

Nickel, Chromium and Iron Levels in the Saliva of Patients with Simulated Fixed Orthodontic Appliances

Rodrigo Matos de Souza^a; Luciane Macedo de Menezes^b

ABSTRACT

Objective: To assess the in vivo release of nickel, chromium, and iron ions into saliva by different metallic brackets.

Materials and Methods: Thirty volunteers wore removable appliances with bonded brackets and were divided according to the brand of brackets: group A, 3M/Unitek (AISI 303); group B, American Orthodontics (AISI 316L); and group C, Dentaurum (AISI 316L). The appliances were worn for 60 days, and saliva samples were collected at the following time points: T1, before placement of the appliance; T2, after 10 minutes; T3, 24 hours; T4, 7 days; T5, 30 days; and T6, 60 days after insertion of the removable appliance. Saliva samples were analyzed for nickel, chromium, and iron by atomic absorption spectrophotometry. Statistical analysis was performed by nonparametric tests (Friedman, Mann-Whitney, Kruskal-Wallis).

Results: Saliva evaluation revealed a large variation in concentration of these ions between individuals. The results also appeared to indicate an increase in nickel and chromium ions immediately after placement of the appliance (T2), but this was statistically significant only for groups B and C. There was no increase in iron levels. A tendency for increases in nickel and chromium concentrations was verified immediately after placement of the appliance, but these values are probably reduced because of biofilm formation regardless of the bracket used.

Conclusion: Nickel and chromium ion concentrations increased immediately after placement of the appliance in the mouth for all study groups. There were no significant differences in the nickel, chromium, and iron levels released by the three groups of appliances at all study periods.

KEY WORDS: Corrosion; Orthodontic brackets; Nickel; Saliva

INTRODUCTION

The biocompatibility of dental alloys has been investigated over the past 20 years. However, studies on this issue have given rise to questions without answers, confirming the need to learn more about the

biocompatibility of these materials. Since this process has not been fully explained, orthodontists may be confused in the selection of biologically safe appliances for their patients.¹

Nickel is the most common cause of contact allergy.² Orthodontic brackets, bands, and archwires are universally made with an alloy, which contains approximately 6% to 12% nickel and 15% to 22% chromium.³ In addition to the allergic issue, carcinogenic, mutagenic, and cytotoxic effects have been assigned to nickel and, to a lesser extent, chromium.

The introduction of metal ions into the human body is an additional risk to health since these ions may be released in different places and at different levels, depending on the characteristics and solubility of the products containing them.⁴ Consequently, biological functions are affected, which may lead to systemic and local effects.⁵

The resistance to corrosion, a fundamental aspect of biocompatibility, may be affected by several factors. The first depends on the manufacturing process, type

^a Postgraduate MS Student, Department of Orthodontics, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

^b Assistant Professor, Department of Orthodontics, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

Corresponding author: Dr Rodrigo Matos de Souza, R. Coronel Pedro Benedit, no. 505 sala 508, Criciúma SC, Brasil Cep: 88801-250

(e-mail: rodrigoortodontia@yahoo.com.br)

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of alloy, and surface characteristics of the piece.³ The second refers to the environment in which the piece is inserted.⁶ The third corresponds to use (aging) of the alloy, which is subject to side effects such as stress, thermal treatment, and recycling of components.⁷

Several *in vitro* tests have demonstrated the corrosion and release of nickel and chromium ions from orthodontic brackets. However, the results of these tests are limited and extrapolation to the clinical situation difficult because the methodologies used are unable to precisely reproduce the highly complex and dynamic oral environment.⁸

On the other hand, nickel release *in vivo* in the oral cavity has been more difficult to demonstrate. The literature includes some *in vivo* studies evaluating the ion release in saliva. Kerosuo et al⁹ evaluated the salivary concentrations of nickel and chromium in patients wearing different types of appliances. The study sample was composed of 47 patients, and four saliva samples were collected: (1) before placement of the appliance, (2) after 2 days, (3) after 1 week, and (4) after 1 month. The mean salivary concentration was 55 ng/mL for nickel and 61 ng/mL for chromium, similar to the values observed before placement of the appliance.

