

## Original Research

**Evaluation and comparison of copper and zinc ion release from copper-oxide and zinc-oxide nanoparticles coated orthodontic brackets- An In vitro Study**Nausheen Mobeen<sup>1</sup>, Sangeetha Duraisamy<sup>2</sup>, K.Ravi<sup>3</sup><sup>1</sup>Post Graduate, <sup>2</sup>Professor, <sup>3</sup>Dean and HOD, SRM Dental College, Ramapuram, Chennai

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**ABSTRACT**

**AIM:** To evaluate and compare the zinc and copper ion release from zinc oxide and copper oxide nanoparticles coated orthodontic brackets in artificial saliva under In vitro condition.

**METHODOLOGY:** 36 preadjusted edgewise 0.022" slot, MBT prescription maxillary first premolar Mini Diamond series stainless steel brackets (Ormco Corporation, Orange County, CA, USA) were divided into two Groups of 18 each. Group A brackets were coated with zinc oxide and group B brackets were coated with copper oxide nanoparticles using spray pyrolysis method. Three brackets from each group were randomly selected and examined under scanning electron microscope (TESCAN, USA. Inc) to confirm the uniformity of coating. 15 brackets from each group were bonded to the human premolar teeth extracted for orthodontic purpose and prepared for the study. The samples were transferred to polyethylene vials filled with 25ml of artificial saliva with a pH of 6.5 and incubated at 37°C. 2ml of artificial saliva was collected from each bottle at 4 different time intervals (immediately after placing the sample, on 7th day, 14th day and 28th day) and evaluated for the presence of zinc and copper ions using an atomic absorption spectrometer (GBC Scientific equipment Ltd. USA). Intragroup comparison of concentration of zinc and copper ions released at different time intervals was done using repeated measures ANOVA and intergroup comparison was done using independent t-test.

**RESULTS:** The concentration of zinc ions released from the group A samples increased from a base line value of 0.01 ppm to 3.01 ppm on the 7th day and decreased thereafter. Repeated measures ANOVA revealed a significant difference between different time intervals and post-hoc pair comparison showed significant difference between all-time intervals except between 14th and 28th day. The concentration of copper ions released from group B samples increased from 0.01 ppm to 0.37 ppm on the 7th day and decreased thereafter. Repeated measures ANOVA and Post-hoc comparison revealed a significant difference in the amount of copper ions released at different time intervals. Independent t-test showed that except for the baseline value the amount of zinc ions released were significantly higher than the amount of copper ions released at all-time intervals. (p value < .001)

**CONCLUSION:** The amount of zinc ions released was significantly greater than the amount of copper ion released with the highest release on the 7th day. The amount of both zinc and copper ions were well below the levels that are toxic to humans at all the time intervals tested.

**KEYWORDS:** Biosafety, Atomic absorption spectrometer, Artificial saliva, Nano coating

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## INTRODUCTION

Nanoparticles are now routinely used in dentistry for improving the properties of composite resins, dental cements and impression materials.<sup>[1-4]</sup> Nanomaterials were also evaluated as coating material on dental implants for improving stability and reducing peri-implantitis.<sup>[4]</sup> Their application is being experimented in various domains of orthodontics, from surface coatings of brackets and arch wires to development of enhanced bonding materials.<sup>[1,5-8]</sup>

Nanoparticles of metals and metal oxides like silver, copper oxide, zinc oxide and titanium oxide have been evaluated as coating materials for orthodontic brackets and arch wires for their ability to reduce formation of whitespot lesions and reducing frictional resistance during sliding of brackets through archwire.<sup>[5, 6, 9-23]</sup>

Currently, copper has been registered as the metal of most superior antimicrobial properties by the American Environmental Protection Agency (EPA).<sup>[24]</sup> Copper oxide and zinc oxide nanoparticles have demonstrated good antibacterial property and the combined effect of zinc oxide and copper oxide coated orthodontic brackets have demonstrated better antimicrobial effect on *S.mutans*.<sup>[13]</sup> When coated on arch wires these metal oxides reduced the frictional resistance produced during the sliding mechanics and also improved the anti-wear properties by protecting the arch wires against oxidation of metal surfaces.<sup>[10,14,20]</sup>

