Estimation of Release of Nickel and Chromium by Indian Made Orthodontic Appliance in Saliva

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ABSTRACT

Introduction: With increasing use of Indian made orthodontic materials, need was felt to know nickel and chromium release from these material.

Materials and Methods: This study was conducted on simulated appliances consisting of brackets (022"Roth, Modern orthodontics, Ludhiana, India), from second premolar to central incisor, buccal tube and 0.019×0.025- inch SS arch wires secured with SS ligatures. Immersion was done in artificial saliva. Samples were analysed to using Atomic Absorption Photospectrometer (GVC ScientificEquipment Pvt. Ltd Australia) at AES Laboratories (P) Ltd., Noida India on 1st, 7th, 14th and 28th day.

Results: SPSS (ver 17, Inc., Chicago, Illinois, USA) was used toperform the statistical analysis. Descriptive statistics i.e. median and 25 and75 percentiles were used. Peak nickel release was on 7th day and subsequently declined over 14th and 28th day. The peak level of chromium concentration was on 14th day, which declined thereafter.

Conclusion: Average daily release of nickel and chromium over a period of one month was 97.368 μg/day and 47.664 μg/day respectively. The estimated release rates were approximately 32% and 16% of the reported average daily dietary.

INTRODUCTION

Most orthodontic brackets are made from AISI type 304L and 316 L stainless steel [1,2]. Such an alloy contains 17–22% chromium and 8–12% nickel with a small amount of manganese, copper, titanium, iron and silicon and has a low carbon content, typically less than 0.03% [2,3]. In addition, wires of other compositions and differing mechanical properties have become available.

With current popularity of arch wires that have a high concentration of nickel and/or titanium such as Nitinol (NiTi) and TMA (TiMo), and with the increasing popularity of recycled brackets, it is conceivable that a greater liability to corrosion products could occur [4] and other health problems have always been of much debate and concern [5,6].

Among the metallic corrosion products that may enter the body, nickel ion has received considerable attention, due to its carcinogenic, allergenic, mutagenic, cytotoxic and genotoxic effects [3]. Various reports have indicated that 4.5% to 28.5% of the population is sensitive to nickel and condition is more common among females [7-9]. Nickel is a strong bioallergen that causes a type IV "delayed" hypersensitivity reaction [10]. Park and Shearer reported an average release of 40μg nickel and 39μg chromium per day from a simulated full-mouth fixed appliance [11]. Nickel sensitization is thought to be increased by mechanical irritation, skin laceration, mucosal injury, increased environmental temperature and exposure to the allergen [1]. These factors may occur during orthodontic treatment. Clinical features of intraoral nickel allergy include stomatitis, perioral rashes, loss of metallic taste, numbness, burning sensation and soreness at the lateral borders of the tongue, severe gingivitis in the absence of plaque or angular cheilitis [12].

After nickel, Cr is believed to cause allergic reaction and is carcinogenic. It has been suggested that chromium content of 16–27% will provide the optimal corrosion resistance for nickel-based alloys [13]. The average dietary intake for chromium has been estimated to be 280 μg/day. For nickel it is 200- 300 μg/day [13,14].

Release of Ni and Cr from orthodontic appliances has been a controversial subject with many studies indicating that the release of ions is significant to cause potential allergic and cytotoxic effect [10,15]. However, other studies refuse these claims. With increasing use of Indian made materials in the present orthodontic scenario, it was felt that there was need to know the nickel and chromium release from these material as well.

AIM AND OBJECTIVES

1. To assess the amount of nickel and chromium release from Indian made orthodontic brackets, bands and arch wires.

2. To examine the effects of immersion time on nickel and chromium release.

MATERIALS AND METHODS

This study was done on simulated fixed orthodontic appliances [Table/Fig-1]. Each represented half of the maxillary arch consisting of five bracket (022"Roth, Modern orthodontics, Ludhiana, India), from second premolar to central incisor, a molar band with a buccal tube, and an arch wire 0.019×0.025- secured with stainless steel ligatures. Inner surfaces of the bands or the bonding surface of the

[Table/Fig-1]: Simulated fixed orthodontic setup

brackets were not covered with the cement or composite Maxillary molar tubes had double buccal tubes with hooks. The arch wire, band material and ligature wire were American Iron and Steel Institute (AISI) type 304 and Bondable brackets were AISI type 316 [Table/Fig-2].

The arch wire length was determined from an ideal typhodont set-up and measured distally from the right first molar tube to the left first molar tube. The teeth were ligated to the arch wires with standard 0.010-inch stainless steel ligature wires. The arch wire was then cut between the two maxillary central incisors such that each half represented aquadrant.