Kocadereli et al¹⁰ evaluated the salivary concentrations of nickel and chromium on 45 patients treated with fixed orthodontic appliances (1) before, (2) after 1 week, (3) after 1 month, and (4) after 2 months. The results of this study did not indicate statistically significant differences between metal concentrations before and after placement of the appliance. Fors and Persson¹¹ compared the salivary concentration of nickel in young patients who did wear and did not wear fixed orthodontic appliances. The average period since appliance insertion was 16 months at the time of sample collection. No significant difference in the nickel content of filtered saliva was found between the test and the control samples; the median values of nickel content were 0.005 and 0.004 $\mu\text{g/g}$ saliva, respectively. On the other hand, a significant difference was found for the filter-retained fraction; the median values for nickel were 25.3 and 14.9 $\mu\text{g/g}$, respectively.

The most significant method for measurement of nickel release before and after onset of orthodontic treatment is salivary analysis since it is the first diluent of the human body and allows long periods of analyses.¹² Thus, the effects of material aging and fatigue on the ion release could be investigated.

Thus, this study investigated the ion release associated with the biodegradation process of three brands of metallic brackets manufactured with different types of steel and techniques. The immersion mean was in the oral environment, thus yielding similar results as those occurring during routine orthodontic treatment.



Figure 1. The appliance used.

MATERIALS AND METHODS

This study was conducted using removable appliances with bonded brackets. These appliances were worn for 60 days by volunteers, who were dental students at the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil. This group was composed of 8 men and 22 women aged 20 to 26 years. This study was approved by the Ethics Committee of PUCRS, and human volunteer informed consent forms were obtained.

The individuals were randomly divided into three study groups with 10 individuals each, according to the different brands of metal brackets used:

- Group A (Dyna-Lock Standard Edgewise, 3M/Unitek, Monrovia, Calif)
- Group B (LG Edgewise, American Orthodontics, Sheboygan, Wis)
- Group C (Discovery Roth, Dentaaurum-Ispringen, BW, Germany)

The appliances used were acrylic plates adapted to the palate containing seven bonded brackets each (Figures 1 and 2), corresponding to the brackets used in a mandibular hemiarch. Brackets were bonded to the plate with a double-face adhesive acrylic foam tape (3M/Unitek). To avoid discomfort to individuals due to the presence of brackets on the palate, a metallic matrix was used for fabrication of undercuts in the plate so that the brackets would be at the same level as the acrylic.

Saliva Collection and Analysis

Saliva samples were collected from each individual at different study periods: T1, before placement of the appliance; T2, after 10 minutes; T3, 24 hours; T4, 7 days; T5, 30 days; and T6, 60 days. At the day of placement and before breakfast, the first and second

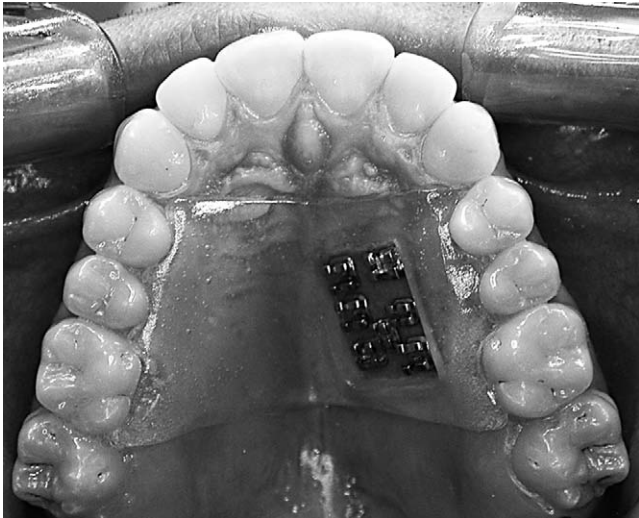


Figure 2. Occlusal view of the appliance adjusted in the mouth.

collections were obtained following these steps: (1) rinsing with deionized water for 30 seconds and spitting, (2) accumulation of saliva in the mouth for 5 minutes, and (3) spitting saliva in a labeled and prepared flask. The patients were asked to wear the appliance all the time and perform further collections at home, at the first hour in the morning, before breakfast, following the same aforementioned procedures. However, they were also informed that they should clean the appliances with water and toothbrush before performing the second step. Samples were kept in a refrigerator until they were processed.

