Original Article

Metal Release and Cytotoxicity of Different Orthodontic Bracket-Wire Combinations: An *In Vitro* Study

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Abstract

Aim: To quantify and compare the metal ions released from different bracket-wire combinations and to assess their cytotoxicity. **Materials and Methods:** A total of 360 fabricated sectional fixed orthodontic appliances were divided into 6 groups. The first three groups consisted of stainless-steel brackets with stainless-steel, snickel-titanium (NiTi), and titanium-molybdenum alloy (TMA) archwires, and the other three groups were fabricated using ceramic brackets (polycrystalline alumina) with stainless-steel, NiTi, and TMA archwires. These appliances were immersed in artificial saliva (pH 6.5 ± 0.5, 37°C), for 1 week, 2 weeks, and 1 month. The nickel and chromium ions released in the artificial saliva were quantified using a flame atomic absorption spectrometer, and cytotoxicity assessment was performed using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay on human cervical cancer cell line. **Results:** The stainless-steel bracket groups displayed a significantly greater release of nickel and chromium ions compared to the ceramic bracket groups ($P < 0.05$). However, no significant differences were identified when comparing the three archwire types within the stainless-steel/ceramic bracket groups. At the end of 1 month, the % cell viability demonstrated by the appliances was in the decreasing order of stainless-steel-TMA > ceramic-stainless steel > stainless-steel-NiTi > ceramic-NiTi > stainless-steel-stainless steel > ceramic-TMA. **Conclusion:** Considerably greater release of nickel and chromium ions was observed from the appliances utilizing stainless-steel brackets compared to those employing ceramic brackets. However, no remarkable difference in the levels of nickel and chromium ions was observed when comparing the three archwires: stainless steel, NiTi, and TMA. In the cytotoxicity assessment, the ceramic-TMA combination displayed the highest level of cytotoxicity, while the stainless-steel-TMA combination displayed the least cytotoxicity.

Keywords: *Ceramic, cytotoxicity, nickel titanium, stainless steel, titanium molybdenum alloy*

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Introduction

Intraoral orthodontic appliances are made from alloys of different metals.[1] Stainless-steel alloys (18-8) serve as the primary alloy in manufacturing orthodontic bands, brackets, and archwires.[2]

The metal alloys in saliva are thermodynamically unstable and have a tendency to change from a solid state to an ionic form.[3] Previous studies have demonstrated negligible levels of cytotoxicity for various orthodontic appliances.[4,5] However, metal ions in direct contact

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with the oral mucosa, while simultaneously being mechanically irritated by orthodontic appliances, have the potential to induce cellular damage in oral mucosa cells, even if they do not reach hazardous levels in susceptible individuals.[6] Nickel is a strong immunologic sensitizer, while chromium ions leached from metal alloys negatively affect some enzyme activity, oxygen consumption, and intracellular adenosine triphosphate levels and are considered mutagenic.[7]

In clinical practice, archwires are employed with brackets that might alter the corrosion rate due to the potential difference between the alloys used. The results of several research on toxicology and ion release of orthodontic materials are inconsistent, making it impossible to make any firm conclusions. Considering this knowledge gap, the present study was designed to compare the metal release and cytotoxicity of different bracket-wire combinations frequently used in clinical practice. The null hypothesis was that there is no difference in the release of nickel and chromium ion in all the wires and brackets combinations and its cytotoxicity.

Materials and Methods

Setting and design

The present *in vitro* observational study was conducted over a period of 2 years in the Department of Orthodontics and Dentofacial Orthopedics, Dr. Z. A. Dental College, Aligarh Muslim University after getting approval from the Institutional Ethical Committee. The observer was trained to follow a standardized protocol. The materials used in the study were stainless-steel brackets (Gemini MBT. 022, 3M Unitek), ceramic brackets (Premium Aesthetic series MBT. 022, Koden), stainless-steel archwires (0.017 × 0.025-in. rectangular, Ormco), nickel-titanium (NiTi) archwires (0.017 × 0.025-in. rectangular, Ormco), titanium-molybdenum alloy (TMA) archwires (0.017 × 0.025-in. rectangular, Ormco), bondable first molar tubes (Metro Orthodontics), stainless-steel ligature wire (Metro Orthodontics), artificial saliva, human cervical cancer cell line (HeLa Cell Line, from National Centre for Cell Science, Pune) and sterilized glass tubes.

