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Evaluation of cytotoxicity of copper oxide and zinc oxide hybrid nanocoated orthodontic brackets

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ABSTRACT

Background: Using a combination of two nanoparticles for coating stainless orthodontic brackets might alter their potential cytotoxicity. The study investigated the cytotoxicity of brackets nanocoated with copper oxide, zinc oxide and a combination of both the particles.

Materials and Methods: Stainless steel orthodontic brackets (Ormco Mini -Diamond series 0.22" slot, MBT prescription) (ORMCO CORP Glendora, California, USA) (n= 31 in each group) were coated with nanoparticles of copper oxide, zinc oxide and a combination of copper oxide –zinc oxide using a spray pyrolysis method. The brackets was assessed for cytotoxicity in mouse fibroblast (L929) using MTT assay with a standard control and a group of uncoated brackets for comparison. The optical density and percentage of cell viability of the 5 groups were compared with ANOVA and Post hoc Tuckey HSD. P value ≤ 0.05 was considered as statistically significant.

Results: The three groups of coated brackets and the uncoated brackets exhibited significantly lesser percentage of cell viability than the control group. The percentage of cell viability in all the four groups was greater than 70%**.** Zinc oxide and copper oxide nanocoated brackets exhibited lesser cell viability than the combination group and the uncoated brackets.

Conclusion: Brackets coated with combination of copper oxide and zinc oxide nanoparticles exhibited lesser cytotoxicity than the brackets coated with copper oxide or zinc oxide nanoparticles.

1. Introduction

Coating of orthodontic bands, brackets and archwires with nanoparticles possessing antimicrobial and solid lubricant properties have been extensively investigated in the past decade.Nanocoated orthodontic attachments demonstrated antibacterial properties and studies have proven that nanocoating could considerably reduce the friction generated during sliding of brackets along archwires.[1–9] Using a combination of two nanoparticles as a hybrid coating method improved the antibacterial properties and may also reduce the adverse effects of the individual particles.[9–11] Copper oxide and zinc oxide nanoparticle coated stainless-steel orthodontic brackets reduced the count of streptococcus mutans in cell culture and a hybrid nanocoating of these metal oxides obtained by mixing them in the ratio of 1:1 by weight was reported to have better antibacterial property than their individual coatings or when mixed with silver nanoparticles.[9–11]

Orthodontic brackets, bands, archwires, ligatures, solder joints, composite resins, mini-implants, and clear aligners are often examined for their toxicity to determine whether they release ingredients that can be harmful to cells.[12–18] The concerns about release of metal ions from the metallic orthodontic brackets have received a lot of attention.[19–21] Various testing methods were used to use to investigate

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the quantity of ions released from components of fixed orthodontic appliance and its potential cytotoxic effects. Amini et al. evaluated the release of nickel into the saliva of patients undergoing fixed orthodontic treatment using an atomic absorption spectrometry to compare it with their siblings of same gender who has not received any orthodontic appliances. They concluded that the components of fixed orthodontic appliances leach metal leading to an increase in their salivary concentration.[19]

Huang et al. evaluated the effect of recycling on the biocorrosion of orthodontic brackets stored in artificial saliva and buffers of various pH. The concentration of nickel, chromium, iron, and manganese released was detected by atomic absorption spectrometry and they confirmed that recycling increases the biocorrosion but the total ions released over a period of 12 weeks did not exceed the recommended daily dietary intake.[20] Kuhta et al. evaluated the effect of the composition of the archwire, the pH of artificial saliva and the duration of immersion on the release of six different type of ions including copper and zinc. They concluded that the ions released were sufficient to cause delayed hypersensitivity reactions and should be considered while selecting type of appliance and archwires in patients with hypersensitivity or compromised oral hygiene.[21]

Mobeen et al. evaluated the release of copper and zinc ions from copper-oxide and zinc- oxide nanoparticles coated orthodontic brackets in artificial saliva using an atomic absorption spectrometry. The findings concluded that leaching of zinc from the zincoxide nanocoated brackets was greater than that of copper from the copper oxide nanocoated brackets but the amount of both zinc and copper ions released over a period of 28 days was well below the levels that are toxic to humans.[22]

