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RESEARCH ARTICLE

IN VITRO RELEASE OF NICKEL AND CHROMIUM FROM NEW STAINLESS STEEL BRACKETS INTO SIMULATED ORAL ENVIRONMENT AT DIFFERENT PH, AND UNDER MANUAL BRUSHING

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ABSTRACT

Objective: To examine the effects of three different parameters—pH value, brands of bracket, and role of brushing—on release of metal ions from orthodontic appliances.

Materials and Methods: Simulated fixed orthodontic appliances which consist of brackets with covered base were immersed in artificial saliva of different pH values (4.2, 6.5 and 7.6) during 28-day period. Three different brands of stainless steel brackets were used: Gemini(3M), Abzil(3M), Orthox(JJ ortho) series. All the brackets embedded in self cure and the effect of brushing on ions release have been evaluated. The quantity of metal ions was determined with the use of a Atomic absorption spectrophotometer.

Results: The release of Nickel and chromium ions were observed. Results showed that (1) The Ni and Cr released from all the brands of brackets; (2)The change in pH had a strong effect on the release of ions; and (3) When compared between brushing and non brushing group maximum amount of Ni and Cr released from brushing group at all the days.

Conclusion: Levels of released ions are sufficient to cause delayed allergic reactions. This must be taken into account when different brand of brackets is selected, especially in patients with hypersensitivity or compromised oral hygiene.

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INTRODUCTION

Metal is a considerable part of an orthodontist's armamentarium. Earlier orthodontists used gold, platinum, Iridium and silver alloys for orthodontic treatment. which were not esthetically pleasing and corrosion resistant, they lacked flexibility and tensile strength, these alloys were inappropriate for complex machining and joining when used as traction bars of that time (Claude G Matasa, 4th edition). Due to this difficulty stainless steel gain popularity, the force per unit activation with stainless steel was greater than gold i.e. high stiffness, excellent work hardening capabilities and low frictional magnitude (Robert P. Kusy, 2002). The austenitic stainless steel commonly used for orthodontic brackets, bands and wires contain approximately 18 percent chromium and 8-percent nickel (Park and Shearer, 1983). Intra-oral fixed orthodontic appliances include brackets, bands, ligatures, springs and arch wires that are made of alloys containing nickel, cobalt and chromium in different percentages (Shin and Oh Chung-Ju Hwang, 2003). The mass and duration of arch wire use is substantially less than that of the stainless steel brackets in full bonded cases (Theodore Eliades *et al*., 2002). The majority of metals used in the orthodontics contain nickel (Grimsdottir *et al*., 1992). The American Iron and Steel Institute type 316L austenitic stainless steel alloy, which is used for bracket manufacturing contains nickel in range of 10% to 14% (Brantley, 2001). The materials used in the manufacturing of base and wing components of metallic brackets are mostly austenitic-type stainless steel alloys (303L, 304L, 316L), and recently titanium has been introduced for the same (Claude G Matasa, 1995). Several studies have

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investigated whether orthodontic appliances release metal ions, through emission of electro-galvanic currents, with saliva as the medium or through continuous erosion over time (Harris *et al*., 1988; Vandekerckhove *et al*., 1998; Fiorenzo Faccioni *et al*., 2003). The oral environment is particularly ideal for the biodegradation of metals, because of its thermal, microbiologic and enzymatic properties (Fiorenzo Faccioni *et al*., 2003). The ion release is influenced by factors such as surface morphology, phase of the metal, galvanic coupling of dissimilar metals, salivary composition, pH, temperature, brushing. An unstable metal may corrode, releasing metal ions into solution (Hera Kim and Jeffery W. Johnson, 1999). The average dietary intake for nickel is 200-300 µg/day (Grandjean, 1984; Cempel and Nikel, 2006) and for chromium 280 µg/day (Skin Notation (SK) Profile Metallic Chromium and other Substances Containing Hexavalent Chromium [Cr(VI)] 2010). Nickel allergies are common (some sources indicate that 16.9% of males and 23.8% of females or even 28%of males and 31.9% of females have them) chromium allergy is estimated at 10% in males and 3% in females (Gunseli Agaoglu *et al*., 2001). The purpose of this study was to determine the amount of leaching of different ions from 3 different brands of orthodontic bracket, At 3 different pH of artificial saliva. To correlate between manual brushing and amount of leaching of different ions from Gemini series (3M) orthodontic brackets at 6.5 pH of artificial saliva.

