Assessment of Nickel Release from Stainless Steel Crowns

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Abstract
Objective: Adverse effects of dental materials, especially metals, have been an important issue in recent decades.

Purpose of Study: The purpose of this study was to determine the amount of nickel released from stainless steel crowns in artificial saliva.

Materials and Methods: In this in-vitro study, 270 stainless steel crowns were divided into five groups, each with nine subgroups. Each group (I to V) was comprised of four, five, six, seven and eight crowns, respectively. Each subgroup was placed in a polyethylene jar containing artificial saliva and held in an incubator at 37°C for four weeks. The amount of released nickel was determined on days 1, 7, 14, 21 and 28, using an atomic absorption spectrophotometer. Wilcoxon Signed-Rank and Kruskal-Wallis with Dunn’s post hoc tests (SPSS software, v. 18) were used for statistical analysis at a significance level of 0.05.

Results: The mean level of nickel on day 1 was more than that of day 7; this difference was statistically significant for all groups (P < 0.05), except for group II (P = 0.086). Also, the mean difference of released nickel between the groups was significant on day 1 (P = 0.006) and was insignificant on day 7 (P = 0.620). The nickel levels were zero on days 14, 21, and 28.

Conclusion: The amount of nickel was below the toxic level and did not exceed the dietary intake.

Keywords: Nickel; Saliva; Stainless Steel

INTRODUCTION
Attention towards adverse effects of dental materials used in dentistry, particularly metals, has been one of the major issues of recent decades [1, 2]. Metals, due to high resistance against corrosion and low price, are the most common materials used in dentistry especially pediatric dentistry [3, 4]; they are used in the manufacturing of various appliances such as space maintainers, stainless steel crowns (SSCs), as well as brackets [5–7]. Nickel has long been known as one of the causes of allergy and sensitivity [8]. This metal is known as an immunological allergen [9, 10] and approximately affects 10 % of the general population [11]. Other reports indicate that 4.5-28.5% of people are sensitive to nickel [12]. Also, nickel has been introduced in some studies as a carcinogenic and mutagenic
agent [10-14]. In addition, clinical symptoms such as allergic dermatitis, asthma, and mucosal ulcers and at the cellular level, toxicity as well as alterations in cellular function have been attributed to this metal [15]. On the other hand, some studies have indicated that placing dental alloys containing nickel in the oral environment has not caused allergies to nickel [16] and its release is much lower than the nutritional intake [5].

The electrochemical corrosion phenomenon occurs in the oral environment [11]; which is suitable for bio-degradation of metals due to enzymatic, microbiological, thermal and chewing conditions [11, 12] and corrosion in this environment results in the release of metals [11].

Presence of toxic metals in the saliva has been reported [17]. At present, among the trace elements, harmful effects of nickel have attracted much attention. Although many studies have assessed nickel release from orthodontic brackets [3, 9, 12, 14] due to the widespread use of SSCs, there is little information on the release of nickel from such crowns and there are major concerns about the biological effects of this release. In one study Keinan et al. assessed the absorption of released metal ions from SSCs by the roots of crowned primary molars. They found that crowns release nickel in oral environment and this ion is absorbed by the roots of primary molars [18]. Bhaskar et al. indicated that the level of nickel release on day 7 after placing space maintainers reaches its maximum and this amount cannot lead to toxicity [5]. Also, Zenelis et al. showed that there is no significant difference in the metal composition of retrieved crowns after intraoral exposure compared with unused crowns [3].

In dental treatments under general anesthesia there is the possibility of restoring up to eight teeth and sometimes more in one session with SSCs. In this study the amount of nickel release from SSCs in artificial saliva was assessed.

MATERIALS AND METHODS
In this in vitro study, 270 SSCs (3M/ ESPE, St. Paul MN, USA) were studied. Initially, the internal surface of crowns was filled with polycarboxylate cement (Aria Dent, ACT, Tehran, Iran) to prevent contact with artificial saliva and then after being fully set, they were placed into the saliva sample. The crowns were divided into five groups, so that code I was the group with four crowns, code II the group with five crowns, code III the group with six crowns, code IV the group with seven crowns, and code V the group with eight crowns. Each group had nine subgroups (A to I). The crowns were randomly assigned to groups and subgroups in different types and sizes.

Subgroups were maintained separately in closed polyethylene jars (Axigen, Union City, CA, USA) containing 20 ml of artificial saliva [synthetic saliva with a pH of 6.43±0.26 consisting of: 0.8 g NaCl, 2.4 g KCl, 1.5 g NaH$_2$PO$_4$, 0.1 g Na$_2$S and 2 g CO(NH$_2$)$_2$] in an incubator at 37°C for four weeks. The samples were placed in the solution on day 0. After day 1 and every seven days they were taken out from the solution and placed in another container with fresh saliva in order to avoid saturation of solution with released ions. All samples were shaken gently during immersion, to ensure bathing all crowns in saliva and to obtain a uniform solution.