Fabrication of sectional fixed orthodontic appliances

The sectional fixed orthodontic appliances were fabricated using the brackets of central incisor, lateral incisor, canine, first premolar, second premolar, and a bondable first molar tube of a single quadrant of the upper arch [Figure 1]. These brackets were ligated to 0.017 × 0.025-in. rectangular archwire of 5 cm length using stainless-steel ligature ties. The archwires were bent at both ends to prevent the brackets from sliding off the archwire.

A total of 360 fabricated appliances were divided into 6 groups of 60 each depending on the type of archwires and brackets by a blinded laboratory assistant, who randomly allocated the sampling units to the respective groups used as follows.

Study groups were group 1—-stainless-steel wire ligated with stainless-steel brackets (SS-SS), group 2—NiTi wire ligated with stainless-steel brackets (SS-NiTi), group 3—TMA wire ligated with stainless-steel brackets (SS-TMA), group 4—stainless-steel wire ligated with ceramic brackets (C-SS), group 5—NiTi wire ligated with ceramic brackets (C-NiTi), and group 6—TMA wire ligated with ceramic brackets (C-TMA). A control group was with simulated artificial saliva without incubated with any orthodontic appliances.

Preparation of artificial saliva

The simulated artificial saliva was prepared using 0.4 g NaCI, 1.21 g KCI, 0.78 g $NaH_2PO_4 \cdot 2H_2O$, 0.005 g $Na_2S.9H_2O$, 1 g urea [$CO(NH_2)_2$], and 1000 mL distilled and deionized water. This formula is the modification of composition used by Gjerdet and Herø,[8] in which $CaCl_2 \cdot H_2O$ is substituted with an equimolar amount of KCL. This was done because $CaCl_2 \cdot H_2O$ in the original formula had an interfering effect on chromium absorbance in the air–acetylene flame.

Quantitative assessment of metal ions

A total of 180 sectional appliances, 30 from each group, were immersed in 50 mL of artificial saliva in 180 sterilized glass tubes and were incubated at 37°C. These glass tubes were color-coded into six groups [Figure 2].

Figure 1: Simulated orthodontic appliances

By the end of 1 week (T1), 2 weeks (T2), and 1 month intervals (T3), 20 mL of artificial saliva was collected from each tube and analyzed for nickel and chromium ions using a flame atomic absorption spectrophotometer. In our study, we used the air–acetylene flame because it has minimal enhancing interfering effect by other metal ions on chromium absorbance.

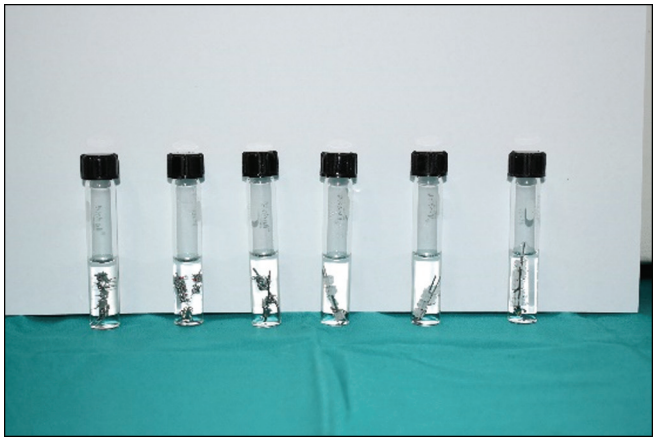

Figure 3: Simulated orthodontic appliances immersed in artificial saliva for cytotoxicity assessment

Figure 2: Simulated orthodontic appliances immersed in artificial saliva for quantitative assessment

Cytotoxicity assessment

A total of 180 sectional appliances, 30 from each group, were immersed in 3 mL of artificial saliva [Figure 3] in 180 sterilized glass tubes and were incubated at 37°C. At T1, T2, and T3, saliva sample from each tube was subjected to cytotoxicity assessment using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay on the HeLa cell line.