Jacoby et al. assessed the in-vitro cytotoxicity of orthodontic bands with and without silver solder in keratinocytes, fibroblasts and kidney epithelial cells using MTT assay and concluded that the cell lines showed decreased viability after exposure to the extracts from bands with solder joints.[12] Retamoso et al. evaluated the cytotoxicity of metal, nickel free metal, ceramic and polycarbonate brackets in mouse fibroblast (NIH/3T3) using a MTT assay and a microplate reader that recorded the optical density at 570nm and concluded that nickel-free brackets had better biocompatibility and polycarbonate brackets were cytotoxic.[14] Bracket identification dyes displayed cytotoxicity at higher concentrations when evaluated with MTT assay and a real-time cell analysis system.[18]

Ersoz et al. assessed the biocompatibility of three different light cure orthodontic resins using a real time cell analysis system in human gingival fibroblasts (HGF) and found that two of the composite resins tested low HGF index when exposed to the resin elutes for a longer period of time.[16] Bahrami et al. evaluated the cell viability of human gingival fibroblasts exposed to orthodontic bands coated with silver or zinc oxide nanoparticles and concluded that the zinc oxide coated bands had the highest biocompatibility.[23]

Even though the antibacterial and frictional properties of nanocoated orthodontic brackets have been investigated extensively, the hybrid coating using a combination of two nanoparticles and their cytotoxicity, which might be presumed to be less toxic than the individual materials, have not been previously examined. The present study, therefore evaluated the cytotoxicity of orthodontic brackets nanocoated with copper oxide, zinc oxide and a combination of both nanoparticles in L929 mouse fibroblasts using an MTT assay.

2. Materials and Methods

This study design was approved by 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 determination was performed using N-Master software (V2.0) and with a power of 80% and alpha error of 5%, the sample size arrived was 31 in each group. There were four groups, Group I brackets were coated with nanoparticles of copper oxide, Group II with nanoparticles of zinc oxide, Group III with a hybrid coating comprising a combination of copper oxide and zinc oxide and Group IV of uncoated stainless steel orthodontic brackets.

In total 136 Ormco Mini -Diamond series 0.22” slot, MBT prescription, stainless steel orthodontic brackets (ORMCO (ORMCO CORP Glendora, California, USA) were used in the study. Group I, II & III were allocated with 35 brackets and group IV with 31 brackets. The group I, II and III brackets were coated with nanoparticles of copper oxide, zinc oxide and a hybrid of copper oxide –zinc oxide using a spray pyrolysis method. Zinc oxide nanoparticles with an average size of 40 nm and copper oxide nanoparticles with an average particle size of 45 nm and 99.9% purity were procured (Ultrananotech, Bangalore, India).

The brackets to be coated were cleaned with deionized water and ethanol at 80̊ C for 30 minutes to get rid of the oxidized layer over the surface. The spray pyrolysis method was used to coat the brackets with the nano particles to achieve uniform coating using the spray pyrolysis equipment (Ho-TH-04, Holmark –optomechtronics Ltd, Kochi, kerala, India) The solution for the coating was prepared by diluting 0.3gms of zinc oxide nanoparticle powder in isopropanol solution for 1.5 hours and stirring well in an ultrasonic bath.[22] The distance between the spray nozzle and face of the bracket was maintained at 15cm and a thin film of zinc oxide nanoparticle was precipitated over the brackets as a uniform coating of 100 nm thickness

at150̊ C temperature and 0.5 Pa pressure. A similar method was used for coating the orthodontic brackets with copper oxide nanoparticles. The hybrid coating of copper oxide –zinc oxide was obtained by mixing the two nanoparticles in the ratio of 1:1 by weight.[9] Four coated brackets from each group were randomly selected and examined under SEM at different magnification to confirm the uniformity of coating and the particle size (Figures 1, 2 and 3). The rest 31 brackets were used for assessing the cytotoxicity.

