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Original Research Article

A spectrophotometric analysis of copper and zinc released from stainless steel brackets coated with a combination of copper oxide and zinc oxide nanoparticles in artificial saliva

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ABSTRACT

Background: Nanocoating stainless steel orthodontic brackets with a combination of copper and zinc oxide nanoparticles might alter the quantity of ions released from them in saliva. The purpose of the study was to evaluate the quantity of copper and zinc ions released from stainless steel brackets coated with a combination of copper oxide and zinc oxide.

Materials and Methods: Stainless steel orthodontic brackets (Ormco Mini -Diamond series 0.22" slot, MBT prescription) (ORMCO CORP Glendora, California, USA) (n= 15 in each group) were coated with nanoparticles of copper oxide (Group I) , zinc oxide (Group II) and a combination of copper oxide –zinc oxide (Group III) nanoparticles using a spray pyrolysis method . The quantity of copper and zinc ions released from these three groups of brackets, when stored in artificial saliva and intubated at 37^0 C was evaluated at 24hrs, 7th day, 14th day and 28th day using an atomic absorption spectrometer.

Results: The three groups of coated brackets released significantly more copper and zinc ions than the uncoated brackets. The copper oxide nanocoated and zinc oxide nano coated stainless steel orthodontic brackets released more copper and zinc ions when compared to the copper oxide - zinc oxide combination nanocoated orthodontic brackets and uncoated brackets. The highest surge of ion release was noted at the 7th day in all the three coated groups for both the ions evaluated.

Conclusion: Brackets coated with a combination of copper oxide and zinc oxide nanoparticles demonstrated reduced levels of copper and zinc ion release in artificial saliva when compared to copper oxide nanocoated brackets and zinc oxide nanocoated brackets.

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1. Introduction

Surface coating of stainless steel brackets with nanoparticles can alter its biological and tribological properties.[1–5] In the recent years nano coating as a noncompliance method to reduce white spot lesions in patients undergoing fixed orthodontic treatment has received a lot of attention.[6–9] Nanocoating improves the antibacterial properties, prevents biofilm formation and adhesion on the surface of substrates.[10,11]

Nanoparticles when used in combination may result in increased antibacterial efficacy with reduction in their individual side effects.[12–14] The antibacterial effect of brackets coated with a combination of silver and zinc oxide nanoparticles against Streptococcus mutans and Lactobacillus acidophilus was greater when compared to the brackets separately coated with silver or zinc oxide.[12] Similarly the combination of copper oxide and

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zinc oxide demonstrated superior antibacterial effect when compared to the individual nano particles.[13] The combination also exhibited superior antibacterial effect against Streptococcus mutans when compared to silver -copper oxide and silver - zinc oxide coating on stainless steel brackets.[14] Nitrogen doped titanium oxide nanocoating of stainless steel brackets demonstrated long term antimicrobial property against Streptococcus mutans.[8] Doping of titanium oxide with nitrogen narrows their photo catalytic band gap from UV spectrum to visible light, eliminating the need for UV radiation.[2]

The orthodontic brackets are constantly subjected to a cyclic variation in temperature, pH and mechanical stresses from oral functions leading to bio-corrosion and leaching of metal ions into the saliva or migration into the adjacent tissues.[15–17] The biosafety of the nanoparticles used for coating the brackets is a major concern as the particles may be released from the surface coating during the prolonged orthodontic treatment. Silver ions were detected in the serum and saliva of white albino rats bonded with orthodontic brackets coated with nano silver particles.[18,19] Presence of copper and zinc ions were observed in artificial saliva incubated at 37^0C with copper oxide and zinc oxide nanocoated brackets.[20]

The literature is replete with studies evaluating the antibacterial and frictional properties of nanocoated brackets, but only few studies have evaluated these effects clinically, because of the lack of information on their cytotoxicity and biocompatibility.[1–14] The cytotoxicity of stainless steel brackets coated with the combination of copper oxide and zinc oxide nanoparticles was as low as the uncoated brackets and significantly lesser than that of brackets coated with the individual particles.[21] This reduction in cytotoxicity may be attributed to the dose dependent cytotoxic nature of copper and zinc and the reduction in quantity of the nano particles used in the combination coating.[12,21] In combination or hybrid coating, the nanoparticles are mixed in 1:1 proportion by weight reducing its quantity in the coating by half.[12–14,21] This may reduce the levels of copper or zinc ions leaching from them when compared to the brackets coated entirely with copper oxide or zinc oxide.