Statistical analysis

The data were collected and analyzed for normality. For normally distributed quantitative data, analysis of variance was used. For the intergroup comparison, the *post hoc* Tukey's test was done. All analysis was done using SPSS [version 22.1]. The level of significance was kept at $P \leq 0.05$. The results were expressed as mean ± standard deviation.

Results

Quantitative assessment of metal ions

The nickel and chromium ions released from stainless-steel bracket groups were significantly greater than the control group ($P = 0.000$). However, the ceramic bracket groups showed a non-significant increase in nickel and chromium levels [Table 1]. On comparing the groups based on the type of archwires used, the groups that employed NiTi archwires (SS-NiTi and C-NiTi) displayed increased nickel release followed by stainless-steel (SS-SS and C-SS) and TMA (SS-TMA and C-TMA) at all time intervals [Table 2]. The chromium release was greater from stainless-steel wire groups followed by NiTi and TMA archwires

Table 1: Comparison of metal ions released among the three archwires used with stainless-steel and ceramic brackets at T1, T2, and T3

	P value									
	Stainless-steel bracket groups				Ceramic bracket groups					
	1-2	2-3	3-1	(1, 2, and 3)-C	4-C	5-C	6-C	4-5	5-6	4-6
Comparison of nickel ions										
T1	1.000	0.916	0.930	0.000*	0.225	0.076	0.532	0.999	0.941	0.998
T2	1.000	0.782	0.904	0.000*	0.944	0.791	0.991	1.000	0.992	1.000
T3	1.000	0.992	0.999	0.000*	0.477	0.358	0.984	1.000	0.845	0.922
Comparison of chromium ions										
T1	0.807	1.000	0.690	0.000*	0.129	0.306	0.563	0.999	1.000	0.976
T2	1.000	1.000	1.000	0.000*	0.888	0.933	0.983	1.000	1.000	1.000
T3	0.991	1.000	0.985	0.000*	0.941	0.974	0.993	1.000	1.000	1.000

*Analysis of variance with *post hoc* Tukey's honestly significant difference test. *P* value ≤ 0.05 is significant

T1 = 1 week, T2 = 2 weeks, T3 = 1 month

Table 2: Concentration of metal ions (in ppm) released from simulated orthodontic appliances (mean ± standard deviation)

	SS-SS (1)	SS-NiTi (2)	SS-TMA (3)	C-SS (4)	C-NiTi (5)	C-TMA (6)	Control (C)
Concentration of nickel ions							
T1	0.211 ± 0.113	0.213 ± 0.089	0.174 ± 0.086	0.082 ± 0.069	0.099 ± 0.072	0.063 ± 0.060	0.000
T2	0.621 ± 0.355	0.647 ± 0.352	0.507 ± 0.264	0.101 ± 0.076	0.138 ± 0.079	0.069 ± 0.027	0.000
T3	0.636 ± 0.354	0.66 ± 0.312	0.597 ± 0.216	0.172 ± 0.057	0.189 ± 0.078	0.072 ± 0.041	0.000
Concentration of chromium ions							
T1	0.230 ± 0.106	0.193 ± 0.074	0.188 ± 0.065	0.071 ± 0.042	0.059 ± 0.036	0.048 ± 0.031	0.000
T2	0.456 ± 0.209	0.446 ± 0.217	0.438 ± 0.209	0.076 ± 0.044	0.068 ± 0.054	0.051 ± 0.022	0.000
T3	0.754 ± 0.334	0.697 ± 0.243	0.691 ± 0.221	0.083 ± 0.027	0.070 ± 0.027	0.055 ± 0.039	0.000