The cytotoxicity was evaluated as per the specifications mentioned in the ISO10993-5 norm and as used in the previous studies.[14,18,24–27] The mouse fibroblast cell line (L929) used in the cytotoxicity evaluation was procured from the National Centre for Cell Science, Pune, India. The Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich Chemie, Germany) was used to culture the cells. The medium was supplemented with 1% penicillin, streptomycin and amphotericin B to prevent bacterial and fungal growth. Fetal bovine serum (10%) and L –glutamine was added to provide nutrition to the growing cells. The cells were incubated in humidified carbon dioxide incubator with 5% Carbon dioxide and 95% humidity at 37°C for 2 days. Once 70% confluence was reached, the confluent cells were detached using 0.025% trypsin and 0.05% EDTA. The cell suspension was shared to the flasks in a laminar flow cabinet equipped with ultraviolet light sterilization and centrifugation followed by pipetting until the target concentration of 2.5 $X10^6$ cell/mL was reached.

The percentage of viable fibroblasts was assessed using the colorimetric MTT assay, which measured the ability of succinate dehydrogenase, present in their mitochondria to reduce the yellow colour tetrazolium dye [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide] to purple colour formazan. The cells were seeded into 4 groups of 31 well plates and incubated. After the cells attained confluency, the autoclaved sterile brackets were placed in the cell plates and incubated at 37°C, in 5 per cent CO 2 -in-air for a period of 2 days. Culture medium with cells and without brackets were also incubated under the same conditions which served as control. Following the intubation, 200 μl of MTT dye for every ml of culture was added to each well and the plates were incubated at 37°C, and5 per cent CO 2 in air for a period of 4 hours. The MTT solution was prepared by dissolving 5mg of the dye in 1 ml of Phosphate buffered saline PBS and filtered through a 0.2 μm filter. After intubation, 300 μl dimethyl sulfoxide was added to each culture well incubated for 30 min to lyse the cell and obtain a homogenous colour. The solution was centrifuged for 2 min to sediment the cells and 100 μl from each well will be transferred to a new plate before measuring the optical density. The experiments were carried out in triplicates to increase the credibility of the results.

This assay is based on the capability of metabolically active fibroblast cells to reduce the yellow colored MTT salt to purple formazan crystals. The intensity of the purple colour obtained is proportional to the quantity of the viable cells and is measured as the degree of absorbance or optical density (OD) at 570 nm using an enzyme-linked immunosorbent assay reader (U2000, Hitachi, and Tokyo, Japan). The cell viability is calculated as the ratio of the optical densities of the experimental wells to that of the control wells.

The mean optical density and the percentage of cell viability was calculated (Table 1) (Figures 4 and 5). Intergroup comparison of optical densities and percentage of cell viability was done with ANOVA and Post hoc Tuckey HSD (Tables 2, 3, 4 and 5). A $P \leq 0.05$ was considered statistically significant.

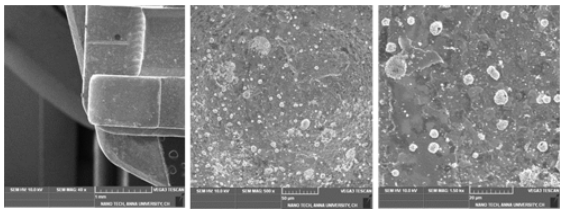

Figure 1: Scanning electronmicroscopic images of copper oxide nanocoated brackets

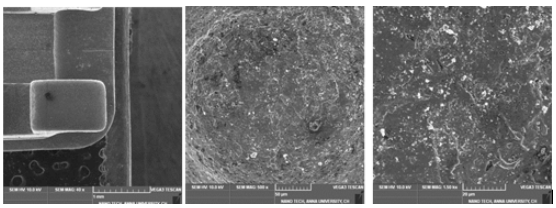

Figure 2: Scanning electronmicroscopic images of Zinc oxide nano coated brackets

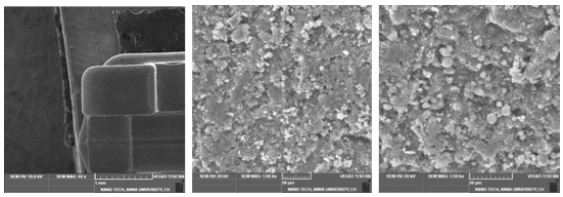

Figure 3: Scanning electronmicroscopic images of copper oxide-Zinc oxide nanocoated brackets