To test this hypothesis, the present in vitro study was designed to evaluate and compare the levels of copper and zinc ions released into the artificial saliva from the stainless steel orthodontic brackets coated with copper oxide, zinc oxide and the combination of both particles at different time intervals.

2. Materials and Methods

The protocol of the current study was approved by the Institutional Review Board and Institutional Ethical Committee of SRM Dental College, Ramapuram, Chennai with an approval number of SRMDC/IRB/2023/PhD/NO.156. The sample size estimated for a power of 80 %, α error of 5 % was 15 in each group. The p value < 0.05 was considered statistically significant. Preadjusted edgewise, 0.022" slot, MBT prescription, stainless steel maxillary Ormco (ORMCO CORP, Glendora, USA) premolar brackets were used for the study.

A total number of 75 brackets were divided in three experimental groups of 20 each and one control group of 15. The study group brackets of 20 each were coated with nano particles of copper oxide, zinc oxide and a hybrid combination of both using spray pyrolysis method (Ho-TH-04, Holmark –optomechtronics Ltd, Kochi, kerala, India).[20,21] Copper oxide nanoparticles of an average size of 45 nm and zinc oxide with an average size of 40 nm with 99.9% purity was used for the nanocoating. (Ultrananotech, Bangalore, India).Scanning Electron Microscopic analysis was performed in 5 brackets randomly chosen from each group to ensure the uniformity of coating. The rest 15 brackets from each group and the 15 uncoated brackets of the control group were used for spectrophotometric analysis.

Sixty healthy human maxillary first premolar teeth, extracted for therapeutic purpose were immediately collected and store in distilled water at room temperature. Teeth with surface cracks, defects, demineralization spots, caries or restoration were not included. The crowns were amputated using a carborundum disc, the pulp chamber was cleaned and filled with a flowable composite resin. The prepared crowns were randomly grouped into 4 groups of 15 each and were bonded with the respective four groups of brackets using standard bonding protocol. 37% phosphoric acid (Anabond Eazetech Etchant Etching Gel), light cure adhesive primer and composite resin (3M Unitek Transbond XT) was used for etching and bonding.

The artificial saliva was prepared using the basic formula given by Gjerdet and Hero and later modified by Barret, Bishara and Quinn.[22,23] The pH was adjusted to the average pH of the oral cavity (6.75) using 10M sodium hydroxide (NaOH). The four group of brackets (Group I: Copper oxide nanocoated brackets, Group II: Zinc oxide nanocoated Brackets, Group III: Copper oxide –Zinc oxide nanocoated brackets & Group 1V: Uncoated brackets)were segregated and autoclaved at 120ºC temperature 15 psi pressure for 20 minutes. The brackets were immersed into 25 ml of artificial saliva in labelled polyethylene bottles and stored at 37^oC. A sample of 2ml of saliva was collected from each of the 60 bottles at the end of 24 hrs, 7^{th}day,14^{th} day & 28^{th} day. After every sample collection, the artificial saliva was replaced in each bottle to avoid the cumulative effect.[24]

A total of 60 samples from the four groups at each time interval were collected and analysed using an atomic absorption spectrometer (GBC-932A plus GBC Scientific equipment Ltd. USA). Three plain samples of artificial

saliva were used as a blind test ("0" sample) to calibrate the spectrophotometer.[24] The group I samples were analysed for the quantity of copper ions , group II for Zinc ions and group III & IV samples for both Copper & Zinc ions. The quantity of ions in ppm from each group were tabulated and subjected to statistical analysis.