To avoid sample contamination, flasks for saliva collection were prepared by (1) washing with Extram in tap water, (2) immersion in Extram in ultrasound for 6 minutes, (3) rinsing with Mili-Q water, and (4) drying in an electric oven at 150°C for 15 minutes. The spit collection method employed for saliva collection required the patient to accumulate saliva in the oral cavity and then spit it into a flask.¹³

Solutions were removed from the glass flasks and evaluated in an atomic absorption thermal electric spectrophotometer with a graphite oven (Analyst 800 Perkin Elmer, Norwalk, Conn) for the presence of nickel, chromium, and iron. A standard calibration curve was employed for each ion analyzed, and each metal was quantified separately. Correction of interference from the matrix was performed with the Zeeman corrector, available in the spectrophotometer. This methodology allowed evaluation and quantification of ions released by the different devices analyzed. The detection limits of nickel, chromium, and iron were 1 µg/L, 0.1 µg/L, and 1 µg/L, respectively.

Statistical Analysis

Normal distribution of data was assessed by the nonparametric Kolmogorov-Smirnov test. Since some

Table 1. Comparison of Release of Nickel Ions (General) Corresponding to the Three Groups, According to the Study Periods

| Time | n | Mean, µg/L | SD, µg/L | Mean Rank | P |
|-------------|----|------------|----------|--------------------|------------------|
| Before (T1) | 28 | 5.26 | 7.14 | 3.32 ^{AB} | .01 ^a |
| 10 min (T2) | 28 | 16.01 | 15.29 | 5.18 ^D | |
| 1 d (T3) | 28 | 11.15 | 24.35 | 3.82 ^A | |
| 7 d (T4) | 28 | 4.67 | 8.33 | 3.32 ^{AB} | |
| 30 d (T5) | 28 | 2.29 | 2.66 | 2.75 ^{BC} | |
| 60 d (T6) | 28 | 1.69 | 1.68 | 2.61 ^C | |

^a Values followed by same letters do not differ from each other.

Table 2. Comparison of Release of Chromium Ions (General) Corresponding to the Three Groups, According to the Study Periods

| Time | n | Mean, µg/L | SD, µg/L | Mean Rank | P |
|-------------|----|------------|----------|--------------------|------------------|
| Before (T1) | 28 | 0.64 | 0.67 | 3.34 ^{AD} | .01 ^a |
| 10 min (T2) | 28 | 1.72 | 2.14 | 4.57 ^B | |
| 1 d (T3) | 28 | 1.66 | 3.03 | 4.04 ^{AB} | |
| 7 d (T4) | 28 | 1.20 | 1.32 | 3.95 ^{AB} | |
| 30 d (T5) | 28 | 0.29 | 0.55 | 2.07 ^C | |
| 60 d (T6) | 28 | 0.52 | 0.43 | 3.04 ^D | |

^a Values followed by same letters do not differ from each other.

Table 3. Comparison of Release of Iron Ions (General) Corresponding to the Three Groups, According to the Study Periods

| Time | n | Mean, µg/L | SD, µg/L | Mean Rank | P |
|-------------|----|------------|----------|-------------------|------------------|
| Before (T1) | 28 | 94.03 | 114.77 | 3.63 ^A | .01 ^a |
| 10 min (T2) | 28 | 98.69 | 100.66 | 4.21 ^A | |
| 1 d (T3) | 28 | 103.58 | 147.16 | 4.02 ^A | |
| 7 d (T4) | 28 | 85.25 | 105.41 | 3.91 ^A | |
| 30 d (T5) | 28 | 48.13 | 39.09 | 3.16 ^A | |
| 60 d (T6) | 28 | 28.31 | 28.52 | 2.07 ^B | |

^a Values followed by same letters do not differ from each other.

variables presented abnormal distribution, nonparametric tests were employed (Friedman, Mann-Whitney, and Kruskal-Wallis).