T1 = 1 week, T2 = 2 weeks, T3 = 1 month, SS-SS = stainless-steel wire ligated with stainless-steel brackets, SS-NiTi = nickel-titanium wire ligated with stainless-steel brackets, SS-TMA = titanium-molybdenum alloy wire ligated with stainless-steel brackets, C-SS = stainless-steel wire ligated with ceramic brackets, C-NiTi = nickel-titanium wire ligated with ceramic brackets, C-TMA= titanium-molybdenum alloy wire ligated with ceramic brackets

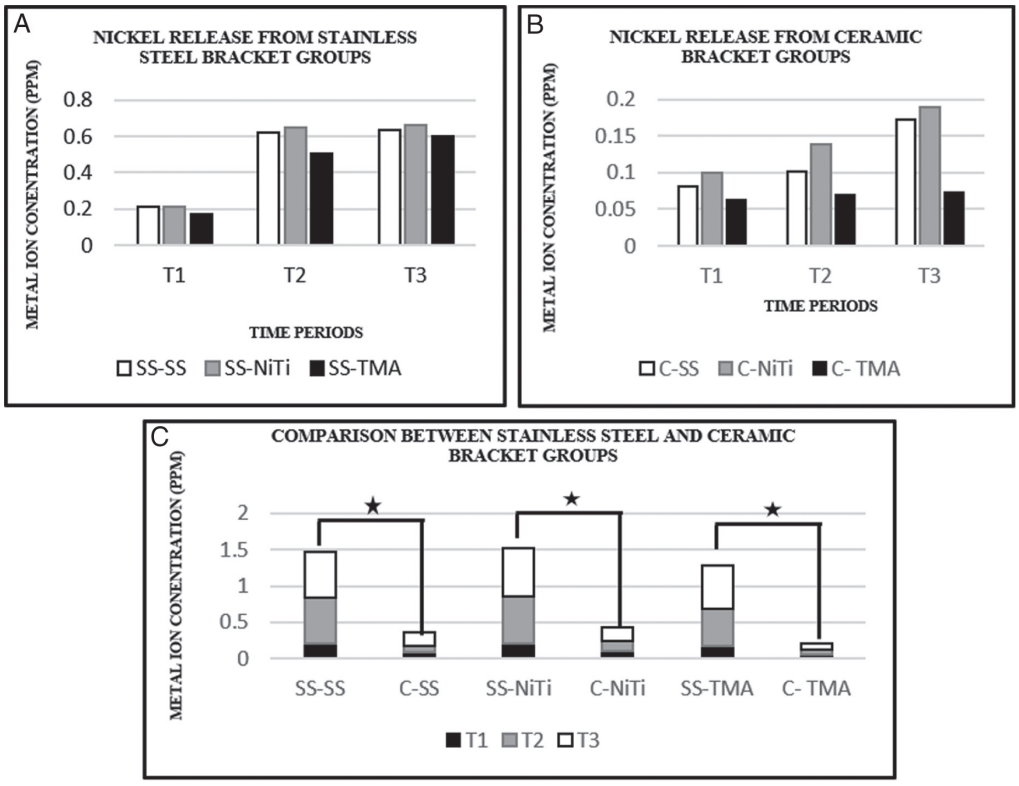

Figure 4: (A) Comparison of nickel release among the stainless-steel bracket groups. (B) Comparison of nickel release among the ceramic bracket groups. (C) Comparison of nickel release between stainless-steel and ceramic bracket groups (indicates $P \leq 0.05$)

[Table 2]. However, the difference was not statistically significant [Figures 4, 5A and B]. On comparing the groups based on the type of brackets used, stainless-steel bracket groups (SS-SS, SS-NiTi, and SS-TMA) showed significantly increased nickel and chromium levels than their ceramic counterparts (C-SS, C-NiTi, and C-TMA) at all time intervals [Figures 4 and 5C].

