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1 Ion release from dental casting alloys as assessed 2 by a continuous flow system: Nutritional and 3 toxicological implications

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8 ABSTRACT

Objectives. The aims of this study were to quantify the metallic ions released by various dental alloys subjected to a continuous flow of saliva and to estimate the nutritional and toxicological implications of such a release.

Methods. Four pieces of three nickel-based, one noble, one high-noble and two copper-aluminum alloys were cast and then immersed in a continuous flow of artificial saliva for 15 days. To simulate three meals a day, casts were subjected to thrice-daily episodes, lasting 30 min each and consisting of pH decreases and salinity increases. After 15 days, the metallic ions in the artificial saliva were analyzed. Data were expressed as averaged release rate: $\mu\text{g}/\text{cm}^2/\text{day}$ of ion released for each alloy. The highest value of 95% CI of each ion was adapted to a hypothetical worst scenario of a subject with 100 cm^2 of exposed metal surface. The results were compared with the tolerable upper daily intake level of each ion.

Results. The copper-aluminum alloys released copper, aluminum, nickel, manganese and iron. The nickel-based alloys essentially released nickel and chromium, while the beryllium-containing alloy released beryllium and significantly more nickel. The noble and high-noble alloys were very resistant to corrosion. The amount of ions released remained far below the upper tolerable intake level, with the exception of nickel, released by beryllium-containing nickel-based alloy, whose levels approach 50% of this threshold.

Significance. The daily amount of ions released seems to be far below the tolerable upper intake levels for each ion.

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1. Introduction

3 There are currently hundreds of alloys available for
4 prosthodontic restorations. The major factors affecting
5 alloy selection are economics, physical properties, casting
6 technique, corrosion and biocompatibility [1,2]. In recent
7 years, there has been increasing concern about the adverse

8 effects, both local and systemic, of prosthodontic alloys [3,4].
9 These adverse effects can be toxic or allergic and are linked
10 to ion release in the organism, as well as to the kind of
11 ions released [5-7]. However, as some elements are essential
12 nutrients involved in biological functions, a minimum daily
13 intake is needed. The tolerable upper intake level is the
highest daily nutrient intake, from food plus other sources

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of supply, which can be safely ingested by the vast majority of individuals without posing any adverse health effects [8]. Given that prosthodontic restorations are a supply source for metal ions, the amount of ion released must be below the tolerable upper intake level.

Ion release from dental alloys has been evaluated mainly by *in vitro* studies, in which the alloy is subjected to different settings: oral bacteria [9], galvanism [10], electrolyte bath [11], oral proteins [12], different pH levels [13] and brushing with toothpaste [14]. In general, the most frequently used method for monitoring the number of elements released from casting dental alloys is a static system. However, to mimic the *in vivo* setting of the oral cavity more closely, a continuous flow system has been employed for studying the fluoride release of glass-ionomer materials [15,16].

The aims of this study were: (1) to identify and quantify the different ions released by various dental alloys subjected to a continuous flow of saliva, thereby reproducing certain *in vivo* conditions, such as the constant flow of saliva and the sudden changes in pH and salinity that occur during meals and (2) to compare the number of ions released over time with the tolerable upper intake level of minerals.

2. Materials and methods

Five current prosthodontic alloys were chosen: three nickel-based alloys, Will-Ceram Litecast[®] (Williams Dental, USA), Litecast B[®] (Williams Dental, USA) and Nibon[®] (Ventura, Spain); one noble alloy, Cerapall 6[®] (Metalor, Switzerland) and one high-noble alloy, Pontor 4CF[®] (Metalor, Switzerland). In addition, two copper-aluminum alloys, Orcast[®] (Ventura, Madespa, Spain) and NPC[®] (Aalbadent, USA), were selected as positive controls due to their highly corrosive properties. Their composition was verified by semi-quantitative analysis using X-ray diffraction, which indicates the presence of elements in a proportion greater than 1% (Table 1).

