

1 Article

2 Detection of Ni, Fe, and Cr released in saliva after 3 prefabricated metal crown placement in children

4 César Tadeo Hernández-Martínez ¹, Raúl Alberto Morales-Luckie ^{2,*}, Norma Leticia Robles-
5 Bermeo ¹, Sandra Isabel Jiménez-Gayosso ¹, Marius Ramírez-Cardona ³, Verónica García-
6 Hernández ³, Edith Lara-Carrillo ¹, Carlo Eduardo Medina-Solís ^{1,4,*}

7 ¹ Advanced Studies and Research Centre in Dentistry "Dr. Keisaburo Miyata" of Faculty of Dentistry at
8 Autonomous University State of Mexico, Toluca, Mexico; cetahm@gmail.com (C.T.H.M.);
9 norle.rob@gmail.com (N.L.R.B.); sajimiga@gmail.com (S.I.J.G.); laracaedith@hotmail.com (E.L.C.)

10 ² Sustainable Chemistry UAEMex-UNAM Research Center of Faculty of Chemistry at Autonomous
11 University State of Mexico, Toluca, Mexico; ramluckie@gmail.com

12 ³ Academic Area of Earth Sciences and Materials of Basic Sciences and Engineering Institute at Autonomous
13 University of Hidalgo State, Pachuca, Mexico; mariusr@uaeh.edu.mx (M.R.C.); v720609@yahoo.com.mx
14 (V.G.H.)

15 ⁴ Academic Area of Dentistry of Health Sciences Institute at Autonomous University of Hidalgo State, Pachuca,
16 Mexico; cemedinas@yahoo.com

17 * Correspondence: cemedinas@yahoo.com (C.E.M.S.); ramluckie@gmail.com (R.A.M.L.); Tel.: +52-771-189-
18 9294 (C.E.M.S.); +52-722-394-5042 (R.A.M.L.)

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20 **Abstract:** Dental caries is a public health problem worldwide according to WHO data. Among
21 treatments in pediatric dentistry, prefabricated metal crowns (PMCs) have been one of the most
22 successful options since they were introduced in cases of considerable tooth destruction. Our
23 objective was to detect the presence and concentration of iron (Fe), chromium (Cr), and nickel (Ni)
24 in saliva of patients who require rehabilitation with PMCs, before and after their placement. A
25 quasi-experimental study was performed in 32 patients who attended dental care in a pediatric
26 dentistry clinic at a public university and who required rehabilitation with PMCs. Parametric tests
27 (ANOVA and Pearson correlation) were performed, and a $p \leq 0.05$ was considered statistically
28 significant. Statistically significant differences were found when comparing the Ni release before, 1
29 week, and 1 month after placing the crowns. Similarly, we observed a positive correlation between
30 the number of crowns and Ni release. No tests were performed for Fe and Cr because the amounts
31 of these metals were less than 0.1 ppb, which was not detectable by inductively coupled plasma
32 optical emission spectrometry (ICP-OES). The levels of Fe, Cr, and Ni released were below toxic
33 health levels. Studies are required to evaluate whether this release has negative effects at cellular
34 levels.

35 **Keywords:** prefabricated metal crowns (PMCs); nickel (Ni); chromium (Cr); iron (Fe); ion release

36

37 1. Introduction

38 Dental caries is a public health problem in most countries. Data from the WHO indicate that this
39 pathology affects around 60–90% of school-age children [1], and dental caries is 32-times more
40 common in children exposed to risk factors such as low socioeconomic status, carbohydrate-rich diet,
41 and parents/guardians with a low educational level [2]. Currently, there are various restorative
42 alternatives in pediatric dentistry, and PMCs have been one of the most successful options when
43 there is considerable tooth destruction [3].

44 PMCs were first described by Engel followed by Humphrey in 1950 [4,5], and since then, PMCs
45 have been used successfully in the restoration of both primary and permanent teeth [5–9]. These
46 restorations have evolved over the years, improving their physical characteristics and clinical
47 management [6]. Currently, the most-used PMCs are made of stainless steel, which are composed of

48 iron (65–74%), chromium (17–19%), nickel (9–13%), and other elements (4%) [5,6] Similarly, there are
49 nickel-chromium crowns, which are composed of 72% nickel, 14–17% chromium, and 6–10% iron,
50 with small amounts of other elements [6].

51 Both nickel and chromium have been widely studied because of their potential health effects; it
52 has been established that these metals can cause allergy, dermatitis, asthma, and toxicity [10]. Studies
53 have shown that the release of Ni and Cr in patients with PMCs is much lower than levels of these
54 elements that are ingested in the daily diet [11]. Special attention has also been paid to these metals
55 because they have carcinogenic and mutagenic potential [12].

56 The objective of this study was to detect the presence and concentration of Fe, Cr, and Ni in
57 saliva of patients who attended rehabilitation at the Clinic of the Pediatric Dentistry Specialty at the
58 Autonomous University, State of Mexico.