MATERIALS AND METHODS

Three different brands of unused brackets (each brands have 87 brackets which divided equally into 3 subgroups) were kept in three different container of artificial saliva whose pH 6.5,4.2 and 7.6. The bracket bases were covered with light cure adhesive Transbond XT, to eliminate the possibility of corrosion from the inner aspect. 50 ml of artificial saliva of three different pH is taken into the polyethylene screw top bottle and the brackets were kept in the saliva for 1,7, 14,21,28 days period of time at room temperature. Again 50 ml of artificial saliva of 6.5 pH is taken in polyethylene screw top bottle, and Gemini series of brackets (29) embedded into self cure acrylic is placed into the saliva for checking the effect of manual brushing and ion release for 1,7,14,21,28 days period of time at room temperature. The brushing is done in circular motion twice daily for 3 min using power toothbrush. To avoid saturation with released ions, artificial saliva solutions were replaced after each sample collection. All the polyethylene bottle is labeled with different pH, and company name with collection date of sample. 5 ml of sample is taken for the analysis, before taking sample the polyethylene screw top bottle with artificial saliva is shake using universal shakers to avoid sedimentation of ions. All samples collected on all days were analyzed at HIMRAL laboratory, Sri ramchandra university, chennai, using AAS (Atomic Absorption Spectrophotometer) Perkinelemer inc. USA, Model: AAnalyst 400.

RESULTS

There is no statistically significant release of Ni and Cr ions between Gemini(3M) and Abzil(3M) groups of bracket at different pH. Orthox (JJ ortho) group shows significant amount of Ni ion release at pH 4.2 compared with pH 7.6. Cr shows no statistical difference in orthox series bracket. The statistically significant increase in the release of Ni and Cr noted between brushing and non brushing group of brackets in Gemini (3M) series.

Table 1. Gemini Oneway descriptive

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Nickel release	pH 4.2	5	.30060	.319897	.143062	-.09660	.69780	.010	.778
	pH 6.5	5	.60180	.483051	.216027	.00201	1.20159	.136	1.285
	pH 7.6	5	.68580	.566431	.253315	-.01752	1.38912	.023	1.410
	Total	15	.52940	.465704	.120244	.27150	.78730	.010	1.410
Chromium release	pH 4.2	5	.05340	.013612	.006088	.03650	.07030	.041	.069
	pH 6.5	5	.04900	.016062	.007183	.02906	.06894	.029	.073
	pH 7.6	5	.11720	.098464	.044035	-.00506	.23946	.013	.249
	Total	15	.07320	.062748	.016201	.03845	.10795	.013	.249

Table 2. Gemini Oneway Anova

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Nickel release	Between Groups	.410	2	.205	.937	.419
	Within Groups	2.626	12	.219		
	Total	3.036	14			
Chromium release	Between Groups	.015	2	.007	2.155	.159
	Within Groups	.041	12	.003		
	Total	.055	14			

Table 3. Gemini Oneway Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) pH	(J) pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Nickel release	pH 4.2	pH 6.5	-.301200	.295864	.580	-1.09052	.48812
		pH 7.6	-.385200	.295864	.421	-1.17452	.40412
	pH 6.5	pH 4.2	.301200	.295864	.580	-.48812	1.09052
		pH 7.6	-.084000	.295864	.957	-.87332	.70532
	pH 7.6	pH 4.2	.385200	.295864	.421	-.40412	1.17452
		pH 6.5	.084000	.295864	.957	-.70532	.87332
Chromium release	pH 4.2	pH 6.5	.004400	.036767	.992	-.09369	.10249
		pH 7.6	-.063800	.036767	.233	-.16189	.03429
	pH 6.5	pH 4.2	-.004400	.036767	.992	-.10249	.09369
		pH 7.6	-.068200	.036767	.194	-.16629	.02989
	pH 7.6	pH 4.2	.063800	.036767	.233	-.03429	.16189
		pH 6.5	.068200	.036767	.194	-.02989	.16629

Table 4. Gemini Oneway Kruskal-Wallis Test

Test Statistics[a,b]

	Nickel release	Chromium release
Chi-Square	2.340	1.087
df	2	2
Asymp. Sig.	.310	.581

a. Kruskal Wallis Test

b. Grouping Variable: pH

Table 5. Abzil Oneway descriptive

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Nickel release	pH 4.2	4	.11450	.103877	.051938	-.05079	.27979	.058	.270
	pH 6.5	5	.20360	.149467	.066844	.01801	.38919	.020	.335
	pH 7.6	5	.42420	.280141	.125283	.07636	.77204	.019	.740
	Total	14	.25693	.227175	.060715	.12576	.38810	.019	.740
Chromium release	pH 4.2	4	.03800	.015875	.007937	.01274	.06326	.021	.059
	pH 6.5	5	.03940	.015241	.006816	.02048	.05832	.019	.059
	pH 7.6	5	.07820	.067210	.030057	-.00525	.16165	.006	.172
	Total	14	.05286	.043636	.011662	.02766	.07805	.006	.172

