mean Zone of Inhibition as compared to Group 1 & Group 3 and the mean differences were statistically significant at p<0.001 & p=0.001 respectively. This was then followed next by Group 4 which showed signicantly higher mean Zone of Inhibition as compared to Group 1 & Group 3 and the mean differences were statistically significant at p<0.001 & p=0.009 respectively. This was then further followed next with Group 3 showing significantly higher mean.

Zone of Inhibition as compared to Group 1 and the mean difference was statistically significant at p=0.04. However, no significant difference was found between Group 2 and Group 4 [p=0.25]. This infers that the mean Zone of Inhibition against Streptococcus at 12 hrs interval was significantly highest in Group 2 followed by Group 4, Group 3 and least in Group 1.

Previous studies mentioned that the antibacterial effect of the copper nanoparticles might be due to interactions with -SH groups of key microbial enzymes, leading to their denaturation.[13,14]

A more recent theory is called "Trojan horse effect", where the acidic lysosomal environment (pH 5.5) is capable of promoting nanoparticles degradation/ corrosion, which converts core metals to ions and therefore toxic substances.[17]

CONCLUSION

- According to the results of the study, there is no statistically significant difference between antimicrobial activity of G2 and G4, therefore the type of adhesive system was not of much significance.
- There is a statistically significant difference on antimicrobial activity once the copper nano particles are incorporated.
- There is a significant difference in the antimicrobial activity between 12 and 24 hours.
- Zone of Inhibition against Streptococcus at 12 hrs interval was significantly highest in Group 2 followed by Group 4, Group 3 and least in Group 1.
- mean Zone of Inhibition against streptococcus in Group 4 at 24 hrs time interval was significantly higher [19.67 ± 1.53] as compared to 12 hrs time interval [14.00 ± 1.00] and the mean difference between 2 time intervals was statistically significant at p=0.003.

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