3. Results

The mean optical density from the MTT assay of the standard control was greater than the three experimental groups and the uncoated bracket group (1.35 ± .023) (p value= 0.00) (Tables 1, 2 and 3) (Figure 4). The lowest optical density was observed in the zinc oxide nanocoated

Table 1: Optical density and % of Cell viability values obtained from MTT assay of Group I (copper oxide nanocoated brackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of copper oxide –Zinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples and the standard control.

Groups (n=31)	Optical density		% of cell viability	
	Mean	SD	Mean	SD
Control	1.35	.023	100.00	.000
Group I	1.041	.12	77.45	8.183
Group II	.99	.082	73.61	6.438
Group III	1.16	.079	85.27	5.732
Group IV	1.20	.066	88.91	4.866

Table 2: ANOVA to compare the optical density obtained from MTT assay of Group I (copper oxide nanocoated brackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of copper oxide –Zinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples and the standard control.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.426	4	.607	96.671	.000
Within Groups	.941	150	.006		
Total	3.367	154			

Table 3: Post hoc Tuckey HSD to compare the optical density obtained from MTT assay of Group I (copper oxide nanocoated brackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of copper oxide –Zinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples and the standard control.

	Mean Difference	Std. Error	Sig.
Control vs Group I	.307161*	.020120	.000
Control vs Group II	.355710*	.020120	.000
Control vs Group III	.192645*	.020120	.000
Control vs Group IV	.150097*	.020120	.000
Group I vs Group II	.048548	.020120	.117
Group I vs Group III	-.114516*	.020120	.000
Group I vs Group IV	-.157065*	.020120	.000
Group II vs Group III	-.163065*	.020120	.000
Group II vs Group IV	-.205613*	.020120	.000
Group III vs Group IV	-.042548	.020120	.219

Table 4: ANOVA to compare the % of cell viability obtained from MTT assay of Group I (Group I (copper oxide nanocoated brackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of copper oxide –Zinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples and the standard control.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13243.148	4	3310.787	100.362	.000
Within Groups	4948.268	150	32.988		
Total	18191.415	154			

Table 5: Post hoc Tuckey HSD to compare the % of cell viability obtained from MTT assay of Group I (copper oxide nanocoated brackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of copper oxide –cinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples and the standard control.

	Mean Difference	Std. Error	Sig.
Control vs Group I	22.554*	1.459	.000
Control vs Group II	26.394*	1.459	.000
Control vs Group III	14.732*	1.459	.000
Control vs Group IV	11.093*	1.459	.000
Group I vs Group II	3.840	1.459	.070
Group I vs Group III	-7.822*	1.459	.000
Group I vs Group IV	-11.461*	1.459	.000
Group II vs Group III	-11.662*	1.459	.000
Group II vs Group IV	-15.300*	1.459	.000
Group III vs Group IV	-3.639	1.459	.097

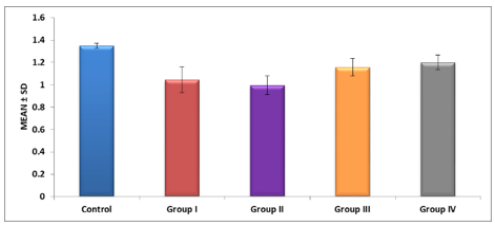

Figure 4: Mean optical density values obtained from the MTT assay of Group I (copper oxide nanocoatedbrackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of copper oxide –Zinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples with the standard control

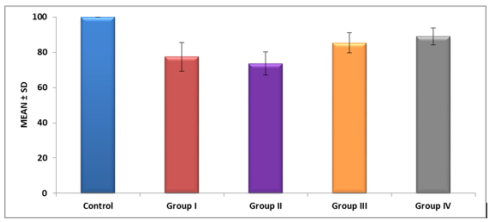

Figure 5: Percentage Cellviability values obtained from MTT assay of Group I (copper oxide nano coatedbrackets), Group II (Zinc oxide nanocoated brackets), Group III (Combination of Copper oxide –Zinc oxide nanocoated brackets), & Group IV (uncoated Brackets) samples and the standard control.

group (.99±.082) (Table 1). The mean value of copper oxide group was slightly higher but was not significantly different from that of the zinc group (1.041±.12.) (p value= 0.00) (Tables 1, 2 and 3).