An orthodontic appliance is constantly exposed to variation in temperature and pH in the oral cavity which may lead to bio corrosion and release of metal ions into the oral cavity.<sup>[25]</sup> Nanocoating of orthodontic brackets may affect its biocompatibility and biosafety and leaching of metal ions from the coating may increase salivary concentration of these ions. Salivary concentration of nano-silver ions was found to be increased in albino rats bonded with nano silver coated orthodontic brackets after one week of bonding.<sup>[5]</sup>

Various metals that are components of orthodontic alloys have been identified to be allergenic, cytotoxic and mutagenic to oral tissue at different concentration.<sup>[26]</sup> Elevated levels of zinc in saliva can result in cell cytotoxicity causing oxidative stress and inflammation to epithelial cells. Studies showed ZnO nanoparticles on ingestion or inhalation caused damage to lungs, liver, kidneys and spleen tissue.<sup>[27]</sup> Increased copper ions level in saliva causes metallic taste in mouth and greenish discoloration on the gingiva. Copper nanoparticles were also reported to cause pathological damage to liver, kidney, and spleen through ingestion, inhalation or direct skin contact.<sup>[15]</sup>

Studies have been reported in the literature evaluating the antibacterial and mechanical properties of zinc oxide and copper oxide nano-coated orthodontic brackets and arch wires but those studies have not evaluated the leaching potential of these coating that may result in increased salivary concentration of zinc and copper ions.<sup>[10,14,19,20]</sup> Hence the current study was designed to evaluate the release of zinc and copper ions from zinc oxide and copper oxide nano-coated orthodontic brackets in artificial saliva using atomic absorption spectrometer under in-vitro conditions.

## MATERIALS AND METHODS:

The current in-vitro experimental research was carried out in the Department of Orthodontics, SRM Dental College, Ramapuram, Chennai. The study design was approved by Institutional Review Board and Ethical Committee with an approval number of SRMDC/IRB/2019/MDS/No.106.

A total number of 36 preadjusted edgewise 0.022" slot MBT prescription Maxillary First premolar Mini Diamond series stainless steel brackets (Ormco Corporation, Orange County, CA, USA) were used in the study. The brackets were divided into two groups of 18 each. The Group A brackets were coated with zinc oxide nanoparticles and Group B brackets were coated with copper oxide nanoparticles. Three brackets from each group were randomly selected for SEM analysis to ensure the uniformity of the coating and the remaining 15 brackets from each group were used for evaluation of zinc and copper ion release.

Zinc oxide nanoparticles with average particle size of 30nm size and copper oxide nanoparticles with average particle size of 45 nm size and 99.9% purity was procured (Ultrananotech, Bangalore, India.). The nanocoating of the brackets was performed with spray pyrolysis method to achieve uniform coating using the spray pyrolysis equipment (HOLMARC, Kochi, Kerala, India) (Fig1).<sup>[13]</sup>

**Figure 1: Spray pyrolysis equipment**



The stainless-steel orthodontic brackets were cleaned with deionized water and ethanol at 80°C for 30 minutes to get rid of the surface oxidized layer. The solution for the coating was prepared by diluting 0.3 g of zinc oxide nanoparticle powder in 100 ml of isopropanol. The solution was stirred well in an ultrasonic bath for 1.5 hours. The brackets were arranged in the platform of the spray pyrolysis equipment and distance between the spray nozzle and face of the bracket was maintained at 15 cm. A thin film of zinc oxide nanoparticle was precipitated over the brackets to obtain a uniform coating of 100 µm thickness at 150°C temperature and 0.5 Pa pressure (Fig 2A). A similar method was used for coating the group B brackets with copper oxide nanoparticles (Fig 2B).