To simulate conditions in the oral cavity, artificial saliva of neutral pH was used. Selection was based on average pH in the oral cavity (6.75) [16]. Artificial saliva was prepared with the formula of Barret, Bishara and Quinn [9] which is a modification of that used by Gjerdet and Hero [17]. All measurements were done with electronic weighing device [Table/Fig-3]. The simulated saliva medium consisted of 0.4 gm NaCl, 1.21 gm KCl, 0.78 gm $NaH_2PO_4.2H_2O$, 0.005 gm $Na_2S.9H_2O$,1 gm Urea {CO $(NH_2)2$} and 1000 ml distilled and deionized water [Table/Fig-4].

The artificial saliva formula was a modification of that used by Gjerdet and Hero, the difference being that their formula included 0.795 gm of $CaCl_2$. H_2O and only 0.4 gm of KCl. The pH values were adjusted with the use of 10 M sodium hydroxide (NaOH). To prevent interferences occurred with the atomization of chromium in the atomic absorption spectrophotometer caused by the presence of the calcium ions it was decided to substitute calcium chloride with potassium chloride. The work of Brown and Merritt [18] indicated an increased corrosion rate when saline solutions contained serum proteins hence protein was also added to the formula. Albumin was selected as the protein component because of its presence in saliva and its ready availability [16].

A total of 20 simulated fixed appliances were used, each one representing maxillary quadrant [Table/Fig-5]. Polyethylene bottles were used for the storage, each containing 5 ml of artificial saliva at the neutral pH (6.75±15) [Table/Fig-6]. Before use, all glassware were rinsed at least three times with deionized water, inverted and allowed to dry. All bottles and laboratory equipment were digested with 65% nitric acid (HNO_3) diluted in water (1:4) to remove any residual organic matter. The bottles and all laboratory equipment were washed in deionized water and dried. All water used in this study was deionized using a 3-stage water purification system (SG Corp, India) to remove any potential metal contamination from the glassware [Table/Fig-7].

The specimens were then rinsed with a mixture of 1:1 ethanol/acetone and air-dried under a cleaned hood, after which the appliances were distributed in polyethylene bottles and were stored at 37°C in an incubator [Table/Fig-8].

At the end of 24 hours, 2 ml of saliva was removed from each of the 20 samples for spectrophotometric analysis. To keep the released ions stable in the solution one drop of 65% nitric acid was added to the samples. The artificial saliva solutions were replaced after each sample collection to avoid saturation with corrosion products. Bottles were then re-stored at 37°C, and similar such 2 ml samples were collected from the 20 bottles containing the appliance immersed in artificial saliva at 7, 14 and 28 days. The samples were analysed to assess the concentration of Ni and Cr ions using ATOMIC ABSORPTION PHOTOSPECTROMETER (GVC scientific equipment pvt. ltd Australia) at the AES LABORATORIES (P) LTD. which is accredited to NABL (T-0176, T-0719, T-0410), recognized by Bureau of India Standards (BIS) and The Export Inspection Council of India (EIC) [Table/Fig-9].

The instrument was calibrated using commercially available nickel and chromium standard stock solution to prepare working

[Table/Fig-2]: Orthodontic materials (Modern orthodontics, Ludhiana). [Table/Fig-3]: Electronic weighing machine. [Table/Fig-4]: Constituents of artificial saliva. [Table/Fig-5]: Twenty Samples in the polyethylene bottles. [Table/Fig-6]: Appliance dipped in the artificial saliva. [Table/Fig-7]: Three stage water purification system. [Table/Fig-8]: Incubator. [Table/Fig-9]: Atomic absorption spectrophotometer. [Table/Fig-10]: Close-up view of auto sampler unit with graphite furnace

standard of 5, 10, 15 and 20 ppb (parts per billion) with distilled and deionized water. Calibration plots were generated at the start of every run using freshly prepared working standards. This instrument allows measurement of extremely low concentrations (ppb) of released nickel and chromium ions. For every metal ions analysed, characteristic wavelengths are generated in a hollow cathode lamp and in turn are absorbed by a vapour of that element. The amount of absorption is proportional to the concentration of the element that is vapourized into the light beam. Twenty µl of the sample was injected directly into the graphite tube from an automated micropipette and sample changer [Table/Fig-10].