The nanocoated brackets exhibited toxic effects at different levels on the fibroblasts when compared with the control group and the uncoated bracket. Cells exposed to copper oxide & zinc oxide nanocoated brackets resulted in lower percentage of cell viability (73.61±6.438) (77.45±8.183) (Tables 1, 4 and 5,) (Figure 5). The greatest value of optical density and cell viability was observed in copper oxide –zinc oxide hybrid nano coated brackets which exhibited toxicity levels similar to that of uncoated brackets. (88.91±4.866.) (85.27±5.732) (p value= 0.00)(Tables 1, 2, 3, 4 and 5.

4. Discussion

Cytotoxicity assays estimate the quantity of cells that are viable and thereby provide a measure of cell death caused by the direct contact of the material or their eluates.[28] Cytotoxicity refers to the chain of events which results in functional and structural damage of a cell after exposure to a substance.[29] The apparent cytotoxicity of a material can be significantly affected by the cell line used for the assay and hence the choice of cells remains a crucial factor.[30] Different cell lines including human gingival fibroblasts, keratinocytes, human endothelial cells, mouse fibroblasts, osteogenic precursor cells from mice and HeLa cells are used to assess the cytotoxicity of nanoparticles in dental field. The mouse fibroblast (L-929) is a permanent cell line with good reproducibility and more sensitivity to toxic effects than human fibroblasts.[27,31] Hence in the current study, the mouse fibroblast (L-929) cell line was used to evaluate the cytotoxicity of orthodontic brackets coated with nano particles of copper oxide and zinc oxide.

Cytotoxity evaluation of a material can be performed on an extract of the test sample or the sample can be directly used. Majority of the previous studies evaluating the toxicity of orthodontic brackets have used the eluates obtained at different time interval.[27] In the current study the brackets were used directly placed in the cell culture to observe the effects on the culture cells. Quantitative methods were used by measuring the reduction of the visible dye and measuring the colour change as the optical density of the medium as they are more reliable than the qualitative methods.[27]

Coating the stainless steel orthodontic brackets with copper and zinc nanoparticles improves the antimicrobial properties and reduce the frictional resistance but their potential cytotoxicity is a major concern. Studies have reported the toxicity of copper and zinc nanoparticles but they are not adequate to understand their cytotoxic potential completely as it is influenced by many factors.[32] Copper and zinc are micronutrients required for normal functioning of the body but when they exceed the tolerance level, they can cause toxic effects in the respiratory tract, nervous, excretory system or gastrointestinal tract based on the portal of entry.[32] Kim et al. demonstrated that laser generated ultra-pure copper nanoparticles possess moderate cytotoxicity to human cells in a cell-dependent manner.[33] The nanoparticles have the tendency to be more toxic than the copper microparticles as they can penetrate the body through skin contact, inhalation, and ingestion and smaller particles at higher concentration exerted maximum toxic effects on the cell viability.[34,35] The dose and size dependent cytotoxicity of copper nano particles have been reported by several studies.[34–38] Zinc oxide nanoparticles produced oxidative stress and exhibited size and dose dependent cytotoxicity in type II alveolar epithelial cells, lung epithelial cells, hepatic cells, skin fibroblasts and astrocytes.[39–44] Hence it became important to assess the cytotoxicity of nanocoated brackets. In the current study zinc oxide nanoparticles with an average size of 40 nm and copper oxide nanoparticles with an average particle size of 45 nm was used to coat the brackets.