The amounts of released nickel were measured on days 1, 7, 14, 21 and 28. The Graphite furnace atomic absorption spectrophotomer (AA-6601G/GFA 6500, Shimadzu, Kyoto, Japan) was used for quantitative assessment of released nickel. Nickel standard solution (100 mg/ml) was prepared through dissolving nickel nitrate (Merck, Frankfurter, Germany) in deionized water. Thinner solutions were prepared on daily basis by diluting the standard solution for calibration of the device. Nickel level of each sample was determined twice and the concentration of nickel below detectable level was considered zero.
Statistical analysis
For data analysis, the Wilcoxon signed-rank test was used to compare the mean released nickel over time (comparison of days 1 and 7). Kruskal-Wallis with Dunn’s post hoc test were respectively used for comparing the mean released nickel in five groups and paired comparison of groups on days 1 and 7. SPSS version 18 (SPSS Inc., Chicago, IL, USA) with a significance level of 0.05 was used for analysis.

RESULTS
The amount of released nickel in different studied groups on day 1 was more than that of day 7. Using the Wilcoxon signed-rank test, a significant difference was observed in the mean released nickel on days 1 and 7 in all groups except for group II (P = 0.086, Table 1). Also, according to the Kruskal-Wallis test a significant difference was observed in the mean released nickel among the five groups on day 1 (P = 0.006); however, such difference was not observed on day 7 (P = 0.620). The Dunn’s post hoc test indicated that the difference in the mean released nickel on day 1 was significant between groups I and IV (P=0.005). Also, significant differences were observed in the mean released nickel on day 1 between group V and other groups (P< 0.05, Table 2). The amount of released nickel on days 14, 21, and 28 was zero.

DISCUSSION
The use of SSCs to restore carious primary molars under general anaesthesia is strongly recommended [19]. These crowns contain chromium (17-19% by weight), nickel (8-10 wt %), manganese (up to 2 wt%), silicon (up

| Groups   | Amount of nickel Mean (SD) | P value * |
|----------|---------------------------|-----------|
|          | Day 1 (ppm)               | Day 7 (ppm) |         |
| Group I  | 0.0112 (0.0031)           | 0.0046 (0.0038) | 0.021*   |
| Group II | 0.0145 (0.0049)           | 0.0069 (0.0094) | 0.086    |
| Group III| 0.0138 (0.0029)           | 0.0057 (0.0045) | 0.011*   |
| Group IV | 0.0161 (0.0048)           | 0.0038 (0.0032) | 0.008*   |
| Group V  | 0.0269 (0.0139)           | 0.0024 (0.0012) | 0.008*   |
| P value b| 0.006*                    | 0.620      |

*P < 0.05.

*a P value was based on Wilcoxon signed-rank test.

*b P value was based on Kruskal-Wallis test

| Paired comparison of groups * | Group II | Group III | Group IV | Group V |
|------------------------------|---------|-----------|---------|---------|
| Group I                      | P =0.252| P =0.101  | P =0.005*| P =0.002*|
| Group II                     | P =0.656| P =0.896  | P =0.041*| P =0.024*|
| Group III                    | P =0.112| P =0.024  | P =0.032*|         |
| Group IV                     | P =0.306|           |         |         |

*P < 0.05.

*a P value was based on Dunn’s post hoc test.
to 1 wt%), and molybdenum (0.6 wt%) [3].

In the oral environment, crowns are immersed in the saliva, which acts as an electrolyte, enhancing corrosion [20]. It has been demonstrated that the release of metal ions is not associated with the percentage of nickel in alloy composition [10, 12] but mostly dependent on resistance against alloy corrosion during intraoral wear. Nowadays, the primary nickel-chromium crowns (containing 72 wt% nickel) are replaced with SSCs containing 8-10 wt% nickel [3].

In the present study, after immersion, the samples were gently shaken. This action, in addition to ensuring that a uniform solution is obtained, will lead to an increase in the release of nickel. Moreover, in order to prevent the release of nickel from the inner surface of crown, all the crowns were filled with cement. Saliva was also refreshed weekly to prevent its saturation.

In our study, the highest amount of released nickel was obtained at 0.0269 ppm; which was insignificant in terms of toxicity (toxic dose: 2.5 mg/ml, lethal oral dose: 50-500 mg/kg body weight [5]). This level of nickel did not exceed the amount of daily intake (200-300 μg), which was consistent with many studies [5, 9, 11, 20]. Of course, for the occurrence of allergic reaction in the mucosa, the antigen should be 5-12 times stronger than what is required to create an allergic reaction on the skin [11, 12]; this amount of nickel can be enough to induce an allergic reaction, due to high haptenic capacity of the released metal [12]. Haptens are small molecules that cannot trigger the immune system reactivity by themselves; but hapten-protein conjugates can act as a trigger. The conjugated haptens become antigens and induce the formation of anti-hapten antibodies. Even antibodies with specificity for metal ions, such as nickel have been produced in this way [21]. We examined the mean nickel released from a maximum of eight crowns; however, in some clinical conditions in which more than eight crowns are cemented simultaneously in the mouth under general anaesthesia, the result might be considerable. Also, no nickel content was detected on days 14, 21, and 28; this could be due to the release of this metal below the detectable level by the device. In most studies, the metal ions are often released only in the early stages and in the initial days [10, 20].