The tube was heated electrically by passing a current of 5.0 mA through it in a programmed series of steps that included 25 seconds at 120^0c to evaporate the solvent, 6 seconds at 950^0c to drive off any volatile organic material and char the sample to ash and 5 seconds at 2100^0c to vapourize and atomize the elements. A beam of electromagnetic radiation from a hollow cathode lamp specific for nickel (232 nm) and chromium (357.9 nm) is passed through the vapourized sample. The metal atoms in the sample absorb some of the radiation. This gives the absorbance of the sample, which enables the calculation of the nickel and chromium concentration in the unknown sample from the standard calibration curves. The concentrations of nickel and chromium were expressed in parts per billion. Each sample was analysed 3 times and the mean value was used. Heating the graphite tube to 2300^0 creadies it for the next sample.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS ver. 17, Inc., Chicago, Illinois, USA) was used toperform the statistical analysis. Descriptive statistics such as median and 25 and 75 percentiles were used. Two–way repeated measures analysis of variance by ranks (Friedman test) was used to test the statistical significance of differences inconcentrations of each metal on 1,7,14 and 28 days of immersion of fixed orthodonticappliances. Post-hoc pair wise comparisons among groups of the same element were calculated using the Wilcoxan signed rank test. Pairwise comparisons of nickel and chromium were also performed using the Mann-Whitney test.

RESULTS

Changes in nickel concentration

At the end of 1st day, median nickel concentration was 17.50 ppb which reached peak on day 7, steadily decreased during the

Days	N	Mean (Ppb)	Std. Deviation	Min	Max	Percentiles		
						25th	50th (Median)	75th
Ni 1	20	17.87	2.02	15.33	22.43	16.35	17.50	19.48
Ni 7	20	35.30	4.30	26.41	40.10	32.65	37.09	38.49
Ni 14	20	28.63	7.03	15.37	39.48	22.28	29.06	33.72
Ni 28	20	15.56	5.10	7.37	22.98	11.34	15.47	21.08

[Table/Fig-11]: Salivary nickel concentrations (Ppb) at different time periods

Test Statistics for nickel	
N	20
Chi – Square	45.18
p – value	0.01

[Table/Fig-12]: Friedman test showing test the statistical significance of differences inconcentrations of Nickel on 1,7,14 and 28 days

Pairs	Ni 7 – Ni 1	Ni 14 – Ni 1	Ni 28 – Ni 1	Ni 14 – Ni 7	Ni 28 – Ni 7	Ni 28 – Ni 14
p – value	0.01	0.01	.067	.001	0.01	0.01

[Table/Fig-13]: Post-hoc pairwise comparisons by Wilcoxan signed rank test for nickel release

subsequent 3-week period. After 1week median salivary nickel concentrations increased to 37.088 ppb. There was a decrease of nickel concentration to 15.468 ppb at the end of 4 weeks [Table/Fig-11].

The chi-square test indicated a statistically significant difference in nickel concentrations released in the artificial saliva with time (p= 0.01) [Table/Fig-12]. Statistically significant differences were observed when comparing 1 day, 7 days, 14 days and 28 days levels of nickel using the Friedman test [Table/Fig-12]. Post-hoc pair wise comparisons of nickel release at different time periods showed statistically significant differences which revealed significant result on 1st day with 7 and 14 days, 7 days with 14 and 28 days, 14 with 28 days except one with 28 days [Table/Fig-13].

Changes in chromium concentration

At the end of 1st day median chromium concentration was 5.481 ppb which showed the greatest increase through day 14 and reduced then on till day 28. Concentration increased to 11.383 ppb at the end of 7 days, 19.310 ppb at the end of 14 days and then reduced to 9.755 ppb at the end of 28 days [Table/Fig-14].

The analysis of variance indicated a statistically significant difference in chromium concentrations released in the artificial saliva with time (p=0.01). Statistically significant differences were observed when comparing 1day, 7 days, 14 days and 28 days levels of chromium using the Friedman test [Table/Fig-15]. Post-hoc pair wise comparisons of chromium at different time periods showed statistically significant differences which revealed significant result on 1st day with 7, 14 days and 28 days, 7 days with 14 days, 14 with 28 days except 7 with 28 days [Table/Fig-16].