The cytotoxicity of brackets coated with the combination of copper oxide and zinc oxide nano particles was noted to be similar to the uncoated brackets and lesser than the copper and zinc oxide coated brackets. This may be attributed to their dose dependent cytotoxic nature and the reduction in quantity of the particles used in the combination

coating as discussed by Zeidan et al. who evaluated the antibacterial effect of a combination of silver and zinc oxide nanoparticles coating of orthodontic brackets.[10] The uncoated brackets exhibited a certain level of reduction in the cell viability which may be due to the release of nickel, chromium, iron and other ions. This is in agreement with the previous studies published in the literature.[12,14,19,21]

The zinc oxide group exhibited the least cell viability and this may be due to the greater leaching tendency of the zinc ions from the coatings as confirmed by Mobeen et al. who noted that the quantity of zinc leached was greater than of copper from the respective coated brackets.[22] They noted that the quantity of zinc and copper ions leached were well below the levels that can elicit systemic toxicity from ingestion in humans until 28th day of immersion.[22] Materials that exhibit cell viability lesser than 70% of the cell viability of the positive control are considered to possess potent cytotoxic activity and cannot be used safely in patients.[27] MTT assay is a sensitive assay with an excellent linearity up to 10^6 cells per well and even a small change in metabolic activity of the cells can generate a large variation in the findings, allowing one to detect cell stress upon exposure to a toxic agent even in the absence of cell death.[45] The percentage of cell viability as calculated from the MTT assay for all the four group of brackets evaluated were greater than 70% of that of the positive control. This suggests that both zinc and copper oxide nanocoated stainless steel brackets can be used safely in patients to reduce the incidence of enamel decalcification and resistance during sliding.