**Figure 2A&B .Zinc oxide and copper oxide nano-coated brackets**

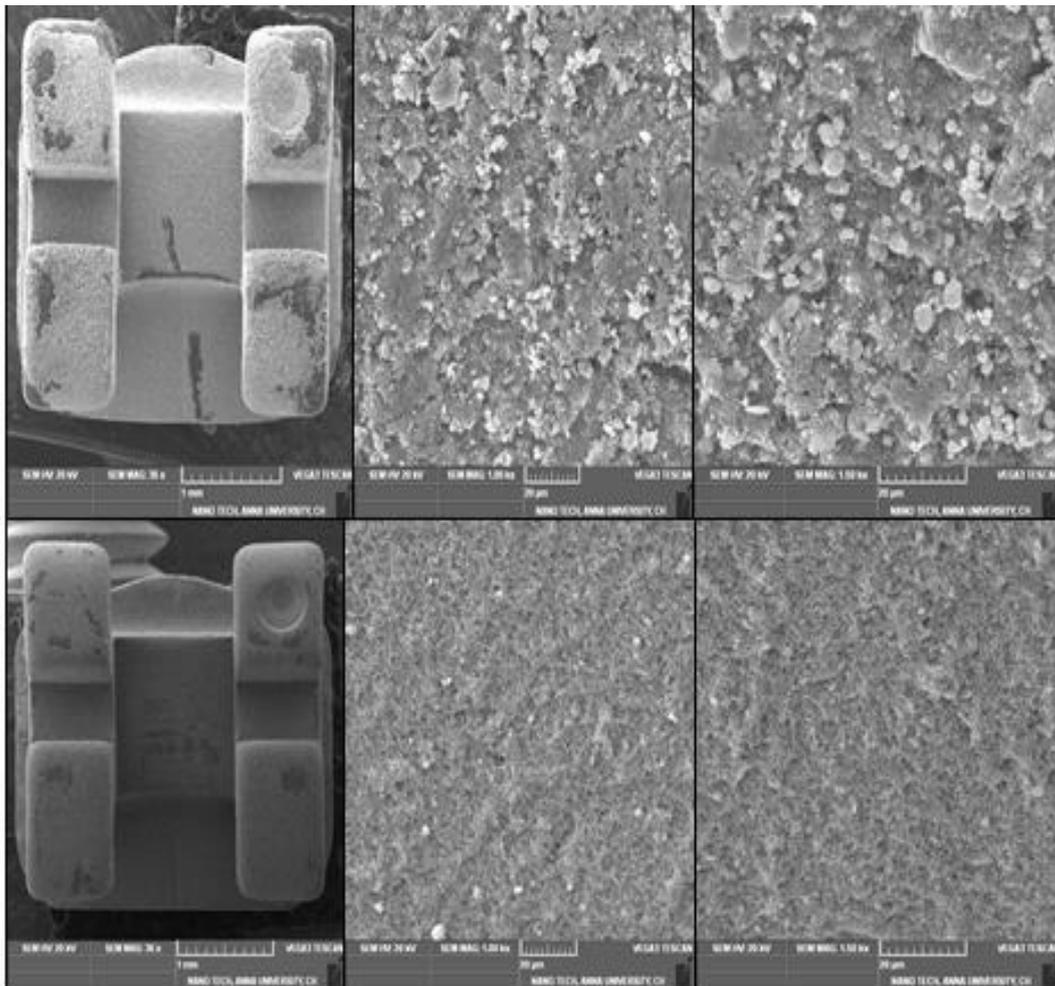


*-Invitro Study*

Three coated brackets from each group were randomly selected to confirm the uniformity of coating using scanning electron microscope (TESCAN, USA. Inc.) The brackets were studied under 36X, 1000X, and 1500X magnifications. The images confirmed a uniformly distributed coating over the surface of the brackets for both zinc oxide and copper oxide nanoparticles coated group (Fig 3A & B).