Comparison between nickel and chromium concentration

Comparison between nickel and chromium concentration was done using Mann Whitney's test. The nickel concentration in the artificial saliva was at a significantly higher level than that of chromium at

Days	N	Mean (Ppb)	Std. Deviation	Min	Max	Percentiles		
						25th	50th (Median)	75th
Cr 1	20	6.57	3.50	2.38	14.26	3.60	5.48	9.20
Cr 7	20	11.10	4.41	4.98	19.62	7.06	11.38	14.49
Cr 14	20	19.12	6.82	7.09	31.29	12.99	19.31	24.64
Cr 28	20	10.88	4.81	4.05	22.54	7.33	9.76	14.05

[Table/Fig-14]: Salivary chromium concentrations (Ppb) at different timeperiods of Chromium release

| Test Statistics for chromium	
N	20
Chi–Square	48.78
p–value	0.01

[Table/Fig-15]: Friedman test showing test the statistical significance of differences inconcentrations of Chromium on 1,7,14 and 28 days.

Pairs	Cr 7 – Cr 1	Cr14 – Cr1	Cr28– Cr1	Cr 14– Cr7	Cr28– Cr7	Cr 28– Cr14
p – value	0.01	0.01	.067	.001	.709	0.01

[Table/Fig-16]: Wilcoxan signed rank testPairwise comparisons of chromium release

Pairs	Mann Whitney U	p
Ni 1 and Cr 1	0.01	0.01
Ni 7 and Cr 7	0.01	0.01
Ni 14 and Cr 14	67	.0.01
Ni 28 and Cr 28	101	.007

[Table/Fig-17]: Mann–Whitney U-Test Pairwise comparisons of nickel and chromium
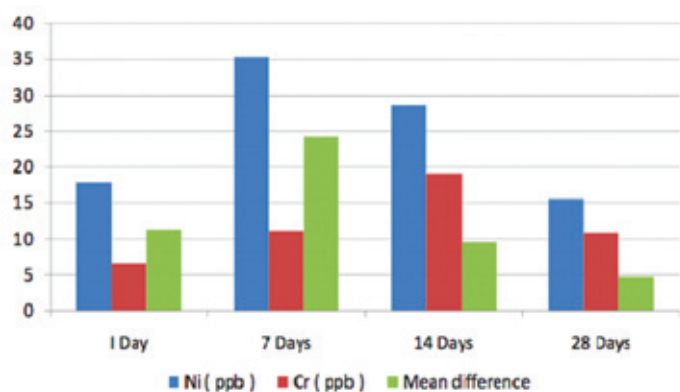
[Table/Fig-18]: Mean difference of nickel and chromium concentrations at different time periods

each time period. There was highly significant difference on day 1,7 and 14 (p =0.01) and only a significant difference at day 28(p=0.008) [Table/Fig-17]. The smallest difference was recorded on day 28 where the nickel concentration averaged 1.43 times the chromium concentration. The greatest difference occurred at day 7 where the nickel levels were 3.18 times those of chromium [Table/Fig-18].

DISCUSSION

Several studies have demonstrated that metal ions released from fixed orthodontic appliances, primarily nickel and chromium, can cause allergic reactions and act as a carcinogen and mutagen [3,4,19,20].

Recently there has been an increasing trend towards using Indian made orthodontic material because of easy availability directly from the manufacturers and cost effectiveness. There is however very less literature that focuses on their biocompatibility. Hence, it was necessary to investigate the release of nickel and chromium concentration from fixed orthodontic material from Indian manufacturers. The material used in this study was made by Modern orthodontics Ludhiana, as these are the commercially available indigenous suppliers following the AISI standards.

The average daily release of nickel from the simulated appliance over a period of one month was 24.342 ppb ± 9.421 SD. The range being 14.921 to 33.763 ppb. After one day mean nickel release was 17.868 ppb. Peak nickel release was on 7th day and subsequently declined over the 14th and 28th day. The results of present study were similar to that Park and Shearer, Menne et al., and Marek and Treharne, Singh DP, Rajasekharan Ajith, who also found that the corrosion of appliance reached the plateau after 7 days and does not increase appreciably thereafter [11,21-24]. The reason for this finding could be explained on the following basis; first the nickel present on the surface of stainless steel may quickly corrode during the first 7 days from the fixed orthodontic appliance used in the study, then the rate of release declined as the surface nickel is depleted. Second corrosion products may have formed on the surface after 7 days slowing the corrosion of nickel.

When the finding pertaining to chromium level was taken into consideration, the average daily release of chromium was 11.916 ppb ± 6.719 SD and the range being 5.197 to 18.706 ppb. After one day mean chromium release was 6.572 ppb. The peak level of chromium concentration was on 14th day which declined thereafter. The result were similar to that Barret & Bishara and Hwang et al., who also found that the corrosion of appliance reached the maximum level after 14 days and does not increase appreciably thereafter [7,9].

