

Quantitative analysis of leaching of different metals in human saliva from dental casting alloys: An *in vivo* study

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Abstract

Background: The issue of biomaterial-derived ionic release in various sites of the human body has attracted the interest of many investigators because of the possibility that debris or degradation products elicit a foreign body reaction or have a role in the induction of pathological processes.

Purpose: The purpose was to evaluate the saliva of denture wearers after insertion of the prosthesis for leaching of metals from metallic denture.

Materials and Methods: Total 20 subjects of age group of 40–60 years including both males (10) and females (10) were selected for the study. Total subjects were divided into 2 groups each containing 10 subjects, Group I (control group): Subjects having dentition intact up to second molar and free of any dental restoration; Group II (study group): Partially edentulous subjects rehabilitated with cast-metal removable partial denture. Saliva samples were taken at three stages that is, 1 h, 24 h and 72 h after the denture insertion from subjects of study group as well as from the control group. Atomic absorption spectroscopy (AAS) was used to estimate the concentration of elemental ions. Obtained data's were analyzed using SPSS (Statistical Package for Social Sciences) version 15.0 statistical analysis software. The values were represented in a number (%) and mean \pm standard deviation.

Results: At 1 h, 24 h and 72 h after the denture insertion in study group, chromium (Cr) had statistically significant higher mean concentration as compared to manganese (Mn) ($P < 0.001$). Cr had maximum concentration (0.1479 + 0.0052) immediately after denture insertion while maximum concentration of Mn (0.1479 + 0.0052) was found 24 h after denture insertion.

Conclusion: Metal-based dentures show maximum leaching immediately after wearing of the prosthesis which decreased significantly over the period of 3 days. Cr and Mn were the metal ions mainly found in saliva of cast partial denture wearer. No concentration of cobalt, molybdenum (Mo) and iron (Fe) was found in saliva of metal base denture wearer. There was a significant change in concentration of elutes in saliva in first 72 h/3 days making time an effective variable was observed.

Key Words: Dental casting alloys, leaching, saliva

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INTRODUCTION

The issue of biomaterial-derived ionic release in various sites of the human body has attracted the interest of many investigators because of the possibility that debris or degradation products elicit a foreign body reaction or have a role in the induction of pathological processes. Thus, research in the broader biomedical literature has focused on the release of substances

from various devices including orthopedic and dental materials.^[1] Faber (1957) suggested that a cast metal base is suitable particularly for lower denture-based.^[2] Taylor (1962) claimed that metal denture bases accurately reproduce the details that have been recorded in the impression without distortion or warpage.^[3]

Traditionally gold alloys have been most popular for the fabrication of metal denture bases. They are well tolerated by the patient and do not corrode in the oral environment. However, search for the economic alternatives lead to the evolution of base metal alloys. The use of cobalt-chromium (Co-Cr) alloys is increasing rapidly especially in the field of partial dentures. The composition of the various alloys varied considerably, but the basic pattern is cobalt (Co) 63–68% and chromium (Cr) 25–30% with addition of minor elements such as molybdenum (Mo), manganese (Mn), carbon (C), iron (Fe), silicon (Si), etc.^[4]

The complexity and diversity of these alloys make understanding their biocompatibility difficult because any element in an alloy may be released and may influence the body. Release of elements from the alloy is nearly always necessary for adverse biologic effects such as toxicity, allergy, or mutagenicity. The biologic response to released elements depends on which element is released, the quantity released, and the duration of exposure to tissues.

Literature regarding the biocompatibility of denture-based materials mainly consists of *in vitro* studies. Although, intra-orally different factors such as saliva characteristics, chewing or thermal and chemical dietary changes may influence the biological behavior of denture base materials. Therefore, this study was done to evaluate the saliva of cast partial denture wearers after insertion of the prosthesis for leaching of metals from Co-Cr base metal alloys. It was hypothesized that there should not be leaching of metallic ions from cast partial denture base in human saliva environment.

MATERIALS AND METHODS

This *in vivo* study was conducted in Department of Prosthodontics, faculty of dental sciences, King George Medical University Lucknow in collaboration with Indian Institute of Toxicology Research (IITR), Lucknow. Total 20 subjects of age group of 40–60 years including both males (10) and females (10) were selected for the study. Subjects had good oral hygiene (S-OHI < 1.3–3) and were free of any dental restoration. Subjects are having history of using any dental prosthesis in past or presently, working in industries related to any metal work, and/or taking any kind of medications affecting salivary flow rate were excluded from the study.