Cytotoxicity assessment

Among the stainless-steel bracket groups, the SS-NiTi displayed a significantly decreased % cell viability than SS-SS and SS-TMA groups at T1 and T2. However, at T3, SS-SS group displayed significantly increased level of cytotoxicity than SS-NiTi. On comparing SS-SS and SS-TMA, SS-SS showed a significant decrease in % cell viability than SS-TMA at T2 and T3 [Tables 3 and 4; Figure 6A].

In the ceramic bracket groups, C-TMA showed a significant decrease in % cell viability than C-NiTi and C-SS, except at T1, when no significant difference was found between C-TMA and C-NiTi. C-NiTi showed a

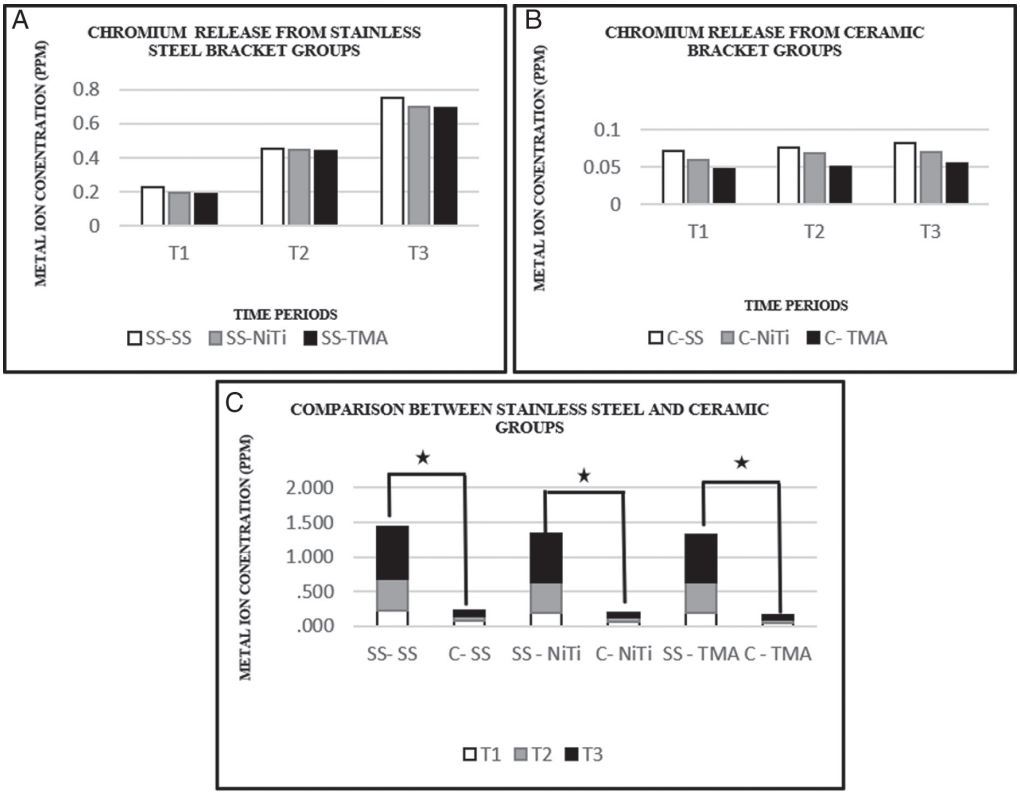

Figure 5: (A) Comparison of chromium release among the stainless-steel bracket groups. (B) Comparison of chromium release among the ceramic bracket groups. (C) Comparison of chromium release between stainless-steel and ceramic bracket groups (indicates $P \leq 0.05$)

Table 3: % Cell viability of the stainless steel and ceramic bracket groups

	SS-SS (1)	SS-NiTi (2)	SS-TMA (3)	C-SS (4)	C-NiTi (5)	C-TMA (6)
T1	93.48 ± 2.28	77.72 ± 4.76	92.76 ± 3.52	77.28 ± 11.47	58.84 ± 9.65	51.28 ± 4.72
T2	77.81 ± 4.79	60.88 ± 2.38	82.40 ± 4.26	70.78 ± 1.11	54.09 ± 1.67	44.90 ± 2.00
T3	45.40 ± 1.77	55.80 ± 6.25	67.40 ± 5.41	61.32 ± 8.40	49.76 ± 13.31	38.32 ± 2.34