For each alloy, 20 rectangular pieces of 1.3 cm × 2.6 cm × 0.04 cm wax (Technowax[®], Protechno, Vilamalla, Spain) were placed in 4 cylinders and then cast with a Ducatron[®] induction casting machine (Ugin Dentaire, Grenoble, France), following the manufacturer's instructions. All casts were subjected to a standardized polishing procedure to obtain a glossy surface with similar average roughness values, which ranged from 0.2 to 0.4 μm analyzed by Profilometry (Perthometer M4P[®], Perthen, Germany). The casts were subsequently put into individual plastic corrosion recipients of 3 cm × 3 cm × 1.5 cm, each containing five pieces of the same cast. Thus, each recipient presented a metal surface of 33.8 cm². Casts were then immersed in a constant flow of artificial saliva (named base saliva) for 15 days by means of a peristaltic bomb (Watson Marlow 302S, 55 rpm). This permitted the artificial saliva to run through the recipients (Fig. 1), regulating a flow of 2.7 mL/h, which corresponds to 10% of real base saliva secretion. Moreover, the casts were subjected to thrice-daily episodes of pH decrease and salinity increase to mimic the changes that occur during meals. This was achieved by means of an electrical valve (Asco Angar[®]) connected to a time programmer that cut off the base saliva flow every 7 h and gave way to modified saliva, with the addition of sodium chloride and

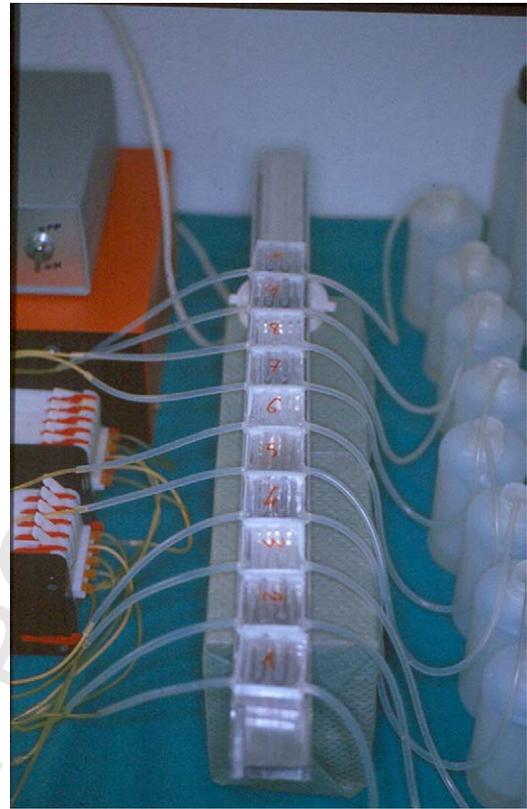


Fig. 1 – Overall view of the peristaltic bomb, the corrosion recipients and the collection containers.

acetic acid (termed meal saliva) for 30 min. “Base saliva” was a solution of Fusayama Meyer artificial saliva [17], containing 0.4 g/L NaCl, 0.4 g/L KCl, 0.69 g/L NaH₂PO₄·H₂O, 0.79 g/L CaCl₂·2H₂O and 1 g/L urea. “Meal saliva” consisted of modified Fusayama Meyer artificial saliva, supplemented by 9 g/L of sodium chloride and 0.1N acetic acid to attain pH 4. These changes were made because most foods are eaten with NaCl supplement and dental plaque pH decreases to approximately 5.5 for 30 min after each meal [18]. Acidity can also be reached through acidic drink or regurgitation [19]. The metal pieces were arranged vertically in corrosion recipients, alternating right and left, so that the saliva flow was forced to zigzag between them, thereby bathing the entire metal surface. Saliva exiting the corrosion recipients was collected in 250 cm³ precipitate glasses covered with parafilm to avoid contamination.

The entire circuit was prepared to prevent the saliva coming into contact with metals other than the experimental alloys. Artificial saliva solutions were, therefore, kept in plastic bottles, the electrical valve was made entirely of Teflon, and the tubes through which the saliva passed were made of silicon, and were connected to the electrical valve by plastic brackets. All connections between the silicon tubes, as well as those between the silicon tubes and the plastic recipients, were made watertight by means of silicone rubber glue (Rhodia CAF3[®], Rhône Poulenc). The metal pieces were stuck to the floor, the lateral wall and the lid of the plastic recipient were stuck with silicon, and the lid of the plastic recipient was stuck to the recipient with an adhesive of rigid plastics

Table 1 – Semi-quantitative analysis of the composition (wt%) of each alloy

Alloy		Ni	Cr	Mo	Al	Si	Sn	Be	Pd	Au	Ag	In	Ga	Zn	Cu	Mn	Fe
WC-Litecast		70.6	15.2	13.0	1												
Litecast B	Nickel-based	78.9	12.0	3.5	4.3			1.7									
Nibon		63.2	18.2	7.1		5.5	3.6										
Cerapall 6	Noble								75.0	6.1	6.4	5.9	6.0				
Pontor 4CF	High-noble								8.7	64.3	20.3	3.7		1.0			
Orcast	Copper–aluminum	4.6			10.6										83.0		1.5
NPG		3.6			8.7									3.0	80.9	1.5	2.1

Bold-face elements show the main component of each alloy.