59 2. Materials and Methods

60 2.1. Study design

61 A quasi-experimental study was performed. Both parents and children were informed about the
62 purpose of the study, and an informed consent document was signed by the child's parent or
63 guardian before participation. The study was performed in accordance with the ethical principles of
64 the Declaration of Helsinki and was approved by the Research and Ethics committee of the
65 Autonomous University State of Mexico, Faculty of Dentistry.

66 2.2 Study participants

67 The present study was performed in patients who attended Pediatric Dentistry Specialty clinic
68 of the Advanced Studies and Research Centre in Dentistry "Dr. Keisaburo Miyata" at the Faculty of
69 Dentistry at the Autonomous University State of Mexico, between January and June 2017. These
70 patients had extensive carious lesions and needed restorative treatment with PMCs.

71 Thirty-two children, from 3 to 9 years old (average age, 5.72 ± 1.73 years), were selected to
72 participate in the study. The inclusion criteria were as follows: a) both sexes; b) required restoration
73 using PMCs; c) systemically healthy; d) informed consent signature; and e) cooperative patients. The
74 exclusion criteria were as follows: a) patients who presented with previously placed PMCs; b)
75 patients with previously placed metal restorations; c) patients in whom it was decided to place free
76 metal crowns; and d) patients with orthopedic appliances.

77 2.3 Process

78 The placement of PMCs was performed by students in the Pediatric Dentistry Specialty at the
79 Autonomous University State of Mexico, Faculty of Dentistry. The process was performed under
80 local anesthesia and followed the restorative dentistry guidelines of the American Academy of
81 Pediatric Dentistry [20]. After establishment of the preoperative occlusion, absolute isolation was
82 achieved using a rubber dam, caries was eliminated and pulp therapy was performed in necessary
83 cases. Subsequently, an occlusal reduction of 1.5 to 2 mm was performed using a wheel-shaped
84 diamond bur. The proximal, vestibular, and lingual/palatal wear was from 1 to 1.5 mm, which was
85 performed with a pencil tip diamond bur. Finally, the PMC (3M, St Paul, MN, USA) was tested,
86 adjusted, and cemented using glass ionomer cement.

87 Three 5-mL samples of unstimulated saliva were taken per patient according to previously
88 established guidelines [21]. The first sample was taken before PMCs rehabilitation, and the other two
89 were taken 1 week after and 1 month after the PMCs placement. The samples were collected around
90 10 am, the patient and his/her parent were instructed to avoid eating, drinking liquids, or chewing
91 gum 1 hour before taking the samples. The patient was instructed to rinse with deionized water and
92 wait 5 minutes in a relaxed position, and then they were asked to avoid movement and to tilt the
93 head at 45° and spit out the collected saliva in a polyethylene bottle until the required quantity was
94 reached; subsequently, the samples were stored at a temperature of 5°C until processing.

95 The samples were pre-digested with 2% nitric acid (HNO₃) and kept refrigerated until analysis.
96 Before analyzing the samples, they were digested using a heating plate. Metal levels were measured

97 in the laboratory of the Academic Area of Earth Sciences and Materials of Basic Sciences and
 98 Engineering Institute at the Autonomous University of Hidalgo State using ICP-OES (OPTIMA 8300
 99 by Perkin Elmer, Madrid, Spain).

100 2.4. Statistical process

101 The statistical package SPSS Version 23.0 (IBM Analytics, New York, USA) was used to perform
 102 the data analysis. In the univariate analysis, frequencies and percentages were reported for the
 103 qualitative variables, and means and standard deviation for quantitative variables. To test for
 104 differences in the included variables, a bivariate analysis was performed, in which parametric tests
 105 were used (ANOVA and Pearson's correlation). A value of $p \leq 0.05$ was considered statistically
 106 significant.

107 3. Results

108 Thirty-two patients participated in the study; they had a mean age of 5.71 years ± 1.72 and 53.1%
 109 (n=17) were women. The average number of PMCs placed in the patients was 5.16 ± 2.56 . The rest of
 110 the variables included in the study are shown in Table 1.

111 **Table 1.** Descriptive analysis of the study variables

Variable	Mean	SD
Age	5.71	1.72
Number of PMCs	5.16	2.56
Ni ¹		
Baseline Ni amount	2	3.59
Week Ni amount	68.48	5.42
Month Ni amount	43.97	9.99
Cr ¹		
Baseline Cr amount	≤ 0.1	0.0
Week Cr amount	≤ 0.1	0.0
Month Cr amount	≤ 0.1	0.0
Fe ¹		
Baseline Fe amount	≤ 0.1	0.0
Week Fe amount	≤ 0.1	0.0
Month Fe amount	≤ 0.1	0.0
	Frequency	Percent
Sex		
Male	15	46.9
Female	17	53.1

112 ¹ ppb

113 A repeated measures ANOVA was performed to evaluate Ni release before, 1 week after, and 1
 114 month after the PMCs had been placed. Table 2 shows the results of Bonferroni's multiple
 115 comparisons for Ni, which showed statistically significant differences between the three
 116 comparisons. The test for Fe and Cr was not performed because the amounts of these metals were
 117 equal to or less than 0.1 ppb, which is not detectable by ICP-OES.