T1 = 1 week, T2 = 2 weeks, T3 = 1 month, SS-SS = stainless-steel wire ligated with stainless-steel brackets, SS-NiTi = nickel-titanium wire ligated with stainless-steel brackets, SS-TMA = titanium-molybdenum alloy wire ligated with stainless-steel brackets, C-SS = stainless-steel wire ligated with ceramic brackets, C-NiTi = nickel-titanium wire ligated with ceramic brackets, C-TMA= titanium-molybdenum alloy wire ligated with ceramic brackets

significant decrease in % cell viability than SS-SS at T1, T2, and T3 [Tables 3 and 4; Figure 6B].

On comparing stainless-steel and ceramic bracket groups, C-SS showed a significant decrease in % cell viability than SS-SS at T1 and T2. At T3, SS-SS showed a significant decrease in % cell viability than C-SS [Figure 7A]. C-NiTi showed a significant decrease in % cell viability than SS-NiTi at T1 and T2. No significant difference was found between them at T3 [Figure 7B]. C-TMA showed a significant decrease in % cell viability than SS-TMA at all time intervals [Figure 7C].

Table 4: Comparison of % cell viability among the three archwires used with stainless-steel and ceramic brackets at T1, T2, and T3

	P value					
	Stainless-steel bracket groups			Ceramic bracket groups		
	1-2	2-3	3-1	4-5	5-6	4-6
T1	0.000*	0.000*	1.000	0.000*	0.160	0.000*
T2	0.000*	0.000*	0.015*	0.000*	0.000*	0.000*
T3	0.029*	0.011*	0.000*	0.011*	0.012*	0.000*

*Analysis of variance with *post hoc* Tukey's honestly significant difference test. $P \leq 0.05$ is significant

T1 = 1 week, T2 = 2 weeks, T3 = 1 month

Discussion

In the present study, the null hypothesis was rejected. Stainless-steel bracket groups displayed significantly increased nickel and chromium ion release than ceramic bracket groups. No significant difference in nickel and chromium release was observed in comparing the three stainless-steel bracket groups. Several previous studies have reported comparable levels of nickel release from

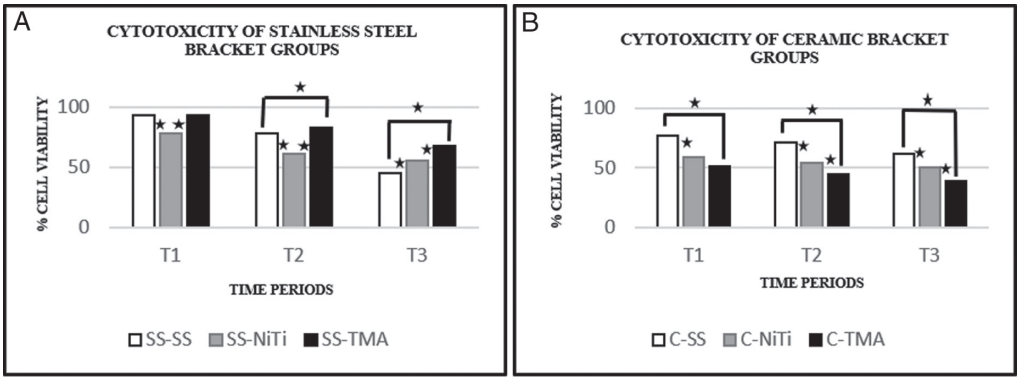

Figure 6: (A) Comparison of cytotoxicity among the stainless-steel bracket groups. (B) Comparison of cytotoxicity among the ceramic bracket groups (indicates $P \leq 0.05$)