RESULTS

Initially, statistical analysis was performed for the three groups (A, B, and C) in combination for analysis of ion release according to the study periods (T1, T2, T3, T4, T5, and T6). The results of the nonparametric Friedman test revealed statistically significant differences ($P < .01$) in the amount of nickel and chromium in saliva during different study periods, with the highest concentrations at 10 minutes after placement of the appliance (T2; Tables 1 and 2). There was also a slight increase in iron after placement of the appliance (T2–T3) but without statistical significance (Table 3). Individual analysis of results for each subject at T1 and T2, which exhibited the greatest difference, revealed that 26 of 30 subjects presented an increased nickel concentration at T2; the concentration was nearly unchanged for the remaining 4 subjects.

Table 4. Comparison Between the Means of Nickel Ions Released in Groups A, B, and C at the Different Study Periods

| Group | n | Mean, $\mu\text{g/L}$ | SD, $\mu\text{g/L}$ | Mean Rank | <i>P</i> |
|-------------|----|-----------------------|---------------------|-----------|----------|
| Before (T1) | | | | | |
| Group A | 10 | 4.85 | 7.47 | 15.95 | .82 |
| Group B | 10 | 5.92 | 7.39 | 17.30 | |
| Group C | 10 | 5.05 | 7.26 | 14.72 | |
| 10 min (T2) | | | | | |
| Group A | 10 | 14.93 | 19.32 | 13.36 | .39 |
| Group B | 10 | 18.79 | 14.35 | 18.70 | |
| Group C | 10 | 14.43 | 12.07 | 16.20 | |
| 1 d (T3) | | | | | |
| Group A | 10 | 6.73 | 14.02 | 14.05 | .40 |
| Group B | 10 | 7.58 | 10.70 | 15.00 | |
| Group C | 10 | 19.60 | 39.07 | 19.15 | |
| 7 d (T4) | | | | | |
| Group A | 10 | 6.71 | 10.63 | 17.20 | .75 |
| Group B | 10 | 2.67 | 3.21 | 14.60 | |
| Group C | 10 | 4.63 | 9.55 | 14.70 | |
| 30 d (T5) | | | | | |
| Group A | 10 | 3.56 | 3.86 | 17.95 | .35 |
| Group B | 10 | 1.33 | 1.72 | 12.45 | |
| Group C | 10 | 1.99 | 1.42 | 16.10 | |
| 60 d (T6) | | | | | |
| Group A | 9 | 1.40 | 1.36 | 13.67 | .07 |
| Group B | 9 | 0.84 | 1.07 | 10.50 | |
| Group C | 10 | 2.70 | 1.96 | 18.85 | |

Comparison of the release of nickel and chromium ions at the different study periods by the nonparametric Kruskal-Wallis test revealed that there were no significant differences between groups A, B, and C for all ions at the different study periods (Table 4–6).

Two subjects lost their appliances before the last saliva collection. This is why Tables 1 through 3 present $n = 28$, compared to $n = 9$ in Tables 4 through 6 for groups A and B at period T6.

DISCUSSION

It is known that metals employed in dentistry, and especially in orthodontics, present some type of corrosion. However, the consequences of products released by corrosion on human health are not clearly defined. Selection of the orthodontic bracket on the basis of the alloy and manufacturing process may be fundamental for biocompatibility. Studies have reported that the two characteristics of alloy and the manufacturing process are the main factors influencing the corrosion of brackets. These data guided the selection of brackets used in this study, which compared stainless steel AISI 316L, the main alloy employed for fabrication of orthodontic brackets,¹⁴ by two manufacturing processes, one piece (group C, Dentaurum) and combined or multiparts (group B, American Orthodon-