One way ANOVA and Post hoc Tuckey HSD for multiple comparisons was used for between group comparisons of Copper (Group I, III & IV) and Zinc (Group II, III & IV) concentration at different time intervals (Tables 1, 2 and 3). Paired sample Test was used for intragroup comparison of copper and zinc ions between different time intervals (Tables 4 and 5). Independent T test was used for intergroup comparison copper and zinc ions at different time intervals (Table 6). The P value ≤ 0.05 was considered as statistically significant.

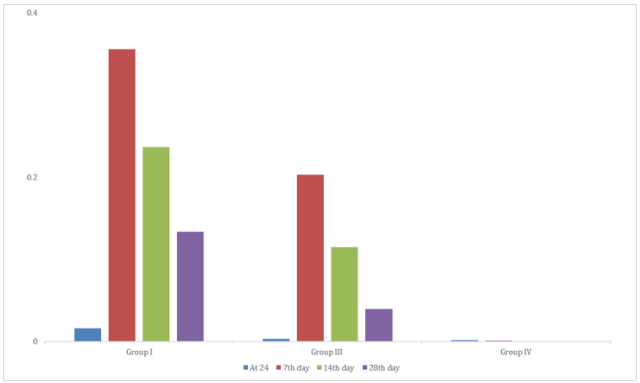

Figure 1: Mean concentration of Copper ions in ppm released from Group I (Copper oxide nanocoated brackets), Group III (Copper oxide –Zinc oxide nanocoated brackets) & Group IV (Uncoated brackets) in artificial saliva at 24 hrs, 7^{th} day, 14th day and 28^{th} day.

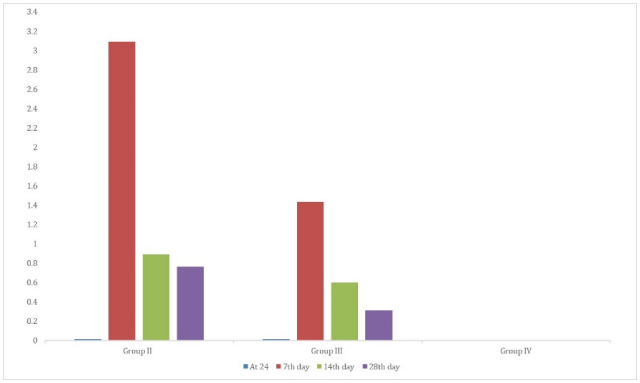

Figure 2: Mean concentration of Zinc ions in ppm released from Group II (Zinc oxide nanocoated brackets), Group III (Copper oxide –Zinc oxide nanocoated brackets) & Group IV (Uncoated brackets) in artificial saliva at 24 hrs, 7^{th} day, 14^{th} day and 28th day.

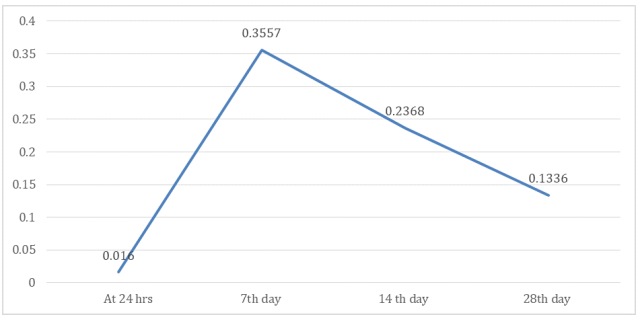

Figure 3: Mean concentration of Copper ions in ppm released from Group I (Copper oxide nanocoated brackets) in artificial saliva at 24 hrs, 7^{th} day, 14^{th} day and 28th day.

