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Chemical and biological evaluation of different commercially available metal orthodontic brackets- An invitro study

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Abstract: *Aims:* This invitro study was done to evaluate the chemical and biological properties of 4 brands of metal orthodontic brackets. *Objectives:* To analyse the chemical composition, the corrosion resistance, Nickel ion release and cytotoxicity of the orthodontic brackets. *Methods:* Four brands of orthodontic brackets namely: Group-1: Gemini(3M Unitek), Group-2: Ecoplus(Chirpans Orthodontics), Group-3: Monalisa(JJ Orthodontics), Group-4: Sapphire(Modern Orthodontics). Composition was analysed by Scanning- electron Microscopy with Energy Dispersive spectroscopy. Corrosion resistance was done by potentiodynamic polarisation in artificial saliva. Nickel ion release from the brackets was analysed by inductively coupled plasma mass spectrometry, at 24 hours, 7, 14, 28 days. Cytotoxicity of the brackets were analysed by Live- dead assay and MTT assay. *Results:* Gemini brackets offered better corrosion resistance and showed the least nickel release among all the groups. Cytotoxicity tests showed that Gemini is the least cytotoxic and Ecoplus is the most cytotoxic. *Conclusion:* The study concluded that Nickel ion is highest in Gemini brackets, but the nickel ion released from Gemini is the least among the four groups and showed better corrosion resistance and least cytotoxic.
Keywords: Cytotoxicity, Corrosion resistance, Nickel ion, Orthodontic brackets, Scanning electron microscopy

Introduction

Stainless steel (SS) is one of the most popular materials used for orthodontic brackets because of its favorable mechanical properties and suitable corrosion resistance. The recent times has seen an emergence of a wide variety of brands manufacturing and marketing stainless steel brackets. Chromium, in the presence of air (oxygen), forms a thin film of chromium oxide which covers the surface of the stainless steel. Chromium oxide, is inert or "passive" by nature, and chromium in the material gives stainless steel its corrosion-resistant properties [1].

Nickel, a main ingredient of orthodontic materials, can cause severe health hazards in biologic tissues. Hypersensitivity is the most common consequence of exposure to nickel-containing products, with incidence ranging from 4.5% to 20% in the literature [2]. A study reported an average release of 40 μg /day from a simulated full mouth fixed appliance. An average of 4.5% - 28.5% of the population have hypersensitivity to nickel. A few reports also show contact stomatitis from nickel in orthodontic patients [3]. Different brands manufacturing stainless steel brackets could differ in their composition, cytotoxicity, Ni-ion release and corrosion resistance, which is the main motive of this study.

Material and Methods

Four brands of metal orthodontic brackets (0.022" slot pre- adjusted edgewise) first premolar brackets of the upper right side were taken. The four brands included:

- Group 1: Gemini (3M Unitek, Monrovia, USA)
- Group 2: Ecoplus (Chirpans Orthodontics, China)
- Group 3: Monalisa (JJ Orthodontics, India)
- Group 4: Sapphire (Modern Orthodontics, India)

Each brand was checked for composition, corrosion resistance, Ni- ion release and cytotoxicity. Composition of 12 samples of each brand were analysed with SEM-EDS. Corrosion resistance of 12 samples of each brand was assessed by potentiodynamic

polarisation device (CH-Analyser). Cytotoxicity of 12 samples of each brand was analysed with a quantitative test: MTT assay, and also Qualitative test: Live–dead assay. Six samples of each brand were immersed in artificial saliva (Fusayama Meyer method) and Nickel ion release was assessed at 24 hours, 7 days, 14 days and 28 days by ICP-MS (Inductively couple plasma mass spectrometry.

Statistical analysis was done by one way Anova and pairwise comparisons with post hoc tukey test.

Results

Composition analysis showed that iron, chromium and nickel were predominantly present in all the four brands. Other trace elements like silicon, aluminium, copper, silver, carbon and oxygen were found in some brands.