With both nickel and chromium, significant changes were found at various time interval (p=0.01). This suggested that both nickel and chromium were not equally dissolved into the artificial saliva. The chromium concentration lagged behind the corresponding nickel concentration. This finding was similar to the study done

by Maja Kuhta [13]. At every time interval (1, 7, 14 and 28 days) the chromium concentration was significantly less than the nickel concentration. In a one month period, the release of nickel and chromium concentration gradually declined after the initial rise with the period of increase being the only variable factor. In case of nickel peak reached on 7th day, whereas in case of chromium it was 14th day. The reason for this could be the ability of chromium to form oxide layer on the surface and slow release in the saliva. The results of our study were similar to that obtained by Barret and Bishara [7] who evaluated nickel and chromium release from fixed orthodontic appliance using atomic absorption spectrophotometer.

The average daily release of nickel concentration in our study was 24.342 µg when only one quadrant of the orthodontic setup was used. Multiplying these values four times to simulate the equivalent release from a fully banded and bonded maxillary and mandibular appliance, increases the nickel release rate to 97.368 µg/day. Similarly for chromium it would be 47.664 µg/day. Our study found the level of nickel andchromium from Indian made orthodontic materials was higher than that of material used in other studies. Study done by Barret & Bishara [7] found the average nickel concentration per day as 26.1 µg and chromium 0.7 µg. Park and Shearer revealed average nickel and chromium release as 40 µg and 36 µg per day respectively [11]. The reason of greater nickel and chromium release could be the fact that the inner surfaces of bands were not covered with cement and mesh surface of brackets were not coated with adhesive in this study. It could also be attributed to the manufacturing process of these materials and the procedure, which was performed during study. Though the levels of nickel and chromium ions released were lesser than the average daily intake (200 – 300 µg/day for nickel and 280 µg/day for chromium) [7,25-28] this small amount of release might still produce hypersensitivity when orthodontic appliances are in place for 2-3 years.

Clinical Implication

The average daily release of metals ions was 97µg for nickel and 47µg for chromium per day for a full-mouth appliance set up. This was below the average dietary intake of nickel and chromium consumed (200–300 µg for Ni and 280 µg for Cr). However, these values were higher compared to orthodontic material used in studies done by MT Costa, MA Lenza, HY Park, TR Shear [4,11]. The clinician should be aware that release of nickel and chromium from orthodontic set up might sensitize patients to nickel and chromium and may cause hypersensitivity reactions in patients with a prior history of hypersensitivity [11]. The clinician should also be able to diagnose sign and symptoms of hypersensitivity due to metal ions. In patients known to be hypersensitive to nickel, the use of nickel free, ceramic, polycarbonate and recently introduced Ti brackets can provide a feasible alternative. Titanium brackets have found to exhibit mechanical properties, corrosion resistance and bond strength equivalent to or better than their stainless steel counterpart [28].

CONCLUSION

1. The nickel and chromium concentration significantly increased after placing the fixed orthodontic setup into the artificial salivary medium.

2. Peak nickel release was on 7th day and subsequently declined over the 14th and 28th day.

3. The average daily release of nickel over a period of one month was 97.368µg/day (Normal Daily intake 200 – 300 µg / day).

4. The peak level of chromium concentration was on 14th day, which declined thereafter. When the finding pertaining to chromium level was taken into consideration the average daily release of chromium was 47.664 µg/day (Normal daily intake 280 µg/day).

5. The estimated release rates from simulated orthodontic appliances areapproximately 32% and 16% of the reported average daily dietary intake fornickel and chromium respectively.

6. At every time interval (1, 7, 14 and 28 Days) the chromium concentration was significantly less than the nickel concentration. In the study the release of nickel is 2.04 times more than chromium.

REFERENCES

[1] Eliades T, Zinelis S, Eliades G, Athanasiou AE. Nickel content of asreceived, retrieved, and recycled stainless steel brackets. *Am J Orthod Dentofacial Orthop*. 2002;122:217–20.

[2] Maijer RI, Smith DC. Biodegradation of the orthodontic bracket system. *Am J Orthod Dentofacial Orthop.* 1986;90:195–98.

[3] Gursoy S, Acar AG, Sesen C. Comparison of metal release from new and recycled bracket-archwire combinations. *Angle Orthod*. 2005;75:92–94.

[4] Costa MT, Lenza MA, Gosch CS, Costa I, Ribeiro-Dias F. In vitro Evaluation of Corrosion and Cytotoxicity of Orthodontic Brackets. *J Dent Res.* 2007;86(5):441-45.