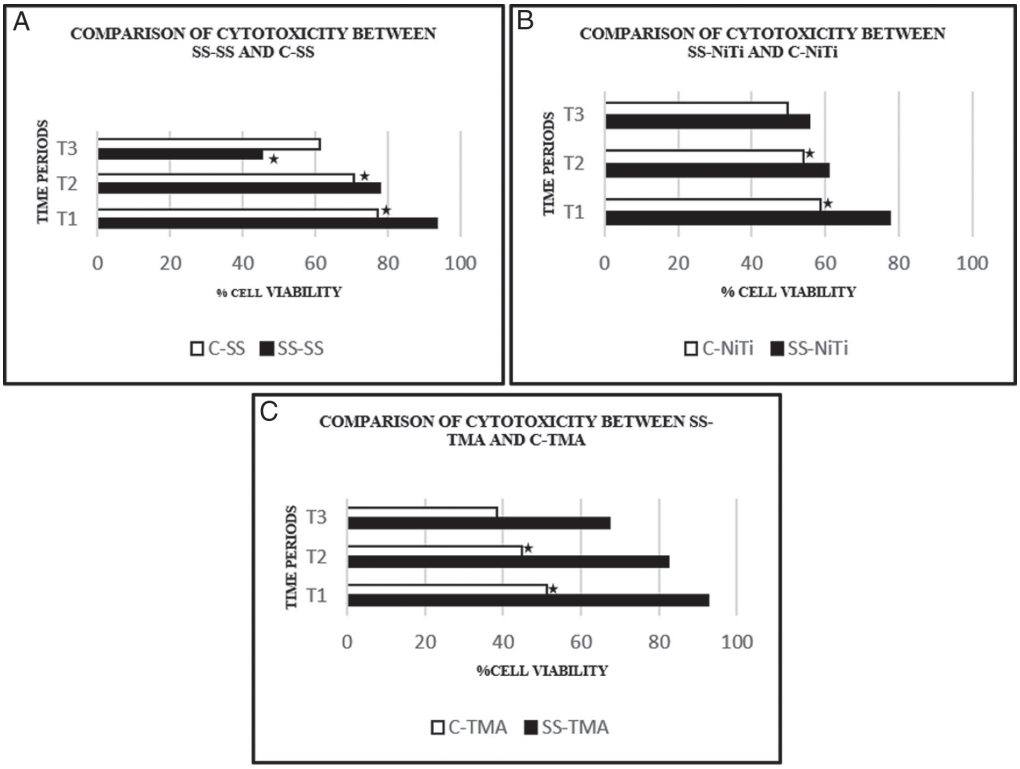

Figure 7: Comparison of cytotoxicity between the stainless-steel and ceramic bracket groups. (A) Cytotoxicity comparison between SS-SS and C-SS. (B) Cytotoxicity comparison between SS-NiTi and C-NiTi. (C) Cytotoxicity comparison between SS-TMA and C-TMA (indicates $P \leq 0.05$)

stainless-steel and NiTi archwires.[9,10] On the contrary, in a study conducted by Hwang *et al.*,[2] the stainless-steel group released increased nickel than the NiTi group. This might have resulted from the heat treatment of stainless-steel archwires that would deteriorate the corrosion resistance property resulting in intergranular corrosion. In the current study, stainless-steel wires were used in "as received" form and were not subjected to any heat treatment.

The metal ions released from the ceramic bracket groups were significantly lesser than the stainless-steel bracket groups due to the decreased overall metal content in the simulated ceramic appliances and decreased incidence of galvanic corrosion with the use of ceramic brackets.

A significant increase in nickel release was observed during first and second weeks from the SS-NiTi and SS-TMA groups and during first week from the SS-SS group, after which it leveled off. Similar findings were observed in previous studies.[9,10] This can be elucidated by two rationales given by Bishara *et al.*[11] First, the depletion of surface nickel during first week. Second, corrosion products may have formed on the surface

slowing down nickel release.[9] The second statement cannot be contemplated in the present study because there was an uninterrupted increase of chromium ions till 1 month from the appliances. A significant increase in chromium release was observed during first and second weeks from all the stainless-steel bracket groups.