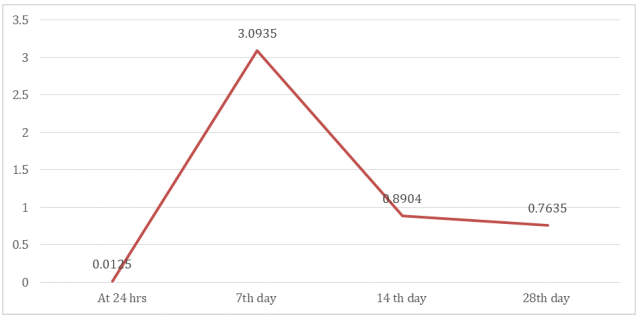

Figure 4: Mean concentration of Zinc ions in ppm released from Group II (Zinc oxide nanocoated brackets) in artificial saliva at 24 hrs, 7^{th} day, 14^{th} day and 28^{th} day.

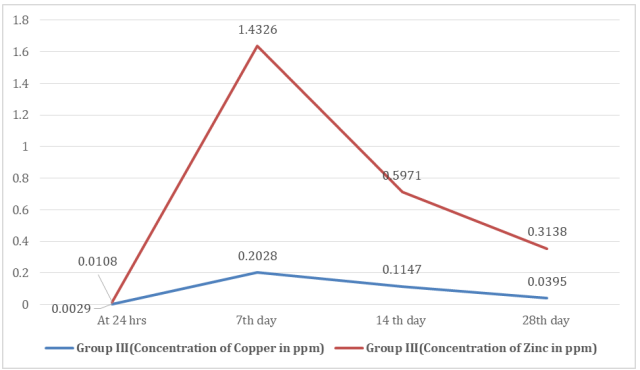

Figure 5: Mean concentration of Copper and Zinc ions in ppm released from Group III (Copper oxide –Zinc oxide nanocoated brackets) in artificial saliva at 24 hrs, 7^{th} day, 14^{th} day and 28^{th} day.

Table 1: Mean concentration of copper (from Group I, III & IV brackets) and Zinc ions ((from Group II, III & IV brackets) in ppm.(Group I: Copper oxide anno coated brackets, Group II: Zinc oxide nanocoated Brackets, Group III: Copper oxide –Zinc oxide nanocoated brackets & Group 1V: Uncoated brackets)

	At 24 hrs	7^{th} day	14 th day	28^{th} day
Group I (Concentration of Copper in ppm)	0.0160 ± 0.0238	0.3557 ± 0.0476	0.2368 ± 0.0301	0.1336 ± 0.0172
Group II (Concentration of zinc in ppm)	0.0125 ± 0.0073	3.0935 ± 0.3916	0.8904 ± 0.1012	0.7635 ± 0.1169
Group III (Concentration of Copper in ppm)	0.0029 ± 0.0039	0.2028 ± 0.0869	0.1147 ± 0.0289	0.0395 ± 0.0332
Group III (Concentration of Zinc in ppm)	0.0108 ± 0.0080	1.4326 ± 0.4563	0.5971 ± 0.0922	0.3138 ± 0.0737
Group IV(Concentration of Copper in ppm)	0.0013 ± 0.0026	0.0008 ± 0.0026	0.0005 ± 0.0007	0.0004 ± 0.0005
Group IV(Concentration of Zinc in ppm)	0.0018 ± 0.0026	0.0015 ± 0.0027	0.0015 ± 0.0013	0.0005 ± 0.0001

Table 2: One way ANOVA to compare the concentration of Copper (Group I, III & IV) and Zinc (Group II, III & IV) between the three groups at 24 hrs, 7 days, 14 days & 28 days.

		Concentration of copper in ppm					Concentration of zinc in ppm				
		Sum of Squares	df	Mean Square	F	Sig.	Sum of Squares	df	Mean Square	F	Sig.
24 hrs	Between Groups	.002	2	.001	5.001	.011	.001	2	.000	11.942	.000
	Within Groups	.008	42	.000			.002	42	.000		
	Total	.010	44				.003	44			
7^{th} day	Between Groups	.950	2	.475	145.101	.000	71.834	2	35.917	297.983	.000
	Within Groups	.138	42	.003			5.062	42	.121		
	Total	1.088	44				76.896	44			
14^{th} day	Between Groups	.419	2	.209	361.802	.000	6.155	2	3.077	492.514	.000
	Within Groups	.024	42	.001			.262	42	.006		
	Total	.443	44				6.417	44			
25^{th} day	Between Groups	.141	2	.070	151.002	.000	4.413	2	2.206	346.632	.000
	Within Groups	.020	42	.000			.267	42	.006		
	Total	.160	44				4.680	44			