The mean value among the four groups for iron analysed by one way ANOVA is statistically significant (Table 1a).

Table-1a: Iron concentration

Group	Mean	Standard deviation	F value	P value
Group 1	70.28	3.70	4.135	0.011*
Group 2	68.35	5.78		
Group 3	68.02	0.94		
Group 4	72.68	2.30		

*Statistical significant at p<0.05

The mean value among the four groups for chromium analysed by one way ANOVA is statistically significant (Table 1b).

Table-1b: Chromium concentration

Group	Mean	Std dev	F value	P value
Group 1	18.92	0.75	11.469	<0.0001**
Group 2	16.15	1.80		
Group 3	18.18	1.05		
Group 4	17.24	1.03		

** statistical high significance p<0.001

The mean value among the four groups for nickel analysed by one way ANOVA is statistically significant (Table 1c).

Table-1c: Nickel concentration

Group	Mean	Std dev	F value	P value
Group 1	8.88	3.56	17.213	<0.0001**
Group 2	4.72	1.03		
Group 3	4.13	0.47		
Group 4	4.26	0.54		

Corrosion Resistance: The polarization curves were plotted in the potential range of +400 mV to -400mV at a scanning rate of 0.01 V/s. The I_{corr} , rate/yr and polarization resistance values obtained are as follows. There is no statistical significance among the brands.

The I_{corr} (current density) for the 4 groups are tabulated as follows (Table 2a). According to one way ANOVA, Group 3 shows the highest current density among the 4 groups.

Table-2a: I_{Corr} values obtained from the four brands

Group	Mean ($\times 10^{-8}$)	Std dev	F value	P value
Group 1	3.41	1.38	1.089	0.364
Group 2	4.28	2.63		
Group 3	4.66	2.61		
Group 4	3.27	2.08		

The rate of degradation of the metal/year is tabulated in Table 2b. One way ANOVA shows that Group 3 the highest rate of degradation/year, which indicates its susceptibility to corrode.

Polarisation resistance among the 4 groups is tabulated in Table 2c. Group 1 shows the highest resistance with indicates its resistance to corrosion. Although there is a difference in value among the 4 groups in case of I corr, Rate/yr, and Rp, it is not statistically significant, according to one way ANOVA.

Table-2b: Rate of degradation of the metal/ year				
Group	**Mean ($x 10^{-3}$)**	**Std dev**	**F value**	**P value**
Group 1	3.54	1.37	1.152	0.339
Group 2	4.73	3.03		
Group 3	5.06	2.79		
Group 4	3.63	2.39		

Table-2c: Polarisation resistance				
Group	**Mean (ohms)**	**Std dev**	**F value**	**P value**
Group 1	5342.45	3218.04	2.224	0.087
Group 2	2854.25	1705.42		
Group 3	3754.40	1662.92		
Group 4	3380.42	2756.75		

Nickel Ion Release: Nickel release over four time durations i.e 24 hours, 7 days, 14 days and 28 days was evaluated. The mean values of the four groups at 24 hours was tabulated (Table 3a) and is statistically significant, according to one way ANOVA. Group 3 shows the highest ion release in 24 hours.

Table-3a: Nickel release over 24 hr				
Group	**Mean**	**Std dev**	**F value**	**P value**
Group 1	3.5	0.83	224.084	0.0001**
Group 2	156.5	18.16		
Group 3	379.16	51.21		
Group 4	51.66	5.81		

Table-3b: Nickel ion release after 7 days				
Group	**Mean**	**Std dev**	**F value**	**P value**
Group 1	4.83	2.13	679.53	0.0001**
Group 2	177	15.19		
Group 3	861.80	58.38		
Group 4	247.16	35.68		

Table-3c: Nickel ion release after 14 days				
Group	**Mean**	**Std dev**	**F value**	**P value**
Group 1	3.5	1.37	86.448	0.0001**
Group 2	137.5	17.16		
Group 3	157.6	22.84		
Group 4	161.83	27.28		

Table-3d: Nickel ion release after 28 days				
Group	**Mean**	**Std dev**	**F value**	**P value**
Group 1	2.5	0.83	93.861	0.0001**
Group 2	109.83	10		
Group 3	132.83	17.64		
Group 4	131.50	23.89		

Cytotoxicity: The results are as follows:

The live dead assay shows live cells as green and dead cells as red. In the study, it was found that Group 2 has shown the maximum number of dead cells as compared to the other brands and is thus shown to be cytotoxic, whereas, Group 1 has the least number of dead cells and thus it is shown to be least cytotoxic.