(Plasticceys®). The entire tubing circuitry, the corrosion recipients, the precipitate glasses and the collection bottles were first washed with 5% nitric acid for 1 h to eliminate any metal presence. They were then rinsed with distilled water, and finally with ultrapure water Milli Q. In addition, saliva from the first 3 h was rejected to attain a perfect cleansing of all circuitry. Each time the corrosion recipients were changed, all the tubing was also replaced.

As the peristaltic bomb had 10 outlets, 7 plastic recipients were connected, each with a different alloy, with the remaining 3 used as blanks as follows: two were connected to plastic recipients containing silicon and Plasticceys® but no metal, and the third was connected directly to the collection bottle. Saliva was collected daily, with 1% nitric acid added to avoid ion precipitation, and poured into a glass bottle.

After 15 days, the saliva collected from each circuit was vortexed and 10 mL were sampled. The presence of metallic ions was analyzed in ppb (ng/mL) by inductively coupled plasma mass spectrometry. The analytical detection limits under these conditions were all below 0.04 µg/mL. All elements constituting the alloys were analyzed. The artificial saliva solutions were also analyzed prior to use.

Data were expressed as the averaged release rate: the mean micrograms of ion released per square centimeter of alloy per day, and the 95% confidence interval (CI) for the mean was also calculated. A non-parametric Kruskal–Wallis test was used to determine significant differences between groups in the volume of artificial saliva collected at the end of 15 days. A *p*-value < 0.05 was considered significant. The quantities released were adapted to a ‘hypothetical worst scenario’ in which a subject had all 32 teeth covered by full metal crowns. This would represent an exposed metal surface of approxi-

mately 100 cm². The results of multiplying the maximum 95% CI value for the average release rate of each ion by 100 were compared with the tolerable upper daily intake level of each ion.

3. Results

The mean of the artificial saliva collected for each group at the end of the 15 days ranged from 696 to 915 mL, and no significant differences were found.

Silica was found in similar proportions in all circuits originating in the silicon tubes, as well as from silicon used to stick the metal pieces.

The number of metallic ions released per surface unit per day is shown in Table 2. The Ni-based alloys essentially released nickel and chromium, but with significant differences between them: the alloy containing beryllium (Litecast B®) released 7 times more ions, mainly nickel, than Will-Ceram Litecast®, and 100 times more ions than Nibon® and moreover, released beryllium. The noble and high-noble alloys proved very resistant to corrosion. The copper–aluminum alloys released mainly copper, iron, aluminum and nickel.

The highest values of the 95% confidence interval for the ions most released from those alloys tested adapted to the hypothetical worst scenario with a subject having all 32 teeth covered by full metal crowns, and the tolerable upper and adequate daily intake levels of each ion are shown in Table 3. The hypothetical values were far below the upper tolerable intake level for each ion, with the exception of nickel, released by the beryllium-containing nickel-based alloy Litecast B®, which gave levels that reached nearly 50% of this threshold.

Table 2 – Mean (95% CI) of averaged release rate of metallic elements in µg/cm²/day

	Nickel-based			Noble	High-noble	Copper–aluminum	
	WC-Litecast	Litecast B	Nibon	Cerapall 6	Pontor 4CF	Orcast	NPG
Ni	0.668 (0.55:0.79)	4.693 (4.17:5.22)	0.042 (0.03:0.05)			0.090 (0.07:0.11)	0.110 (0.09:0.13)
Cr	0.024 (0.02:0.03)	0.014 (0:0.04)					
Al	0.025 (0:0.07)	0.048 (0:0.14)	0.042 (0:0.12)			0.141 (0.05:0.23)	0.161 (0.14:0.18)
Be		0.363 (0.31:0.41)					
Cu						0.050 (0.01:0.09)	0.364 (0.26:0.47)
Mn							0.063 (0.06:0.06)
Fe						0.182 (0.12:0.25)	0.169 (0.02:0.32)
Zn		0.020 (0:0.06)	0.006 (0:0.02)	0.007 (0:0.02)	0.027 (0:0.08)	0.029 (0:0.08)	0.053 (0.05:0.06)
Mo	0.076 (0:0.21)						