Thirty healthy human maxillary first premolar teeth extracted for orthodontic reasons without enamel cracks, decalcification spots, surface defects, caries or restoration were collected and stored in distilled water at room temperature. The roots of the teeth were amputated using micromotor and carborundum disc, the pulp was extirpated, and chamber sealed with flowable composite resin (TE-Econom Flow, Ivoclar, Vivadent).

**Figure 3A&B. SEM images of Zinc oxide and copper oxide nanoparticles coated orthodontics brackets at different magnification (36X, 1000X, 1500X) showing uniform coating**



The nano coated brackets belonging to group A and group B were bonded to the prepared maxillary premolar crowns using standard bonding protocol by a single operator. The buccal surface of premolar was conditioned with 37% phosphoric acid (Anabond Eazetech Etchant Etching Gel) for 30 sec, rinsed with water for 20 seconds and dried with oil-free compressed air for 20 seconds. The light cure adhesive primer (3M Unitek Transbond XT Light Cure Adhesive Primer) was applied on the etched enamel surface and cured for 20 seconds. The brackets were placed on the middle third of the enamel parallel to the long axis using composite resin (3M Unitek Transbond XT Light Cure Adhesive). Excess composite around the margins of the bracket was removed with a dental explorer and photo-polymerized for 20 seconds.

**Figure 4A&B. Group A samples (Zinc oxide nano-coated brackets) and Group B (Copper oxide nano-coated brackets) immersed in artificial saliva.**



The group A & group B samples were segregated separately and sterilized in an autoclave at 120°C temperature 15 psi pressure for 20 minutes. Each sample belonging to the two study groups were transferred to labelled polyethylene vials filled with 25ml of artificial saliva at neutral pH and intubated in an incubator at 37°C (Fig4A&B). Artificial saliva was prepared using Barret, Bishara and Quinn formula, with a composition of 0.4 g NaCl, 1.21 g KCl, 0.78g NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O, 0.005 gm Na<sub>2</sub>S.9H<sub>2</sub>O, 1 gm Urea {CO (NH<sub>2</sub>)<sub>2</sub>} in 1000 ml of deionized water. The pH of the artificial saliva was adjusted to (6.5±.15) using 10M sodium hydroxide (NaOH).<sup>[25]</sup>

Two ml of artificial saliva was collected from each of the 30 vials immediately after placing the sample, on 7<sup>th</sup> day, 14<sup>th</sup> day and 28<sup>th</sup> day respectively. After every sample collection the vials were topped with 2 ml of artificial saliva to replace the quantity collected for the analysis. The samples were analyzed for the presence of zinc ions in Group A samples and copper ions in Group B samples using an atomic absorption spectrometer (GBC Scientific equipment Ltd. USA) (Fig 5).

**Figure 5. Atomic absorption spectrometer**



The quantity of zinc and copper ions measured from the Group A and Group B samples at different time intervals were tabulated and subjected to statistical analysis. The analysis was performed using IBM SPSS software version (IBM SPSS, Armonk NY:IBM Corp). Descriptive statistics including mean and standard deviation and standard error mean were calculated for group A and B. Intragroup comparison for each group was done using repeated measures ANOVA and intergroup comparison was done using independent t test.

*-Invitro Study***RESULTS:**

The mean concentration of zinc ions released from the group A brackets increased from a negligible baseline value of 0.01ppm to 3.01 ppm on the 7th day. The concentration decreased subsequently to 0.86 ppm on the 14th day and 0.91 ppm on the 28th day (Table 1). Repeated measures ANOVA revealed a significant difference in the amount of zinc ions released at different time intervals (Table 1). The post-hoc pair comparison showed significant difference between base line value and the concentration of zinc ions on 7th, 14th and 28th day of evaluation (Table 2). A negligible increase in the levels was noted at the 28th day but the difference was not statistically different from that of the 14th day (Table 2).