In our study, by increasing the number of crowns on day 1 the mean nickel release increased. Group II was an exception in this regard. However, considering the large size of the crowns in this group compared to other groups, this finding may be acceptable. On this day, the highest amount of release was seen in group V. In comparing the mean amount of nickel released on day 1, a significant difference was observed between groups I and IV, as well as group V with all groups. It means that increasing the number of crowns to seven, makes a significant difference in the mean amount of released nickel compared with the use of one crown; while eight crowns created a cumulative effect leading to a significant difference with all previous groups in the mean amount of nickel released.

Also, on day 7, the mean amount of nickel release was not proportional to the number of crowns. This means that in comparing groups II, III, IV and V, increasing the number of crowns decreased the mean amount of nickel. In the present study, the analyses of saliva samples were conducted at separate times; which did not reveal a continuous release pattern. We believe that the amount of nickel release might be proportional to the number of crowns on days before day 7 and due to high levels of nickel in groups with more crowns, metal gradually deposits on the surface of crown by day 7 and this sediment is proportional to the number of crowns at any time before day 7. There was no significant difference between groups on the mean amount of nickel release; which indicates that on day 7 of immersion the number of crowns had no significant effect on the mean amount of released nickel.
nickel. Also, in comparing the mean amount of released nickel in terms of time, it was found that the reduction was significant from day 1 to 7. In group II, this status was relatively close to the level of significance. This release kinetics may be interpreted by an initial release of ions on the metal surface and then slowing down of this process by formation of a stable oxide layer; which protects the surface against corrosion [4, 12].

Apart from the release of ions, wash-out of the wear products in the oral cavity is also of critical importance in biologic capability of metallic materials. The abrasion mechanism created during intraoral corrosion of crowns includes leaching of wear products into the oral cavity. This fact may affect the biological capability of crowns. Worn fragments increase the daily intake of heavy elements from crowns leading to increased risk of biological consequences [3]. Complete comparison of the findings obtained in our study and other in vitro studies is not possible due to methodological differences such as storage medium, sample size and the study variables. Keinan et al. measured the uptake of metal ions released from SSCs by the cementum of deciduous teeth. In their study, there was a significant difference in nickel level in the cementum of crowned and uncrowned teeth; which was indicative of a large amount of nickel in the cementum of crowned teeth. In the mentioned study, the amount of metal ions adsorbed by the cementum was 5-6 times higher in crowned than uncrowned teeth [18]. The findings of their study are in agreement with those of the present study indicative of the release of nickel from SSCs.

The retrieval analyses are in vitro studies that examine in vivo aged samples. In an analysis of retrieved crowns by Zinelis et al, no changes were seen in the composition of elements. This study indicated that neither nickel nor any other element could be released under clinical conditions and in other words the crowns are not prone to corrosion [3].

They showed that clinical conditions revealed no nickel release from SSCs, which is in contrary to our study result. Bhaskar et al. suggested that nickel released after placing space maintainers reaches its maximum on day 7 [5]. Although the concentration of nickel in the above mentioned study and our research declined after the first peak, in our study, the maximum nickel release occurred on day 1. This difference could be due to factors such as the type of examined material (stainless steel bands or crowns), the number of materials used in each saliva sample and the composition of artificial saliva. In both studies, the amount of nickel was much lower than the patient’s nutritional intake.

Although in the present study the factors involved in the release of nickel from crowns (number of crowns and duration of immersion) were assessed, on the whole it must be stated that in vitro experiments have many shortcomings in simulating the oral conditions and the amount of released nickel in this study and other researches cannot be directly attributed to in vivo conditions. Factors such as temperature, quantity and quality of saliva, dental plaque, salivary protein level and pH as well as physical and chemical properties of food and liquids, abrasion caused by chewing and general and oral health can affect corrosion in the oral cavity [9]. Because of these facts and given that the SSCs are also used to restore young permanent molars, it is logical to complete the findings of this study with an in vivo evaluation and to examine the release effects of this element on general health of children.

Also, in vitro and in vivo evaluation of nickel release from SSCs after they are trimmed in length, which is common in the clinical setting, is suggested.

CONCLUSION
From this study, we concluded that restoring up to 8 primary teeth with SSCs cannot cause toxicity in terms of salivary nickel release.
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