In the MTT assay, it is also the reflecting the same results as the qualitative analysis which is statistically significant, with Group 1 showing the maximum amount of cell viability, with Group 2 showing the least cell viability (Table 4).

Table-4: Cell viability among the four groups				
Group	**Mean**	**Std dev**	**F value**	**P value**
Group 1	102.9339	6.6134	260.3136	0.0001**
Group 2	33.6729	6.5816		
Group 3	37.8188	8.1364		
Group 4	47.7246	6.1034		

Discussion

In this study, all the 4 brands showed predominantly, Iron, Nickel and chromium. Gemini (3M) brackets showed the presence of silicon and traces of aluminium. Ecoplus (Chirpans orthodontics) has shown presence of silicon, with traces of copper and carbon. Monalisa (JJ orthodontics) showed silicon with traces of copper and oxygen. Sapphire (Modern orthodontics) has shown traces of copper and silver. Stainless steel's high resistance to corrosion is mostly due to the significant amount of chromium present. Chromium oxide forms a passive layer over the surface of the steel, preventing oxygen from penetrating the alloy. Nickel forms salts that prevent chromium salts from forming, which leaves more chromium to form the passive layer. Nickel also provides firmness and ductility to stainless steel [4] and acts as an austenite stabilizer, making the austenitic form more stable at lower temperatures [5-6].

Since this study is an invitro test, artificial saliva proposed by Fusayama et al was used as the electrolyte for the corrosion test. Marek reported that Meyer and Nally examined the behaviour of several dental alloys in natural saliva, Ringer solutions, and five different synthetic saliva, indicated that, among those tested, that proposed by Fusayama et al. produced results most closely approximating those in natural saliva [7]. In this study, the corrosion resistance of the four brands of orthodontic brackets were analysed by using a potentiodynamic polarization device. Gemini brackets exhibited the highest polarisation resistance(Rp), followed by Monalisa brackets, then Sapphire brackets, with the least Rp shown by Ecoplus brackets which indicates that among the four companies, Ecoplus brackets shows the highest tendency to corrode. There could be many reasons for corrosion to occur on stainless steel brackets. Some of them are temperature, salivary pH, bacterial flora, enzyme activity and proteins, surface roughness of the metal and loading stress on the metal, presence of ions like fluoride and chloride.

According to Fraunhofer, stainless steel exhibits pitting corrosion in chloride media [8]. The artificial saliva in which the brackets were tested for corrosion resistance contained chloride, which could explain the corrosion of the stainless steel brackets invitro. Studies have shown that stainless steel will release nickel ions after corrosion occurs, a disadvantage with stainless steel bracket corrosion concerns patients with allergies to nickel and other specific substances [9]. Of known metals, nickel is the most allergenic. Nickel sensitivity has an incidence between 10 to 20% of the population and nickel is also the most common metal associated with contact dermatitis in orthodontics [10]. Common oral manifestations of a nickel allergy include a burning sensation, glossitis, gingivitis, gingival hyperplasia, metallic taste [11-13]. Kerosuo et al (1997) demonstrated, in vitro, that metal brackets experiencing orthodontic forces release more nickel and chromium than brackets free of orthodontic force [14].