5. Conclusion

Within the limitations of the study, it can be concluded that the nanocoating of copper and zinc oxide nanoparticles did not increase the toxicity levels of the stainless steel brackets beyond the critical levels above which the materials are considered potentially cytotoxic. The hybrid coating with a combination of copper and zinc oxide nanoparticles mixed in the ratio of 1:1 resulted in reduced cytotoxicity than separate coating of the same nano materials. Their toxicity levels were as low as that of uncoated brackets.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Metin-Gursoy G, Taner L, Akca G. Nanosilver coated orthodontic brackets: In vivo antibacterial properties and ion release. *Eur J Orthod*. 2017;39:9–16.
2. Salehi P, Babanouri N, Peikar MR, Zare F. Long-term antimicrobial assessment of orthodontic brackets coated with nitrogen-doped titanium dioxide against Streptococcus mutans. *Prog Orthod*. 2018;19(1):35.
3. Shah AG, Shetty PC, Ramachandra CS, Bhat NS, Laxmikanth SM. In vitro assessment of photocatalytic titanium oxide surface modified stainless steel orthodontic brackets for antiadherent and antibacterial properties against Lactobacillus acidophilus. *Angle Orthod*. 2011;81(6):1028–63.
4. Ameli N, Asadi S, Ghorbani R, Mohebi S, Hans M. Comparative antibacterial efficacy of orthodontic brackets coated with titanium dioxide, copper oxide, and hydroxyapatite-silver nanoparticles against Streptococcus mutans. *Middle East J Rehabil Health Stud*. 2022;9(2):119536.
5. Mayma N, Sangeetha D, Ravi K. Evaluation of antimicrobial property of nanochitosan coated orthodontic brackets against Streptococcus mutans and Lactobacillus acidophilus - an in vitro study. *APOS Trends Orthod*. 2023;13:82–90.
6. Kachoei M, Eskandarinejad F, Divband B, Khatamian M. The effect of zinc oxide nanoparticles deposition for friction reduction on orthodontic wires. *Dent Res J*. 2013;10(4):499–505.
7. Singh PI, Sharma D, Shukla VK, Chaturvedi NK. The effect of various nanoparticle coating on the frictional resistance at orthodontic wire and bracket interface: A systematic review. *J Orthod Sci*. 2022;11:1–7.
8. Shah PK, Sharma P, Goje SK. Comparative evaluation of frictional resistance of silver-coated stainlesssteel wires with uncoated stainless-steel wires: An In vitro study. *Contemp Clin Dent*. 2018;9(2):331.
9. Ramazanzadeh B, Jahanbin A, Yaghoubi M, Shahtahmassbi N, Ghazvini K, Shakeri M. Comparison of antibacterial effects of ZnO and CuO nanoparticles coated brackets against Streptococcus mutans. *J Dent (Shiraz)*. 2015;16(3):200–5.
10. Zeidan NK, Enany NM, Mohamed GG, Marzouk ES. The antibacterial effect of silver, zinc-oxide and combination of silver/zinc oxide nanoparticles coating of orthodontic brackets (an in vitro study). *BMC Oral Health*. 2022;22:230.
11. Duraisamy S, Anandan N, Kannan R. Evaluation of antibacterial effect of hybrid nano-coating of stainless steel orthodontic brackets on Streptococcus mutans – An in vitro study. *J Pharm Bioall Sci*. 2024;16:S1784–91.
12. Jacoby LS, Junior R, Campos VDS, and MD. Cytotoxic outcomes of orthodontic bands with and without silver solder in different cell lineages. *Am J Orthod Dentofac Orthop*. 2017;151(5):957–63.
13. Santos RL, Pithon MM, Martins FO, Romanos MT, Ruellas AC. Cytotoxicity of latex and non-latex orthodontic elastomeric ligatures on L929 mouse fibroblasts. *Braz Dent J*. 2010;21(3):205–10.
14. Retamoso LB, Luz TB, Marinowic DR. Cytotoxicity of esthetic, metallic, and nickel-free orthodontic brackets: cellular behaviour and viability. *Am J Orthod Dentofac Orthop*. 2012;142:70–4.
15. Alves CB, Segurado MN, Dorta MC, Dias FR, Lenza MG, Lenza MA. Evaluation of cytotoxicity and corrosion resistance of orthodontic mini-implants. *Dental Press J Orthod*. 2016;21(5):39–46.
16. Ersoz M, Malkoç S, uç¨uk E, Bozkurt BS, Hakki SS. Biocompatibility evaluation of orthodontic composite by realtime cell analysis. *Hum Exp Toxicol*. 2016;35(8):833–8.
17. Eliades T, Pratsinis H, Athanasiou AE, Eliades G, Kletsas D. Cytotoxicity and estrogenicity of Invisalign appliances. *Am J Orthod Dentofac Orthop*. 2009;136(1):100–3.
18. Celebi F, Altun A, Bicakci AA. Cytotoxicity of bracket identification dyes. *Angle Orthod*. 2019;89(6):426–31.
19. Amini F, Jafari A, Amini P, Sepasi S. Metal ion release from fixed orthodontic appliances–an in vivo study. *Eur J Orthod*. 2012;34(1):126–56.
20. Huang TH, Yen CC, Kao CT. Comparison of ion release from new and recycled orthodontic brackets. *Am J Orthod Dentofac Orthop*. 2001;120(1):68–75.
21. Kuhta M, Pavlin D, Slaj M, Varga S, Varga ML, Slaj M, et al. Type of Archwire and Level of Acidity: Effects on the Release of Metal Ions from Orthodontic Appliances. *Angle Orthod*. 2009;79:102–10.
22. Mobeen N, Duraisamy S. Ravi Evaluation and comparison of copper and zinc ion release from copper-oxide and zinc- oxide nanoparticles