Table 6. Abzil Oneway Anova

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Nickel release	Between Groups	.235	2	.118	2.970	.093
	Within Groups	.436	11	.040		
	Total	.671	13			
Chromium release	Between Groups	.005	2	.002	1.392	.289
	Within Groups	.020	11	.002		
	Total	.025	13			

Table 7. Abzil Oneway Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) pH	(J) pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Nickel release	pH 4.2	pH 6.5	-.089100	.133499	.787	-.44966	.27146
		pH 7.6	-.309700	.133499	.095	-.67026	.05086
	pH 6.5	pH 4.2	.089100	.133499	.787	-.27146	.44966
		pH 7.6	-.220600	.125864	.230	-.56054	.11934
	pH 7.6	pH 4.2	.309700	.133499	.095	-.05086	.67026
		pH 6.5	.220600	.125864	.230	-.11934	.56054
Chromium release	pH 4.2	pH 6.5	-.001400	.028427	.999	-.07818	.07538
		pH 7.6	-.040200	.028427	.367	-.11698	.03658
	pH 6.5	pH 4.2	.001400	.028427	.999	-.07538	.07818
		pH 7.6	-.038800	.026802	.352	-.11119	.03359
	pH 7.6	pH 4.2	.040200	.028427	.367	-.03658	.11698
		pH 6.5	.038800	.026802	.352	-.03359	.11119

Table 8. Abzil Oneway Kruskal-Wallis Test

Test Statistics[a,b]

	Nickel release	Chromium release
Chi-Square	2.697	1.016
df	2	2
Asymp. Sig.	.260	.602

a. Kruskal Wallis Test

b. Grouping Variable: pH

Table 9. Orthox Oneway descriptive

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Nickel release	pH 4.2	4	.06950	.120528	.060264	-.12229	.26129	.004	.250
	pH 6.5	5	.21840	.172908	.077327	.00371	.43309	.031	.388
	pH 7.6	4	.44100	.183308	.091654	.14932	.73268	.190	.613
	Total	13	.24108	.212951	.059062	.11239	.36976	.004	.613
Chromium release	pH 4.2	5	.04160	.014398	.006439	.02372	.05948	.025	.064
	pH 6.5	5	.12060	.212453	.095012	-.14320	.38440	.007	.500
	pH 7.6	5	.06620	.063543	.028417	-.01270	.14510	.005	.151
	Total	15	.07613	.123597	.031913	.00769	.14458	.005	.500

Table 10. Orthox Oneway Anova

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Nickel release	Between Groups	.280	2	.140	5.307	.027
	Within Groups	.264	10	.026		
	Total	.544	12			
Chromium release	Between Groups	.016	2	.008	.496	.621
	Within Groups	.198	12	.016		
	Total	.214	14			

Table 11. Orthox Oneway Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) pH	(J) pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Nickel release	pH 4.2	pH 6.5	-.148900	.108990	.394	-.44768	.14988
		pH 7.6	-.371500*	.114886	.022	-.68644	-.05656
	pH 6.5	pH 4.2	.148900	.108990	.394	-.14988	.44768
		pH 7.6	-.222600	.108990	.152	-.52138	.07618
	pH 7.6	pH 4.2	.371500*	.114886	.022	.05656	.68644
		pH 6.5	.222600	.108990	.152	-.07618	.52138
Chromium release	pH 4.2	pH 6.5	-.079000	.081143	.606	-.29548	.13748
		pH 7.6	-.024600	.081143	.951	-.24108	.19188
	pH 6.5	pH 4.2	.079000	.081143	.606	-.13748	.29548
		pH 7.6	.054400	.081143	.785	-.16208	.27088
	pH 7.6	pH 4.2	.024600	.081143	.951	-.19188	.24108
		pH 6.5	-.054400	.081143	.785	-.27088	.16208

*. The mean difference is significant at the .05 level.

Table 12. Orthox Oneway Kruskal-Wallis Test

Test Statistics[a,b]

	Nickel release	Chromium release
Chi-Square	6.953	.366
df	2	2
Asymp. Sig.	.031	.833

a. Kruskal Wallis Test

b. Grouping Variable: pH

Table 13. Gemini brushing and non brushing group t-test

Group Statistics

	pH	N	Mean	Std. Deviation	Std. Error Mean
Nickel release in brushing and non brushing group(Gemini series)	brushing	5	.64160	.515056	.230340
	Non brushing	4	.15650	.107323	.053662
Chromium release in brushing and non brushing groups(Gemini series)	brushing	5	.42200	.529452	.236778
	Non brushing	5	.03940	.014100	.006306