3. Results

The mean concentration of copper and zinc ions released into the artificial saliva from the different group of brackets during each time period is presented in Table 1 & Figures 1, 2, 3, 4 and 5. The highest concentration of ion release (3.0935 ± 0.3916 ppm) (was noted in zinc at 7^{th} day in Group II (Zinc oxide nano particles coated brackets), whereas the lowest concentration of ion release was noted for copper ions (0.0004 ± 0.0005 ppm) in uncoated brackets at 28^{th} day (Table 1).

The amount of copper and zinc released from the coated and uncoated brackets were significantly different from each other at all the four time intervals tested except between the combination coated and uncoated brackets at 24 hrs for copper (P value = 0.952) and between the zinc coated brackets and combination coated brackets at 24hrs for Zinc (P value =0.753) (Tables 1 and 2). The amount of copper ions released from Group III were significantly lesser than that of from Group I brackets and greater than that of uncoated brackets at all the time intervals tested except at 24 hrs where the difference was not statistically significant (Tables 1, 2 and 3). Similarly the amount of zinc ions released from the Group III brackets were significantly lesser than that of Group I brackets and greater than that of uncoated brackets except at 24 hrs where the between

Table 3: Post hoc Tuckey HSD for multiple comparisons of concentration of Copper (Group I, III & IV) and Zinc (Group II, III & IV) between the three groups at 24 hrs, 7 days, 14 days & 28 days.

	Concentration of copper in ppm			**Concentration of zinc in ppm**		
		Mean Difference & Standard error	Sig.		Mean Difference & Standard error	Sig.
At 24 hrs	Group I & III	0.0132 ± 0.0051	.036	Group II & III	0.0017 ± 0.0024	.753
	Group I & IV	0.0147 ± 0.0051	.017	Group II & IV	0.0106 ± .0024	.000
	Group III & IV	0.0015 ± 0.0051	.952	Group III & IV	0.0090 ± 0.0024	.001
At 7^{th} day	Group I & III	0.1529 ± 0.0209	.000	Group II & III	1.6609 ± 0.1268	.000
	Group I & IV	0.3549 ± 0.0209	.000	Group II & IV	3.0920 ± 0.1268	.000
	Group III & IV	0.2019 ± 0.0209	.000	Group III & IV	1.4310 ± 0.1268	.000
At 14^{th} day	Group I& III	0.1221 ± 0.0088	.000	Group II & III	0.2933 ± 0.0290	.000
	Group I & IV	0.2363 ± 0.0088	.000	Group II & IV	0.8889 ±0.0289	.000
	Group III & IV	0.1142 ± 0.0088	.000	Group III & IV	0.5956 ± 0.0289	.000
At 28^{th} day	Group I & III	0.0941 ± 0.0079	.000	Group II & III	0.4497 ± 0.0291	.000
	Group I & IV	0.1332 ± 0.0079	.000	Group II & IV	0.7630 ± 0.0291	.000
	Group III & IV	0.0391 ± 0.0078	.000	Group III & IV	0.3133 ± 0.0291	.000

Table 4: Paired Sample T test for comparison of concentration of Copper at 24hrs, 7th day, 14th day & 28^{th} day

	Group I			**Group III**			**Group IV**		
	T	**df**	**Sig. (2-tailed)**	**t**	**df**	**Sig. (2-tailed)**	**t**	**df**	**Sig. (2-tailed)**
24hrs & 7^{th} day	-22.570	14	.000	-8.858	14	.000	.529	14	.605
24hrs & 14^{th} day	-34.248	14	.000	-15.005	14	.000	1.277	14	.222
24hrs & 28^{th} day	-16.111	14	.000	-4.152	14	.001	1.562	14	.140
7^{th} day & 14^{th} day	7.662	14	.000	4.514	14	.000	.370	14	.717
7^{th} day & 28^{th} day	15.918	14	.000	7.300	14	.000	.569	14	.578
14^{th} day & 28^{th} day	14.694	14	.000	6.919	14	.000	.807	14	.433