Studies have shown that the metal ions, such as Fe, Cr, and Ni, are released from orthodontic appliances in artificial saliva because corrosion phenomena are much higher than those in saline solutions [2]. In a previous study by Behroozi et al to evaluate the ion release following corrosion of five different bracket-archwire combination (Dentaurum, 3M, Ortho Organizer, Cobas and O.R.G), it was found that the Cobas bracket had the most ion release among the tested brackets, while Ortho Organizer and ORG performed favorably. There was no significant difference between Dentaurum and 3M. Nickel release however was more pronounced in Cobas followed by 3M, ORG, Ortho Organizer and Dentaurum [15].

In this study, Nickel ion showed a peak after 7 days, which gradually declined by day 28 in all the four brands. Gemini brackets showed the least nickel ion release among the four brands. Another feature that was noted among the brands, was that nickel ion release increased by the end of one week among all the brands, but when we consider any one particular brand, the rate of increase or decrease is not consistent. This is in accordance with a previous study done by Sahoo et al to determine in vivo release of nickel and chromium ions in conventional and self-ligating brackets in unstimulated saliva at four time intervals [16] Nickel and chromium released into saliva from conventional and

self-ligating brackets progressively increased from days 1-7 and then decreased at day 30. It has been shown in a study that there is no proportional relation between the release of nickel ions and the nickel content of orthodontic brackets and wires [17].

Satija et al noted a significant increase in Ni and Cr level in saliva and it reached the highest level in 1st week [18]. This was similar to the results of Park and Shearer who evaluated conventional brackets, and reported that the nickel and chromium releases reached a plateau after 6 days [19]. Barrett et al in an in-vitro study found that nickel release reached a maximum after 1 week and then diminished [20]. Kerosuo et al suggested that nickel and chromium concentrations of saliva are not significantly affected by fixed orthodontic appliances during the first month of treatment [14]. Another study was done by Gjerdet et al., who also did not find any differences in nickel amounts in saliva before and 3 weeks after insertion of fixed appliances. Gjerdet et al found, however, a significantly increased nickel concentration in saliva samples taken immediately after placement of the appliances in a group of six cases [13].

Sahmali et al. investigated the effects of dental alloys containing Ni on the level of this element in the serum, liver, kidney, and oral mucosa of guinea pigs. Statistically significant differences were found between liver and oral mucosa Ni content in the experimental and control groups [21]. The cytotoxicity from a corroded metal orthodontic appliance is an important issue. Corrosion releases metal ions into the oral cavity that are ingested into the gastrointestinal system. Locally, the released ions may adversely affect the oral tissues by inhibiting enzyme or mitochondrial activity and damaging DNA, as has been demonstrated in vitro. Moreover, chromium and nickel ions may induce type IV hypersensitivity [22]. In this study, cytotoxicity of the orthodontic brackets were assessed by a qualitative test(live- dead assay) and a quantitative test(MTT assay).The test showed that Gemini brackets were the least cytotoxic and Ecoplus was the most cytotoxic. A previous study was done by Eliades et al indicated no ionic release for the nickel-titanium alloy aging solution, whereas measurable nickel and traces of chromium were found in the stainless steel bracket-aging medium [22].

In this study, Monalisa brackets showed the highest nickel ion release and Ecoplus brackets have shown more cytotoxicity. This could be attributed to the fact that in this study, nickel ion release was checked in artificial saliva and cytotoxicity was checked on cultured fibroblast cells. Standard quality products thus ensure a safer and better treatment of the patients with the least side effects. This study showed that standardization plays a very important role in the manufacturing of orthodontic brackets. Furthermore research by in vivo studies could guarantee a better insight to the results obtained from this study.

Conclusion

From the present invitro study on orthodontic brackets, the following conclusion has been drawn:

1) Nickel concentration is highest in Gemini brackets as compared with the others.
2) The corrosion resistance measured shows that Monalisa is the least corrosion resistant and Gemini brackets are highly resistant to corrosion, but these values are not statistically significant.
3) Gemini brackets showed the least ion leach among the four brands. Gemini brackets showed the highest cell viability and therefore is least cytotoxic and Ecoplus brackets showed the least cell viability and hence, is the most cytotoxic.

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