Total subjects were divided into two groups each containing 10 subjects:

- Group I (control group): Dentulous subjects without any dental restoration
- Group II (study group): Partially edentulous subjects with Kennedy's class II in the mandibular arch and rehabilitated with cast-metal removable partial denture (RPD) [Figure 1]. Ethical clearance and informed consent were taken from each subject participating in the study.

Cast partial RPD framework for each subject of Group II was fabricated using Co-Cr alloy (Wironit, BEGO Bremer Goldschlägerei Wilh. Herbst GmbH and Co., Germany) by the conventional technique.

After recovering the castings from the investment, they were blasted with 50 μ aluminum oxide particles at 80 psi and were ultrasonically cleaned. Polishing of cast partial RPD frameworks was first done electrolytically in an electrolytic polishing device. Oxides are removed evenly from all surfaces, but it did not result in high gloss. Finally, mechanical polishing was done using rubbers and brushes for polishing with paste to obtain a high gloss. For the purpose of standardization, it was confirmed that the weight of the cast partial RPD framework was kept same (20 g) for all the subjects. Cast Framework try-in was done in patient's mouth [Figure 1]. Jaw relations were recorded. Teeth setting and try-in were done. After flasking and curing with heat-cured resin, cast partial RPD was finished, polished and inserted in patient's mouth [Figure 2]. Postoperative instructions were given. All the subjects were instructed to wear the prosthesis 12 h/day.

Co-Cr alloy (trade name)	Composition*
Wironit	Co - 64.0%, Cr - 28.65%, Mo - 5.0%, Si - 1.0%, Mn - 1.0%, C - 0.35%

*As given in MSDS of alloy by manufacturer. MSDS: Material safety data sheet

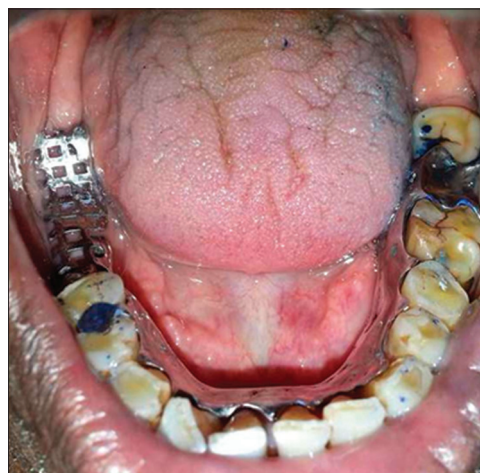


Figure 1: Cast Framework try-in done in patient's mouth

Patients were asked to spit every 30 s into preweighed screw-capped containers for 5-min period.^[5] The containers were re-weighed to estimate the salivary flow rate, assuming that 1 ml of saliva weighed 1 g. Samples were taken at three stages:

- Stage I: 1 h after the insertion - sample x
- Stage II: 1-day after the insertion - sample y
- Stage III: 3 days after the insertion - sample z

All the saliva samples were collected at the same time every day. Three samples were taken for each patient. Samples were sent immediately after collection to IITR Lucknow in an insulated box containing ice cubes to maintain the temperature below 0°C and were stored at -20°C in a refrigerator before starting the analysis.

Saliva sample was transferred to a conical flask and digested on a hot plate with 1% of HNO₃. After dry-up, 1% HNO₃ was added to make up 10 ml volume. After that, sample was transferred in a test tube to atomic absorption spectrophotometer (Awanta sigma; GBC, Australia), as shown in Figure 3, for estimation of metal ion concentration.^[6] Atomic absorption spectroscopy was used to estimate the concentration of elemental ions that is, chromium (Cr), manganese (Mn), cobalt (Co), molybdenum (Mo) and iron (Fe) leaching from Co-Cr alloy in saliva of cast partial denture wearer. With AAS, it is possible to determine about 70 elements (mainly metals) at very low concentrations. The sample is atomized at a very high temperature (2500°C–3000°C), and the free atoms have line spectrum. It means that they can only absorb the energy of light at discrete energy levels according to the excitations of electrons. Only a light with a concrete wavelength belongs to each of these excitation energies and when this light is absorbed it is missing from the continuous spectra of the electromagnetic radiation that is, a black line appears in the absorption spectrum of the atom. Only the examined atoms can absorb it. As a result of absorption, the intensity of light decreases, this is proportional

to the number of the examined atoms being present. That makes very sensitive quantitative measurements possible. Following the table shows the metal ions for which saliva sample was tested and their corresponding wavelength of detection:

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 15.0 statistical analysis software. The values were represented in a number (%) and mean \pm standard deviation.