Table 5: Paired sample t test for comparison of concentration of zinc between 24 hrs, 7th day, 14th day & 28^{th} day

	Group II			**Group III**			**Group IV**		
	t	**Df**	**Sig. (2-tailed)**	**t**	**df**	**Sig. (2-tailed)**	**t**	**df**	**Sig. (2-tailed)**
24 hrs & 7th day	-30.455	14	.000	-12.181	14	.000	.244	14	.811
24 hrs & 14th day	-33.791	14	.000	-25.479	14	.000	.354	14	.728
24 hrs & 28^{th} day	-24.328	14	.000	-15.929	14	.000	1.884	14	.081
7th day & 14^{th} day	20.280	14	.000	7.018	14	.000	.000	14	1.000
7th day & 28^{th} day	21.539	14	.000	8.950	14	.000	1.271	14	.224
14th day & 28^{th} day	3.654	14	.003	8.696	14	.000	2.958	14	.010

Table 6: Independent sample T test for comparison of concentration of Copper and Zinc at, 7th day, 14th day & 28^{th} day

	Group I & II			Group III			Group IV		
	t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)
24 hrs	-			-3.469	28	.002	-.491	28	.627
7^{th} day	-26.880	28	.000	-10.253	28	.000	-.767	28	.450
14^{th} day	-23.973	28	.000	-19.345	28	.000	-2.583	28	.015
28^{th} day	-20.654	28	.000	-13.141	28	.000	-.439	28	.664

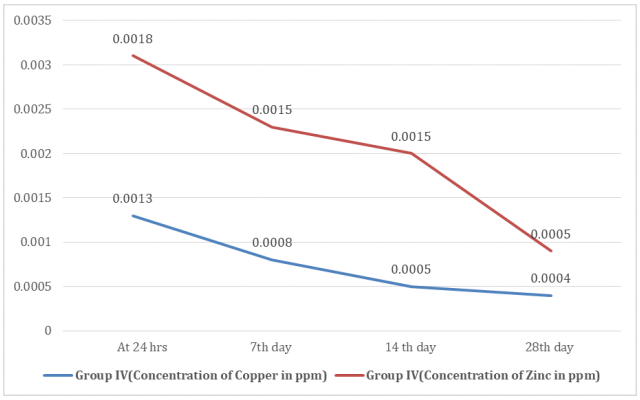

Figure 6: Mean concentration of Copper and Zinc ions in ppm released from Group IV (Uncoated brackets) in artificial saliva at 24 hrs, 7th day, 14th day and 28th day.

group II & Group III values were not significant, significant (Tables 1, 2 and 3).

The largest quantity of Copper and Zinc release was noted on the 7^{th} day for the both individually coated and combination brackets and from there the levels decreased significantly. At the end of 28 days the ion concentration was significantly lesser than that of the 7^{th} & 14^{th} day but was greater than the 24 hours value (Tables 1, 4 and 5) (Figures 1, 2, 3, 4, 5 and 6). In uncoated brackets the amount of both the ions evaluated were not significantly different between the time intervals except for zinc, where the ion levels decreased significantly from 14^{th} day to the 28 th day (Pvalue = 0.010) (Tables 1, 4 and 5). The amount of Copper released was significantly less than that of Zinc at all the time intervals tested for coated brackets whereas in uncoated brackets the values were not significantly different. (Table 6)

4. Discussion

The quantity of the metal ions leaching into the oral cavity is a major concern in the biocompatibility of orthodontic appliances and had been evaluated extensively, yet studies related to the leaching of ions from the nanocoatings of various components of orthodontic appliance is limited.[1–14,20] Attempts had been made to reduce the toxicity of the metals or metal oxides used for nanocoating by using a combination method, where two different nanoparticle mixed in equal proportion by weight was used for coating substrate surfaces.[12,13] However previous studies have suggested that the amount of released metal ions is not always proportional to the content of metal in the alloy.[22,24,25] Hence the present study compared the amount of copper and zinc ions released from the copper oxide, zinc oxide and copper oxide –zinc oxide nanocoated orthodontic stored in the artificial saliva incubated at 37°C at four different time intervals.