Table 14. Gemini brushing and non brushing group independent sample test

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper
Nickel release in brushing and non brushing group(Gemini series)	Equal variances assumed	2.963	.129	1.828	7	.110	.485100	.265399	-.142470	1.112670
	Equal variances not assumed			2.051	4.429	.103	.485100	.236508	-.147233	1.117433
Chromium release in brushing and non brushing groups(Gemini series)	Equal variances assumed	10.702	.011	1.615	8	.145	.382600	.236862	-.163605	.928805
	Equal variances not assumed			1.615	4.006	.181	.382600	.236862	-.274667	1.039867

Table 15. Gemini brushing and non brushing group Mann-Whitney Test

Test Statistics[b]

	Nickel release in brushing and non brushing group(Gemini series)	Chromium release in brushing and non brushing groups(Gemini series)
Mann-Whitney U	2.000	.000
Wilcoxon W	12.000	15.000
Z	-1.960	-2.611
Asymp. Sig. (2-tailed)	.050	.009
Exact Sig. [2*(1-tailed Sig.)]	.063[a]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: pH

DISCUSSION

The fate of the elements found in the human body has attracted the interest of investigators because of the apparent clinical importance of the information. Most of the steel based alloys used in orthodontics contain nickel and chromium. These elements give stainless steel its ductility and corrosion resistance. The focus of this experiment was to compare release of Ni and Cr from 3 different pH solution. The effect of brushing on release of Ni and Cr also studied. The saliva concentration for nickel and chromium in this study showed a large variation. Despite the scatter of values the metal concentrations detected were altogether very low generally less than the daily dietary intake on an average. 3 different pH of artificial saliva has been taken, as oral cavity pH varies according to our diet; the Two pH values (6.5 and 7.6) were in the range of the human natural salivary pH, whereas the pH 4.2 was investigated to simulate a condition that only occasionally occur when people feed on acidic foods or drinks (eg, lemon juice, coke) (Jensdottir *et al*., 2006). Geis –Gerstorfer (1994) reported corrosion occurs continuously in the mouth and release of ions take place due to abrasion by foods, liquids and tooth brushes.

In this study, nickel and chromium were released on all days and at different pH. The maximum nickel released at 7th day and gradually decrease on 14, 21 and 28 days of time period. The pattern of nickel release is similar to the finding of Barrett and Bishara (1993), Bhaskar (1997), Hwang (2001) study. In the brushing group the release of nickel and chromium is much higher than the non brushing group. Maximum nickel released at 7th day and gradually decrease with time, while chromium shows gradual rise in concentration with time. Chromium leach out gradually increases with time, maximum leach out reported at 28 days at different pH. This finding is corresponding to the finding of Barrett and Bishara (Barrett *et al*., 1993), Hwang *et al.* (2001), Sfondrini *et al.* (2008). The release of nickel and chromium increases with decrese in pH. Maximum release reported at pH 4.2, followed by 6.5 and 7.6. This finding is correspond to the finding of Hwang (Chang-Ju Hwang *et al*., 2001), Sfondrini (2008), and Huang (2001). The possible explanations for this behavior can be that, the nickel present on the surface of the stainless steel may quickly corrode during the first 7 days of the experiment, then the rate of release drops off as the surface nickel is depleted. Contrary to nickel, the rate of chromium corrosion did not decrease after day 7. As stated earlier, the more plausible assumption is that the nickel concentration on the surface of the appliances is being depleted at a faster rate than that for chromium. When patients undergoing orthodontic treatment for more than 24 months showed lower nickel induced cell proliferation than those less than 24months as reported by Marigo *et al.* (2005). This data suggest that the longer the treatment continues, the lower the nickel induced Peripheral Blood Mononuclear Cell (PBMC) Proliferation index; suggesting that oral tolerance might develop in this context. The present study identified the effects of changes in pH on the different brands of brackets, and role of brushing on the release of metal ions. Bracket manufacturers are free to change raw materials and processing techniques without complying with any standards or regulations. They are not selected using scientific criteria, instead considering availability, cost, and ease of processing. This has brought to the market attachments, which are not acceptable.

The need for better steel appears to be more pronounced among the one piece than among the multipartite brackets. Improving bracket performance can also help protect patient's health. However even such small amount of release produce sensitivity when the orthodontic appliances are in place for 2-3 years, because symptoms can develop several years later. Therefore nickel hypersensitivity should be observed on a long-term basis. High priority should be given to the development of in-vitro tests, in particular "long-term assays", which better simulate the biological interactions of metallic component in vivo. Furthermore, long-term clinical trials in vivo are needed to ensure the performance of bracket setup in vivo.

Conclusion

Results of this investigation led to the following conclusions:

1. Orthox series brackets released more nickel ion compared to Gemini and Abzil series of bracket.
2. Chromium release is not significant in all the 3 brands of bracket.
3. With decrease in pH, ions release increases.
4. Brushing causes more release of Ni and Cr ions.

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