Table 3 – Comparison between the highest values of the 95% confidence interval for the ions most released from those alloys tested, the maximum daily release in a hypothetical subject with 32 full crowns, and the dietary reference intakes

Alloy		Results		Dietary reference intakes		
		Highest value of 95% CI ($\mu\text{g}/\text{cm}^2/\text{day}$)	Maximum daily release in a worst scenario ($\mu\text{g}/\text{day}$)	Adequate intake ($\mu\text{g}/\text{day}$)	Upper tolerable level ($\mu\text{g}/\text{day}$)	Functions
Ni	Litecast B	5.22	522	Unknown	1000	No clear biological function in humans
Cr	Litecast B	0.04	4	20–45	Unknown	Helps to maintain normal blood glucose levels
Al	Orcast	0.23	23	See text	See text	
Be	Litecast B	0.41	41	See text	See text	
Cu	NPG	0.47	47	900–1300	10000	Component of enzymes in iron metabolism
Mn	NPG	0.06	6	1800–2600	11000	Involved in the formation of bone, as well as in enzymes involved in amino acid, cholesterol and carbohydrate metabolism
Fe	NPG	0.32	32	8000–27000	45000	Component of hemoglobin and numerous enzymes
Zn	Pontor 4CF/Orcast	0.08	8	8000–12000	40000	Component of multiple enzymes and proteins.
Mo	WC-Litecast	0.21	21	45–50	2000	Regulation of gene expression Co-factor for enzymes involved in catabolism of sulfur amino acids, purines and pyridines

4. Discussion

As expected, high-noble and noble alloys showed the least ion release over 15 days, whereas beryllium-containing Ni-based alloy released the maximum amount of ions from the materials tested. It is very difficult to compare these results with other studies that used other alloys and different methods because the number of elements released are significantly affected by the alloy type [20] as well as by the composition and the pH of the corrosion liquid [13,19]. In the present study, the amount of nickel released from beryllium-containing nickel-based alloy was very similar ($4 \mu\text{g}/\text{cm}^2/\text{day}$ approximately) to the amount found in other studies in which a beryllium-containing nickel-based alloy was immersed either in saline [12] or phosphate-buffered saline [14], but 20 times less than when this kind of alloy was immersed at pH 2.3 and saline [21]. The amount of nickel released from WC-Litecast, a non-beryllium-containing nickel-based alloy, was practically identical ($0.6 \mu\text{g}/\text{cm}^2/\text{day}$) to that observed by Denizoglu et al. [22], who used Meyer saliva at pH 4. Noble and high-noble alloys are in general much more resistant to corrosion and release a very low amount of zinc [14,23] as in the present study.

The continuous flow system allows one to mimic the characteristics of the oral cavity, with its constant secretion of saliva and periodic changes in salinity and acidity coinciding with meals. In general, an advantage of using a continuous flow system is that the saturation limit is unlikely to occur. In the case of ions released from dental alloys, although there is no risk of reaching the saturation limit due to their very low release levels, the concentration profile could vary because the dissolved material is removed continuously from

the metal surface by the flow of saliva. In fact, the results of the present study were similar to those of other authors who used static systems [14,21,22]. Ion composition and pH of the corrosive solution are of great significance, especially for nickel-based alloys [13,19,24]. Nevertheless, noble alloys and especially high-noble alloys are not significantly affected by low pH [13] or by different electrolytes [11]. The release of ions into cell culture medium from some high-noble, noble and base-metal casting alloys was investigated over a period of 10 months [20]. Although higher initial rates were suspected, they were not verified and metal ions were constantly released during this entire period. In appraisal of the safety of the ion release compared with the tolerable upper daily intake level, it is desirable to measure ion release in those situations when rates can be high. If higher initial rates exist, the current results for the first 15 days correspond to this higher release. One of the limitations of the present study was that ion release was only measured after 15 days; therefore, the daily release profile is unknown and the data refer to the average release rate.