In our present study the combination nanocoated orthodontic brackets released less copper and zinc ions than that of the individually coated brackets in artificial saliva stored at 37°C. This may be a plausible explanation for the reduced cytotoxicity of these combination brackets as reported in our previous study.[21] The greatest release of both copper and zinc ions from group I, group II & group III brackets was during the 7th day followed by a gradual decline towards the end of the study period. This kinetics of ions release cannot be due to the cumulative effect or the saturation of the artificial saliva with ions because the whole solution was changed every time after collecting the sample at the stipulated time period.[24] This pattern coincides with the pattern of release of metal ions from orthodontic appliances in artificial saliva as demonstrated in previous studies.[20,23,26] The gradual decline following the initial surge of ion release may be due to formation of a stable oxide layer that prevents further ion release.[23–27]

The quantity of copper and zinc ions measured from the samples of the present study are insignificant from a toxicological standpoint (Table 1). The mean amount of ions released from the coated brackets over the 28 days study period when projected to 20 brackets is well below the daily recommended dietary intake level of copper and Zinc.[28,29] The recommended dietary allowance of copper is 900 μg/day for adults with an upper intake limit of 10,000 μg/day and for zinc it is 8-11 mg/ and 40 mg/day respectively. Hence a systemic toxic effect from these coated brackets is unlikely. However the ions released could cause local effects in the mucous membrane or allergic reactions. Though the antigenic potential of an allergen has to be 5 to 12 times stronger for it to cause an allergic reaction in the oral mucous membrane when compared to skin, hypersensitive reactions to components of fixed orthodontic treatment is not a rare phenomenon.[30]

The quantity of zinc ions was greater than the quantity of copper ions at all-time intervals tested in coated brackets. This was similar to the finding observed in our previous study where copper oxide and zinc oxide nanocoated stainless steel brackets were evaluated.[20]

The similar pattern was observed in the copper oxide –zinc oxide coated orthodontic brackets. A logical explanation could be the stability of copper oxide coating on a stainless steel substrate when compared to zinc oxide coating and should be further evaluated before arriving into concrete conclusions. Despite the fact that stainless steel brackets donot contain copper or zinc in their alloy composition, a negligible amount of copper and Zinc ions were noted in the samples collected in uncoated bracket group (Table 1 & Figure 6). The source of the ions could be from the solder or brazing material used during the manufacturing of the stainless steel bracket which contains copper and zinc.[24,31] The quantity of the ions displayed a sharp decline with time reaching to the levels of 0.0004 ± 0.0005 ppm for copper & 0.0005 ± 0.0001 ppm for zinc at 28^{th} day (Table 1).

The study was conducted under invitro conditions and cannot be directly applied to the clinical scenario, however the results of the study will be useful for relative comparison of the quantities of ions released from the different group of brackets and also to determine effect of type of coating and time on the quantity of ions released. The release of ions from the nanocoatings cannot be prevented completely. However the variables affecting the biodegradation of the coatings can be identified in future studies, so that the release of ions can be kept well within the upper limit of their biological tolerance.

5. Conclusion

Brackets coated with combination of copper oxide and zinc oxide nanoparticles released lesser quantity of copper and zinc ions into the artificial saliva when compared with the copper or zinc oxide nanocoated brackets . The coated brackets released more ions than uncoated brackets and the highest surge of ion release was noted at the 7th day in all the three coated groups for both ions evaluated.

6. Source of Funding

None.

7. Conflict of Interest

None.

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