OBSERVATION AND RESULT

Only Cr and Mn were isolated; none of the other trace elements could be isolated on any time interval in saliva of subjects rehabilitated with cast partial denture. Furthermore, none of these ions that is, Cr and Mn were detected in saliva of subjects acting as control. Before the assessment of data, the distribution was checked for normality using Kolmogorov–Smirnov test and was found to be asymmetric and not normal ($P > 0.05$). Hence, a nonparametric analytical plan was used.

Table 1 compares the pattern of change in Cr and Mn metal levels at different time intervals during the study. At 1 h, 24 h and 72 h after the denture insertion in study group, Chromium (Cr) had statistically significant higher mean concentration as compared to Manganese (Mn) ($P < 0.001$). A gradual decrease in mean Cr levels was observed from 1 h after denture insertion to day 3 intervals with a maximum value at 1 h and minimum value at day 3 intervals. However, for Mn, the mean value was maximum at day 1 and minimum at day 3 intervals. Table 2 shows a comparison of change in mean levels of Cr and Mn with time using Kruskal Wallis test (nonparametric ANOVA). It indicates a significant difference in metal ion concentrations at different time intervals for

Metal	Cr	Mn	Co	Mo	Fe
Wavelength (nm)	357.9	279.5	240.7	313.3	248.3



Figure 2: Cast partial RPD inserted in patient's mouth



Figure 3: Atomic absorption spectrophotometer

both Cr ($\chi^2 = 20.589$; $P < 0.001$) as well as Mn ($\chi^2 = 23.396$; $P < 0.001$).

Between time-interval comparison using Mann–Whitney U-test [Table 3] revealed that except for day 1 and day 3 comparisons, all the between time interval comparisons for Mn was found to be significant statistically ($P < 0.05$). The order of concentration for two metals was as follows: Cr: 1 h > day 1 \approx day 3 Mn: Day 1 > 1 h > day 3.

DISCUSSION

There are sizeable literatures on *in vitro* release studies of dental casting alloys, but experiments using human saliva are rare, and few *in vivo* studies have been reported.^[7] Obtained results from this *in vivo* study rejected the null hypothesis and showed that there is leaching of metal ions from cast partial denture frameworks in human saliva. Cr and Mn were the metal ions mainly found in saliva of the denture wearer.

Biting stresses on dentures can be extremely high, temperatures may fluctuate between 25°C and 45°C and pH may change instantaneously from acidic to alkaline. The warm and moist oral environment, which is also enzyme and bacteria rich, is conducive to further decay.^[8] Allergic and/or inflammatory

reactions from dental casting alloys have been ascribed mainly due to leaching of metal ions from denture-based material; for sensitization to occur, these metal ions must presumably be released from the prosthesis into saliva, in which it is conveyed to the oral mucosa or, after being swallowed, to the gastro-intestinal tract.^[9] Prosthesis was inserted on the same day of curing to minimize any changes (physical and chemical) occurring due to storage of prosthesis.^[10] Saliva samples were collected 3 times that is, 1 h, 1 day and 3 days after insertion of the denture as these timings coincide with postinsertion recall visits of the patients.

For the detection of release of elemental ions, atomic absorption spectrophotometry was employed. Atomic absorption spectrophotometer (AAS)^[7] was used as opposed to inductively coupled plasma mass spectroscopy because of simplicity, speed and economy of AAS technique. This technique has following advantage over other techniques: High sensitivity (10–10 g [flame], 10–14 g [nonflame]), good accuracy (relative error 0.1% ~ 0.5%), and high selectivity. A resonance line source is required for each element to be determined.^[11]

After running the saliva sample in AAS, release of Cr, Mn was found primarily over the period of 3 days while Co and Mo despite being one of the main components were not found in the samples [Table 1]. It has been seen that two factors affect the degree of release of one or more metal ions: Alloy type and saliva pH.^[12] When exposed to the oral environment for prolonged periods of time dental alloys are susceptible, in varying degrees, to tarnish. This tendency to tarnish is usually attributed to chemical or electrochemical corrosion and is responsible for the release of elemental ions.^[13] *In vivo*, numerous biological factors, including organic acids and enzymes which are produced by oral micro-organisms or are contained in food, may contribute to alloy corrosion. It must be emphasized that the *in vitro* data do not necessarily reflect the *in vivo* clinical conditions.^[14]