In the present study, the cancer cell line was preferred to other cell lines for cytotoxicity assessment because HeLa cell lines are metabolically active cells and can provide a better understanding of the variables being studied. In contrast to the notion that ceramic brackets are inert in nature,[12] the ceramic bracket groups in the present study demonstrated higher levels of cytotoxicity than the metallic bracket groups. In hydrolysis testing, ultra-low-temperature sintering ceramics showed greater solubility than conventional high-temperature sintering ceramics.[13] Ceramic brackets were also believed to be capable of inducing reversible cellular alterations in buccal epithelial cells and human gingival fibroblasts,[14] supporting our assertion that they are not inert. When exposed to a corrosive environment, alumina, which was thought to be a very stable material, generated nanoparticles and microparticles.[13]

During first and second weeks, among the stainless-steel bracket groups, SS-NiTi showed significantly increased cytotoxicity than SS-SS and SS-TMA. This corresponds to the increased release rate of nickel and chromium in SS-NiTi during first and second weeks. But, at the end of 1 month, the SS-SS group displayed an increased level of cytotoxicity than the SS-NiTi and SS-TMA groups. This can be explained by the conspicuous release of chromium from SS-SS till T3. Although we analyzed the quantities of nickel and chromium in the current study, additional metal ions released by the appliances should also be considered when evaluating the overall cytotoxicity. For instance, iron that constitutes 50% of the composition of stainless steel is a redox-active metal and is considered to be potentially toxic.[15]

A decreased level of cytotoxicity C-SS group than the other two ceramic bracket groups can be explained by a reduced amount of galvanic corrosion in the bracket-wire combination. The increased potential difference between nickel and titanium in NiTi archwires would have initiated galvanic corrosion within the NiTi archwire. C-TMA (38.32% at T3—moderate cytotoxicity according to Ahrari *et al*.[16]) displayed an increased level of cytotoxicity than the other two groups. This could be due to the release of molybdenum from these wires. Molybdenum exhibited moderate to severe cytotoxicity against osteogenic sarcoma cell lines and has the least safe ion concentration of 0.008 ppm.[17]

This is further supported by a study by Yanisarapan *et al*,[18] in which the TMA group showed an increased level of cytotoxicity than the stainless-steel and NiTi groups. Molybdenum from archwire and aluminum/ Al_2O_3 from ceramic brackets might well have coupled to cause this upsurge in cell death. A study conducted to determine the cytotoxic effect of Al_2O_3 and TiO_3 nanoparticles against HeLa cell lines concluded that these nanoparticles possess an anticancer effect and inhibit cell growth.[19]

The study's limitations include the fact that it was conducted *in vitro*, they do not reflect the actual circumstances of the oral cavity. Thus, *in vitro* cytotoxicity testing outcomes cannot be directly extrapolated to clinical circumstances. It would only discuss a few biological aspects of orthodontic appliances and their subcomponents. Additional future studies are required.

Conclusion

The sectional appliances that utilized stainless-steel brackets displayed significantly increased nickel and chromium release than the appliances with ceramic brackets. However, no considerable difference in metal release was observed when comparing the three archwires (stainless steel, NiTi, and TMA). At the end of 1 month, the % cell viability demonstrated by the appliances were in the decreasing order of stainless-steel-TMA > ceramic-stainless steel > stainless-steel-NiTi > ceramic-NiTi > stainless-steel-stainless steel > ceramic-TMA.

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The project was self-financed.

Conflicts of interest

The authors have no conflict of interest.

Authors contributions

All Authors were involvement in conduct of the experimental portion of the study. All Authors were involved in reviewing the data and its analysis, and preparation of the manuscript.

Ethical policy and institutional review board statement

The study was approved by the Institutional Ethical Committee (Ref. IECJNMC/529) of the Faculty of Medicine, Aligarh Muslim University.

Patient declaration of consent

Not applicable, as this was an invitro study with no human subjects being involved.

Data availability statement

The data is available with the corresponding author in the Department of Orthodontics and Dentofacial Orthopedics, Dr. Z. A. Dental College, Aligarh Muslim University, India – 202001.

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