**Table 1. Repeated measures ANOVA for intra group comparison of concentration on zinc ions in artificial saliva from group A samples between four different time intervals.**

Days	Mean Salivary Zinc Level (ppm)	Std. Deviation	Min	Max	Percentile		
					25th	50th	75th
Baseline	0.01	0.91127	0.010	0.010	0.01	0.01	0.01
7th day	3.00667	0.231352	2.650	3.5	2.95	3.00	3.00
14th day	0.86933	0.177421	0.5	1.00	0.75	0.95	1.00
28th day	0.91127	0.225451	0.099	0.990	0.95	0.96	0.99

N	15
P value	0.001

The mean concentration of copper ions released from the group B samples increased from a baseline value of 0.01 ppm to 0.37 ppm on the 7th day. The concentration decreased to 0.26 ppm on the 14th day and to 0.14 ppm on the 28th day (Table 3). Repeated measures ANOVA revealed a significant difference in the amount of copper ions released at different time intervals (Table 3). The post-hoc pair comparison showed significant difference between base line value and the concentration of copper ions on 7th, 14th and 28th day of evaluation (Table 4). The concentration of zinc and copper ions released in the artificial saliva at different time intervals were compared using an independent T test (Table 5). The baseline values for both zinc and copper were not different significantly, but at further time intervals the level of zinc ions released from the zinc oxide nano coated brackets were significantly higher than the amount of copper ions released from copper oxide nano coated brackets (Table 5).

**Table 2. Post-hoc comparison of concentration on zinc ions in artificial saliva from group A samples between four different time intervals using Bonferroni tests.**

Ion release	Ion release	Mean Difference	Std. Error	P value	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
BASELINE	7TH DAY	-2.997*	.060	.000	-3.180	-2.813
	14TH DAY	-.859*	.046	.000	-1.000	-.719
	28TH DAY	-.901*	.058	.000	-1.080	-.723
7TH DAY	14TH DAY	*2.137*	.066	.000	1.934	2.341
	28TH DAY	2.095*	.083	.000	1.841	2.350
14TH DAY	28th day	*-.042	.079	1.000	-.286	.202

**Table 3. Repeated measures ANOVA for intra group comparison of concentration on copper ions in artificial saliva from group B samples between four different time intervals .**

Days	N	Mean of copper levels (ppm)	Std. Deviation	min	max	Percentiles		
						25th	50th	75th
Baseline	15	0.010	0.00	0.010	0.010	0.01	0.01	0.01
7th day	15	0.36667	0.120396	0.220	0.500	0.25	0.35	0.5
14th Day	15	0.25867	0.047789	0.120	0.300	0.25	0.29	0.29
28th day	15	0.14333	0.099043	0.010	0.25	0.01	0.20	0.20

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N	15
P value	0.001

**Table 4. Post-hoc comparison of concentration on copper ions in artificial saliva from group B samples between four different time intervals using Bonferroni test.**

Ion release	Ion release	Mean Difference	Std. Error	P value	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
BASELINE	7th day	-.357*	.031	.000	-.452	-.261
	14th day	-.249*	.012	.000	-.287	-.211
	28th day	-.133*	.026	.001	-.212	-.055
7th day	14th day	.108*	.032	.026	.011	.205
	28th day	.223*	.038	.000	.106	.341
14th day	28th day	.115*	.026	.004	.035	.196

**Table 5. Independent t test for inter group comparison of concentration of zinc ions and copper ions in the artificial saliva from Group A & Group B samples at the four different time intervals**

Days	Brackets	N	Mean Zinc & copper ion levels (ppm)	Std. Deviation	Mean Difference	P value
BASELINE	Group A	15	0.01	0.00	0.00	1.0
	Group B	15	0.01	0.00		

7TH DAY	Group A	15	3.00667	0.231352	2.64	<0.001
	Group B	15	0.36667	0.120396		
14TH DAY	Group A	15	0.86933	0.177421	0.610667	<0.001
	Group B	15	0.25867	0.047789		
28TH DAY	Group A	15	0.91127	0.225451	0.767933	<0.001
	Group B	15	0.14333	0.099043		

## DISCUSSION

Zinc oxide and copper oxide nanoparticles coated orthodontic brackets and arch wires have shown to reduce the count of cariogenic bacteria in experimental set up due to their antimicrobial properties.<sup>[13-14,20]</sup> The nanocoating may affect the properties of the brackets including biocompatibility and bio safety. Ions leaching from these coating may result in increased salivary concentration of zinc and copper ions.