118 **Table 2.** ANOVA with Bonferroni multiple comparisons for Ni

		Difference of means	p value ¹
Baseline Ni amount	Week Ni amount	-66.48	<0.001
Baseline Ni amount	Month Ni amount	-41.97	<0.001
Week Ni amount	Month Ni amount	24.51	<0.001

119 ¹ Analysis of variance of repeated measures (ANOVA) p value ≤ 0.008333 .

120 Table 3 shows the results of the Pearson correlation test where the amount of Ni detected, and
 121 the total number of PMCs placed in the mouth were compared. There was a slight negative
 122 correlation between the number of crowns and the baseline Ni values ($r = -0.342$, $p = 0.056$). Both
 123 week and month a strong positive correlation was found, obtaining the following values respectively:
 124 $r = 0.969$ ($p = 001$) and $r = 0.926$ ($p = 001$). Which indicates that the release of metals is related to the
 125 number of crowns placed. As with the repeated measures ANOVA test, neither the Cr nor Fe test
 126 were performed due to the same reasons.

127 **Table 3.** Pearson correlation between PMCs number and metal release.

Variable	PMCs number	p value ¹
Baseline Ni amount	$r = -0.342$	0.056
Week Ni amount	$r = 0.969$	<0.001
Month Ni amount	$r = 0.926$	<0.001

128 ¹ Pearson correlation

129 4. Discussion

130 PMCs are used extensively in pediatric dentistry because of their successful use in restorations
 131 [3]; however, when exposed to the intra-oral environment, they can corrode and release metal ions
 132 [15]. Its components include the presence of Ni and Cr. These metals have been studied because of
 133 their controversial effects on health [16]. Ni is integrated into steel alloys to stabilize the austenitic
 134 phase, maintain polish, and decrease its ductility; similarly, Cr increases corrosion resistance [17].
 135 Previous studies suggest that it is preferable to use salivary samples from patients who require oral
 136 rehabilitation with PMCs instead of an *in vitro* model, because of the advantage represented by
 137 studying this release in a dynamic environment [12].

138 As in other studies [11,16,18,19], the amount of Ni detected was less than the amount ingested
 139 in the daily diet, which is in the range of 300–500 ppm per day [15,18]. In contrast to other studies, in
 140 the present study, there was no presence of Fe or Cr [11], which may be because the technique used
 141 is not sensitive enough. The release of Ni by PMCs is greater 1 week after placing the PMCs; similarly,
 142 we observed that the amount decreased after 1 month [16]. Systemic studies should be performed to
 143 evaluate if this amount released is constant or lower at this level.

144 The results show that the amounts of metals released by the PMCs are not toxic to health (toxic
 145 dose: 2.5 mg/mL, oral lethal dose: 50–500 mg/kg) [17]. However, the risk of developing allergic
 146 reactions has been well documented [16,20]. Therefore, it is necessary to evaluate the effects of these
 147 metals at the local level, because, although the amounts are small, they can cause allergic reactions
 148 [21].

149 5. Conclusions

150 The present study showed that the amounts of Ni, Fe, and Cr are significantly lower than those
 151 ingested in the daily diet. It is recommended to conduct studies to evaluate if this release has negative
 152 effects at the cellular level.

153 **Limitations:** The present study has certain limitations that should be taken into account for future research.
 154 Measuring the release of metals only in saliva does not provide the exact concentration in the body, and it is
 155 recommended to take samples of urine or nails. Similarly, the use of more sensitive devices such as the ICP-MS
 156 is widely recommended, because in our study, we could not detect quantities lower than 0.1 ppb.

157 **Author Contributions:** César Tadeo Hernández-Martínez, Raúl Alberto Morales-Luckie, Norma Leticia Robles-
 158 Bermeo, Edith Lara-Carrillo and Carlo Eduardo Medina-Solís conceived, designed the experiments, and
 159 contributed reagents and materials; Marius Ramírez-Cardona and Verónica García-Hernández performed the
 160 experiments; César Tadeo Hernández-Martínez, Raúl Alberto Morales-Luckie, Sandra Isabel Jiménez-Gayosso,
 161 and Carlo Eduardo Medina-Solís analyzed the data and wrote the first draft of the manuscript. All the authors

162 were involved in the conceptualization of the paper, analysis, critical review, interpretation of the results, and
163 made intellectual contributions, and they also accepted the final version.

164 **Conflicts of Interest:** The authors declare no conflicts of interest.

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