Table 5. Comparison Between the Means of Chromium Ions Released in Groups A, B, and C at the Different Study Periods

| Group | n | Mean, $\mu\text{g/L}$ | SD, $\mu\text{g/L}$ | Mean Rank | <i>P</i> |
|-------------|----|-----------------------|---------------------|-----------|----------|
| Before (T1) | | | | | |
| Group A | 10 | 0.55 | 0.41 | 16.41 | .94 |
| Group B | 10 | 0.67 | 0.63 | 16.35 | |
| Group C | 10 | 0.70 | 0.96 | 15.20 | |
| 10 min (T2) | | | | | |
| Group A | 10 | 1.48 | 2.16 | 14.50 | .55 |
| Group B | 10 | 2.40 | 2.84 | 18.55 | |
| Group C | 10 | 1.30 | 1.11 | 15.10 | |
| 1 d (T3) | | | | | |
| Group A | 10 | 0.96 | 0.69 | 15.95 | .39 |
| Group B | 10 | 1.03 | 1.23 | 13.25 | |
| Group C | 10 | 3.06 | 5.02 | 18.80 | |
| 7 d (T4) | | | | | |
| Group A | 10 | 0.68 | 0.75 | 12.65 | .25 |
| Group B | 10 | 1.41 | 1.77 | 14.75 | |
| Group C | 10 | 1.51 | 1.21 | 19.10 | |
| 30 d (T5) | | | | | |
| Group A | 10 | 0.26 | 0.19 | 18.35 | .32 |
| Group B | 10 | 0.12 | 0.10 | 12.55 | |
| Group C | 10 | 0.50 | 0.92 | 15.60 | |
| 60 d (T6) | | | | | |
| Group A | 9 | 0.40 | 0.44 | 11.89 | .50 |
| Group B | 9 | 0.59 | 0.51 | 15.39 | |
| Group C | 10 | 0.56 | 0.38 | 16.05 | |

Table 6. Comparison Between the Means of Iron Ions Released in Groups A, B, and C at the Different Study Periods

| Group | n | Mean, $\mu\text{g/L}$ | SD, $\mu\text{g/L}$ | Mean Rank | P |
|-------------|----|-----------------------|---------------------|-----------|-----|
| Before (T1) | | | | | |
| Group A | 10 | 97.20 | 126.48 | 14.68 | .51 |
| Group B | 10 | 45.39 | 26.45 | 14.70 | |
| Group C | 10 | 139.19 | 143.27 | 18.75 | |
| 10 min (T2) | | | | | |
| Group A | 10 | 113.71 | 136.73 | 14.95 | .84 |
| Group B | 10 | 69.41 | 47.33 | 15.85 | |
| Group C | 10 | 111.44 | 96.70 | 17.30 | |
| 1 d (T3) | | | | | |
| Group A | 10 | 59.35 | 25.07 | 16.45 | .97 |
| Group B | 10 | 109.75 | 122.90 | 16.00 | |
| Group C | 10 | 146.06 | 227.91 | 15.50 | |
| 7 d (T4) | | | | | |
| Group A | 10 | 92.81 | 117.91 | 15.45 | .70 |
| Group B | 10 | 74.79 | 49.64 | 17.20 | |
| Group C | 10 | 88.15 | 138.71 | 13.85 | |
| 30 d (T5) | | | | | |
| Group A | 10 | 49.58 | 36.21 | 17.20 | .50 |
| Group B | 10 | 34.08 | 18.35 | 12.85 | |
| Group C | 10 | 60.74 | 53.64 | 16.45 | |
| 60 d (T6) | | | | | |
| Group A | 9 | 30.70 | 29.29 | 16.56 | .14 |
| Group B | 9 | 12.64 | 7.90 | 10.00 | |
| Group C | 10 | 40.26 | 34.81 | 16.70 | |

tics) and brackets fabricated with the AISI 303 alloy in one piece (group A, 3M UNITEK). Combination of these three groups allowed isolated comparison between the types of alloy, 316L and 303, and the manufacturing process in a one piece or multipart technique (brazing).