Release of Cr was maximum (0.1479 µg/ml) in sample taken 1 h after insertion and it decreased further over 1-day (0.0622 µg/ml) and was minimum (0.0586 pg/ml) on the 3rd day of prosthesis wearing. The difference in the metal ion concentration of Cr was significant ($P < 0.001$) over the period of 3 days [Table 1]. As most of the Cr ions have leached immediately after wearing of the prosthesis, this may be because surface layer of these alloy mainly have oxides of Cr as protective layer causing passivation and as time of prosthesis wearing increases this layer decreases.^[14] However, Cr is not mutagenic, it is carcinogenic.^[9] However, even the maximum value of Cr concentration, as found in study, does not exceed daily dietary intake of Cr (Wataha, 2000).^[15]

Table 1: Comparison of change in metal levels (microgram/milliliter) on different time intervals

Interval	Cr		Mn	
	Range	Mean±SD	Range	Mean±SD
1 h after insertion	0.14-0.155	0.1479±0.0052	0.004-0.006	0.0049±0.0010
Day 1	0.055-0.069	0.0622±0.0048	0.005-0.008	0.0065±0.0011
Day 3	0.052-0.064	0.0586±0.0041	0.00-0.002	0.0006±0.0007

SD: Standard deviation

Table 2: Analysis of variance (Kruskal–Wallis test) for concentration of metal ions at different time intervals

Time	Cr		Mn	
	n	Mean rank	n	Mean rank
1 h after	10	25.50	10	17.15
1-day after	10	12.60	10	23.85
3 days after	10	8.40	10	5.50
Total	30		30	
Significance of difference (Kruskal–Wallis test)				
χ^2		20.589		23.396
P		<0.001		<0.001

Table 3: Between time-interval comparison of metal concentrations (Mann–Whitney U-test)

Comparison	Cr		Mn	
	Z	P	Z	P
1 h versus day 1	3.791	<0.001	2.735	0.009
1 h versus day 3	3.800	<0.001	3.868	<0.001
Day 1 versus day 3	1.598	0.123	3.882	<0.001

It was noted that the release of Mn was maximum (0.0065pg/ml) after 1-day as seen and it decreased significantly ($P < 0.001$) after 3rd day [Table 1]. Despite being only 1% of total alloy composition, it was found in saliva. Although the literature shows that the relative amount of released metals does not reflect their relative weight or volume portion in the alloy.^[15]

It was found in the study that the overall concentration of both the metals that is, Cr and Mn decreased significantly after 3 days of prosthesis wearing. However, the concentration of chromium was significantly higher than Mn at all the time intervals, making Cr the main leaching metal ion. This may be due to much higher content of the Cr in alloy than Mn and Cr being more reactive than Mn.^[16]

Despite being main component, Co and Mo were not found in saliva over 3 days period. It has been shown that for Co-Cr- Mo dental alloys Cr forms the protective oxide layer that prevents the corrosion of alloy. This passivating effect is further enhanced by the Mo.^[14] As seen in study concentration of Co and Mo is not found initially it may be found as the usage period of partial denture increases.^[12,17]

Maximum release of elemental ions from cast partial denture was seen 1 h after insertion [Table 3]. Conditioning cast dental restorations before placing them intra-orally might reduce the release of elements inside the oral cavity.^[14] *In vitro* studies have shown some conditioning procedures to be useful because they remove the initial (labile) elements.^[13] Previous *in vitro* studies have used various conditioning times and types of conditioning media.^[17] The release of elements into conditioning media varies among alloys and elements. Previous work showed that in alloys conditioned for longer periods, the degree of element release becomes lower over time.^[14,18]

It was found in the study that significant leaching was found in saliva from denture base materials, but as for every study, this study also has certain limitations. The time period over which leaching was studied that is, 72 h was less. As because of biodegradation of metal denture-based over the time, certain elutes which were not found in saliva initially may become detectable with further use of the prosthesis. A further cytotoxic evaluation of elutes leaching in the concentration found in the study is required to complete the study.

CONCLUSION

Metal-based dentures show maximum leaching immediately after wearing of the prosthesis which decreased significantly over the period of 3 days. Cr and Mn were the metal ions mainly found in saliva of cast partial denture wearer. No concentration of cobalt (Co), molybdenum (Mo) and iron (Fe) was found in saliva of metal base denture wearer. There was a significant change in concentration of elutes in saliva in first 72 h/3 days making time an effective variable was observed.

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