[5] Kocadereli I, Ataç A, Kale S, Özer D. Salivary nickel and chromium in patients with fixed orthodontic appliances. *Angle Orthod*. 2000;70:431–34.

[6] Janson GR, Dainesi EA, Consolaro A, Woodside DG, de Freitas MR. Nickel hypersensitivity reaction before, during, and after orthodontic therapy. *Am J Orthod Dentofacial Orthop*. 1998;113:655-60.

[7] Barret RD, Bishara SE, Quinn JK. Biodegradation of nickel and chromium in vitro. *Am J Orthod Dentofacial Orthop*. 1993;103:8–14.

[8] Huang TH, Yen CC, Kao CT. Comparison of ion release from new and recycled orthodontic brackets. *Am J Orthod Dentofacial Orthop*. 2001;120:68–75.

[9] Hwang CJ, Shin JS, Cha JY. Metal release from simulated fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop*. 2001;120:383–91.

[10] Kolokitha OE, Chatzistavrou E. A Severe Reaction to Ni-Containing Orthodontic Appliances. *Angle Orthod*. 2009;79:186-92.

[11] Park HY, Shearer TR. Invitro release of nickel and chromium from simulated orthodontic appliances. *Am J Orthod Dentofacial Orthop*. 1983;84(2):156-59.

[12] Bass JK, Fine H, Cisneros GJ. Nickel hypersensitivity in the orthodontic patient. *Am J Orthod Dentofacial Orthop.* 1993;103:280-85.

[13] Kuhta M, Pavlin D, Slaj M, Varga S, Lapter-Varga M, Slaj M. Type of Arch wire and Level of Acidity: Effects on the Release of Metal Ions from Orthodontic Appliances. *Angle Orthod.* 2008;79(1):102–10.

[14] Sfondrini MF, Cacciafesta V, et al. Chromium Release from New Stainless Steel, Recycled and Nickel-free Orthodontic Brackets. *Angle Orthod*. 2008;79(2):361-67.

[15] Talic NF, Al Mudhi A, Al Enzy A, Al Qahtani F. Prevalence of nickel hypersensitivity among Saudi dental patients in the Riyadh area: a preliminary study. *Rev Clín Pesq Odontol*. 2007set/dez;3(3):159-63.

[16] Afonsky D. Saliva and its relation to oral health. Montgomery: University of Alabama Press; 1984. Pp.101-05.

[17] Gjerdet NR, Herø H. Metal release from heat-treated orthodontic arch wires. *Acta Odontol Scand*. 1987;45(6):409-14.

[18] Brown SA, Merrit K. Electrochemical corrosion in saline and serum. *J Biomed Mater Res*. 1980;14:173-75.

[19] Costa M. Molecular mechanisms of nickel carcinogenesis. *Toxicol Appl Pharmacol*. 1997;143:152-66.

[20] Wataha J C, Sun ZL, Hanks CT, Fang DN. Efeect of nickel ions on expression of intercellular adhesion. *J Biomed Mater Res*. 1997;36:145-51.

[21] Menne T, Brandrup F, Thestrup-Pedersen K, et al. Patch test reactivity to nickel alloys. *Contact Dermatitis*. 1987;16:255-59.

[22] Marek M, Treharne RW. An invitro study of the release of nickel from two surgical implant alloys. *Clin Orthop*. 1982;167:291-95.

[23] Singh DP, Sehgal V, Pradhan KL, Chandna A, Gupta R. Estimation of nickel and chromium in saliva of patients with fixed orthodontic appliances. *World J Orthod*. 2008;9(3):196-202.

[24] Ajith R, Anil G, Satheesh K, Ramshad AR, Ajay P, Neha G. Release of Nickel and Chromium Ions from Different Brands of Stainless Steel Brackets. An Invitro Study. *IOSR Journal of Dental and Medical Sciences*. 2014;13(7):63-67.

[25] International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol. 23. Chromium and chromium compounds. Leon, France: IARC, 1980: pp. 205-324.

[26] Grandjean P. Human exposure to nickel. *IARC Sci Publ*. 1984;53:469-85.

[27] Schroeder HA, Balassa JJ, Tipton IH. Abnormal trace metals in man— nickel. *J Chron Dis.* 1962;15:51-65.

[28] Eliades T, Athanasiou AE. In Vivo Aging of Orthodontic Alloys: Implications for corrosion potential, Nickel Release, and Biocompatibility. *Angle Orthod*. 2002;72:222-37.

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