The large variability in ion concentration among individuals is a common finding when examining the release of metal ions in saliva.^{9,10,15-17} This variation may be related to several factors since saliva does not present a constant composition and may be different among individuals or even among periods for the same individual. The physical properties, amount, and composition of saliva are influenced by factors such as diet, period of the day, and psychic conditions.¹⁵

A removable appliance was used in this study to avoid direct bonding of brackets on the teeth of study subjects, thus reducing the risk of damage to the individuals. This obviously involves the risk of improper utilization by the subjects, but all subjects were dental students at the Dental School of PUCRS and were well informed of the importance of correct utilization of the appliance before inclusion in the sample.

Moreover, because of the low concentration of metals investigated the procedures used for collection, preparation, and analysis of saliva samples presented risk of accidental contamination.^{10,15}

In general, the results of this study revealed an increase in nickel and chromium levels in saliva of individuals after placement of metallic brackets in the oral cavity, as previously reported in the literature.¹⁵⁻¹⁷ The highest concentrations of nickel and chromium were reached 10 minutes after placement of the appliance (T2), significantly higher than those observed without the appliance (T1; Tables 1 and 2). This result was also observed by other *in vivo* studies,¹⁵ in which significant differences in nickel levels were found only at this period.

The tendency for stabilization of the amount of metals released after time, as observed in this study (Tables 1 and 2), suggests that orthodontic appliances reached a passive state since metals placed in the mouth present immediate alteration of their surface characteristic because of biofilm formation.¹⁸ This factor may reduce the corrosion *in vivo*.¹⁵ This also explains why Tables 1 and 3 demonstrated lower amounts of nickel and iron at T6 compared to T1. This variation probably occurred because the appliance no longer influenced the quantity of ions in the oral cavity, and thus, other factors would affect the variations in salivary ion concentration.

Iron presented a different picture since there was no increase in its levels. This result was unexpected since iron is the basic element of stainless steel and thus should present the greatest variation. This may be ex-

plained by the fact that high amounts of iron ion are present in the oral cavity, even before placement of the appliance.

Even though there were some differences among groups A, B, and C when individually analyzed, statistical analysis comparing the release of nickel, chromium, and iron among groups revealed similar outcomes for all groups at all study periods (Tables 4-6). This is in agreement with the findings of other authors,^{9,15} who studied the release of metals in saliva and observed no differences between brackets, even when appliances with welded parts (eg, the quad-helix) were analyzed. Once again, the biofilm formed on the metallic surface probably influences this, leading to a similar release of ions from different types of steel.

These results disagree with some *in vitro* studies since most laboratory investigations revealed differences in ion release among different appliances.^{6,7,19-23} The brackets in groups B and C are composed of AISI 316L steel, whereas the brackets in group A are fabricated with AISI 303 steel. *In vitro* studies on the 316L alloy observed that it was more resistant to corrosion.⁶ However, even though the brackets in group B are fabricated with a more resistant alloy, they present a welded area between the body and base of the brackets, which tends to increase susceptibility to corrosion.^{3,19,21,24}

Comparisons with *in vitro* studies are necessary, yet limited, because of variations in methodology. The first variable is the immersion solution; the most often solutions used are 0.05% or 0.9% saline solution or artificial saliva with different compositions. The storage method may also vary between static and dynamic media and according to changes of solution to avoid saturation. Moreover, the periods of time and the brackets used vary widely among different studies. Despite that, most studies report similar outcomes, whereby the ion release tends to be constant after a certain period.^{6,22,25,26} However, there is no consensus as to the duration necessary before a metallic device reaches this passive state.

The similar results observed for the three brackets demonstrates the difficulty of extrapolating the results of *in vitro* studies to the clinical routine since nearly all such studies indicate that different results should be found among the different brackets. Such results were not observed in the present study. The high complexity of the oral environment may have led to a similar ion release by the different brackets.

CONCLUSIONS

- There is a large variability among individuals in the concentrations of nickel, chromium, and iron ions in saliva.

- There is an increase in nickel and chromium ions immediately after placement of the appliance in the mouth.
- There was no alteration in iron levels after placement of the appliance.
- There were no significant differences among the nickel, chromium, and iron levels released by the three groups at all study periods.

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