The elevated level of zinc in serum has been reported to suppress immunity and cause anaemia and increased salivary content may result in oxidative stress to oral epithelial cells leading to inflammation and cytotoxicity.<sup>[27,29]</sup> Increased salivary copper levels can cause greenish discoloration of gingiva and ingestion of toxic levels of copper can cause gastrointestinal illness damaging the spleen and liver resulting in Wilson's disease.<sup>[15, 30]</sup>

The current study evaluated the concentration of zinc and copper ions released from coated brackets in artificial saliva at different time intervals over a period of 28 days using atomic absorption spectrometer. Zinc and copper are not a component of austenitic stainless steel used for manufacturing orthodontic bracket. Hence the amount of zinc and copper ions identified in the salivary sample must have been from the nano coating.

In the present study the concentration of both zinc and copper ions in the artificial saliva immediately after placing the specimens was 0.01 ppm and the peak concentration was noted on day seven. The zinc oxide nanocoating leached more ions than the copper oxide nanocoating at all-time intervals tested showing better stability of the copper oxide nanocoating.

Zinc is an essential micronutrient and acts as a catalyst for many enzymes that facilitate protein folding and aids in the regulation of gene expression. The Recommended Dietary Allowance of zinc for adult women is 8 mg/day and men is 11 mg/day and the tolerable Upper Intake Level for adults is 40 mg/day. The maximum concentration of zinc ions released from one zinc oxide nano-coated bracket in our present study as on day seven was 3.0 ppm (0.003 mg) which can be roughly extrapolated to a release of 72 ppm (0.072 mg/day) zinc from a full bonded orthodontic appliance of 24 attachments .

Similarly, copper is a micronutrient and is a component of many metalloenzymes acting as oxidases to achieve thereduction of molecular oxygen. The Recommended Dietary Allowance of copper for adult men and women is 900µg/day and the tolerable Upper Intake Level for adults is 10,000 µg/day (10 mg/day).<sup>[29]</sup> The average amount of copper released from one copper oxide nano-coated bracket was 0.36ppm (0.0003 mg/day) which can be roughly extrapolated to a release of 8.64 ppm (0.0086 mg/day) copper from a full bonded orthodontic appliance of 24 attachments. Thus, the amount of zinc and copper ions released from a fully bonded orthodontic appliance of nanocoated attachments might be far below the recommended Dietary Allowance and tolerable Upper Intake

Level for adults and hence these nanocoated brackets can be used inpatients without the concern of increasing the salivary concentration of these ions to unsafe levels.

### **LIMITATION AND FUTURE SCOPE**

The current study evaluated the release of zinc and copper ions under in-vitro conditions in artificial saliva. The variations in oral PH, temperature and wear and tear from oral functions and brushing which may alter the quantity of ions released from the brackets cannot be reproduced accurately in an experimental setup. Clinical studies evaluating the ion release from these coated brackets may be useful in determining the biosafety and stability of these coating during intra oral use for an extended period of time.

When proven safe zinc oxide and copper oxide nanoparticles coated orthodontic brackets could be a viable method of reducing white spot lesions and frictional resistance during sliding in patients undergoing fixed orthodontic treatment

### **CONCLUSION**

The concentration of zinc and copper ions released from zinc oxide and copper oxide nanocoated orthodontic brackets in artificial saliva under Invitro conditions increased from a negligible baseline value to a peak concentration on the 7<sup>th</sup> day and decreased subsequently on 14<sup>th</sup> and 28<sup>th</sup> day.

The levels of zinc ions were significantly greater than the levels of copper ions at all the time intervals tested except for the baseline value.

### **SOURCE OF FUNDING**

None

### **CONFLICT OF INTEREST**

There is no conflict of interest.

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