The physical properties and the biocompatibility of alloys depend on their composition and microstructure. In general, multiphase alloys are more prone to corrosion than monophase alloys, due to a galvanic effect between areas of different composition inside the alloy [23]. In fact, the same alloy can show different susceptibility to corrosion in different structural conditions created by heat treatment [25]. There are many factors that may change the final properties of the alloys, such as heating and cooling processes during casting, impurities [26] and the porcelain-fused-to-metal firing procedures. These may alter the surface oxides and corrosion properties of nickel–chromium alloys depending on their chemical composition [27].

In the present study, a beryllium-containing nickel-based alloy released the highest amount of nickel. The corrosion resistance of nickel-chromium alloys depends on the formation of a thin layer of oxides on the metal surface, resulting from an initial corrosion, thereafter acting as a protective layer. This phenomenon, known as passivation, generates a characteristic curve of ion release, with a high initial release, which drops after a time [21]. Nevertheless, this passivation layer can be disrupted under various conditions, including bruxism [28]. Even in very low proportions, beryllium is known to form a eutectic phase Ni-Cr-Be, which is susceptible to undergo a preferential corrosion, thereby releasing nickel and beryllium [21,29,30]. Nibon alloy had 15 times less corrosion than Will-Ceram Litecast. This is probably because Nibon contains 18 wt% chromium, which remains within the recommended range (16–27%) for reducing corrosive effects [31,32]. Will-Ceram Litecast and Litecast B contain 15.2 and 12 wt% chromium, respectively.

In living systems some metals have biological functions. While iron is essential in relatively high concentrations, other elements, such as zinc, copper, nickel, cobalt, molybdenum and perhaps chromium are only essential in trace amounts, since at higher concentrations they are very toxic. Some metals, such as mercury, lead, cadmium and uranium, have no clear biological function and are toxic even at very low levels [33].

Even in the worst scenario, with all 32 teeth covered by full metal crowns, the results remain far below the upper tolerable intake level for each ion, with the exception of nickel released by nickel-based alloy containing beryllium Litecast B[®], which gives levels near to 50% of this threshold. Some studies have suggested that non-sensitized persons can develop a tolerance to nickel through continual exposure to it at a mucosal surface, such as the situation with dental braces [34]. A double-blind, placebo-controlled clinical study demonstrated that oral nickel exposure elicits cutaneous nickel-allergic reactions in nickel-sensitive individuals in a dose-dependent manner [35]. It is not known whether a constant release in the oral cavity of minute amounts of nickel is harmful or beneficial to the patient. At the population level, oral intake of small amounts of nickel may help reduce overall sensitivity to nickel prevalence in humans. At the individual level, however, once allergic contact dermatitis has been diagnosed, a reduced oral intake of nickel would be advisable in certain cases [36].

In the worst-case scenario, 41 µg of beryllium a day would be released from Litecast B. The primary route of human exposure to beryllium is inhalation of vapor or particles, an exposure associated with increased incidence of lung cancer and a number of other diseases, from contact dermatitis to chronic granulomatous lung disease. Beryllium may also be ingested in drinking water or contaminated foodstuffs but as only 1% of ingested beryllium enters the bloodstream, this is not thought to be a particularly dangerous mode of exposure. Beryllium can also be inhaled and ingested from cigarette smoke or can enter the body through cuts in the skin [37,38]. Therefore, the ADA Council recommends that practitioners do not use alloys containing beryllium in the fabrication of dental prostheses, a precaution to protect not the patient, but the dental technician. In the same hypothetical worst case, the

maximum daily release of aluminum would be about 23 µg, in this case by Orcast[®]. Aluminum is ingested in amounts of 4–6 mg every day in food and beverages. In certain circumstances, such as the use of aluminum-containing antacids, some people may ingest a thousand-fold greater amount than the average daily consumption. Normally, however, the digestive tract is an effective barrier against gastro-intestinal aluminum absorption, with most of what is ingested excreted wholly unabsorbed in the feces [39].

It is nevertheless difficult to predict the clinical behavior of an alloy from in vitro studies, since such factors as changes in the quantity and quality of saliva, diet, oral hygiene, polishing of the alloy [40], the amount and distribution of occlusal forces [28], or brushing with toothpaste can all influence corrosion to varying degrees [14].

5. Conclusions

Under the conditions of the present study, the average daily release of ions from the alloys tested fell far below the tolerable upper intake level recommended for each ion.

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