coated orthodontic brackets - An In vitro Study. *Int J Orthod Rehabil*. 2022;13(3):10–21.
23. Bahrami R, Pourhajibagher M, Badiei A, Masaeli R, Tanbakuchi B. Evaluation of the cell viability and antimicrobial effects of orthodontic bands coated with silver or zinc oxide nanoparticles: An in vitro study. *Korean J Orthod*. 2023;53(1):16–25.
24. Kao CT, Ding SJ, Min Y, Hsu TC, Chou MY, Huang TH. The cytotoxicity of orthodontic metal bracket immersion media. *Eur J Orthod*. 2007;29(2):198–203.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55–63.
26. Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. *J Immunol Methods*. 1986;94(1-2):57–63.
27. International Organization for Standardization. International standard ISO 10993-5, Biological evaluation of medical devices. 1992. Part 5: Tests for cytotoxicity: in vitro methods. Geneva (SH;. p. 1992–1992.
28. Murray PE, Lumley PJ, Ross HF, Smith AJ. Tooth slice organ culture for cytotoxicity assessment of dental materials. *Biomaterials*. 2000;21:1711–1721.
29. Aldridge WN. The biochemical principles of toxicology. *Exp Toxicol*. 1993;5:56–78.
30. Wataha JC, Hanks CT, Sun Z. Effect of cell line on in vitro metal ion cytotoxicity. *Dent Mater*. 1994;10:156–161.
31. Preclinical evaluation of biocompatibility of medical devices used in dentistry-test methods for dental materials. *International Standards Organisation*. 1996;.
32. Ingle AP, Duran N, Rai M. Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. *Appl Microbiol Biotechnol*. 2014;98(3):1001–1010.
33. Kim YS, Shin S, Park SM, Hah SS. Comparative toxicity studies of ultra-pure Ag, Au, Co, and Cu nanoparticles generated by laser ablation in biocompatible aqueous solution. *Bull Korean Chem Soc*. 2012;33:3265–3268.
34. Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, et al. Acute toxicological effects of copper nanoparticles in vivo. *Toxicol Lett*. 2006;163:109–120.
35. Prabhu BM, Ali SF, Murdock RC, Hussain SM, Srivatsan M. Copper nanoparticles exert size and concentration dependent toxicity on somatosensory neurons of rat. *Nanotoxicology*. 2010;4(2):150–160.
36. Xu P, Xu J, Liu S, Ren G, Yang Z. In vitro toxicity of nanosized copper particles in PC12 cells induced by oxidative stress. *J Nanopart Res*. 2012;14:906–907.
37. Laha D, Pramanik A, Maity J, Mukherjee A, Pramanik P, Laskar A, et al. Interplay between autophagy and apoptosis mediated by copper oxide nanoparticles in human breast cancer cells MCF7. *Biochim Biophys Acta*. 2013;(13):355–358.
38. Siddiqui MA, Alhadlaq HA, Ahmad J, Al-Khedhairy AA, Musarrat J, Ahamed M. Copper oxide nanoparticles induced mitochondria mediated apoptosis in human hepatocarcinoma cells. *PLoS One*. 2013;8(8):69534–69534.
39. Xie Y, Williams NG, Tolic A, Chrisler WB, Teeguarden JG, Maddux BL, et al. Aerosolized ZnO nanoparticles induce toxicity in alveolar type II epithelial cells at the air-liquid interface. *Toxicol Sci*. 2012;125:450–461.
40. Wang Y, Aker WG, Hwang HM, Yedjou CG, Yu H, Tchounwou PB. A study of the echanism of in vitro cytotoxicity of metal oxide nanoparticles using catfish primary hepatocytes and human HepG2 cells. *Sci Total Environ*. 2011;409:4753–4762.
41. Wang J, Deng X, Zhang F, Chen D, Ding W. ZnO nanoparticleinduced oxidative stress triggers apoptosis by activating JNK signalling pathway in cultured primary astrocytes. *Nanoscale Res Lett*. 2014;9:117–117.
42. Kermanizadeh A, Gaiser BK, Hutchison GR, Stone V. An in vitro liver model-assessing oxidative stress and genotoxicity following exposure of hepatocytes to a panel of engineered nanomaterials. *Part Fibre Toxicol*. 2012;9:28–28.
43. Hsiao IL, Huang YJ. Effects of various physicochemical characteristics on the toxicities of ZnO and TiO2 nanoparticles toward human lung epithelial cells. *Sci Total Environ*. 2011;409:1219–1228.
44. Yuan JH, Chen Y, Zha HX, Song LJ, Li CY, Li JQ, et al. Determination, characterization and cytotoxicity on HELF cells of ZnO nanoparticles. *ColloidsSurf B Biointerfaces*. 2010;76:145–150.
45. Kumar P, Nagarajan A, Uchil PD. Analysis of Cell Viability by the MTT Assay. *Cold Spring Harb Protoc*. 2018;1:6.

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