

Effect of Mouthwash on Release of Elements from Dental Casting Alloys: An In-vitro Study

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ABSTRACT

Introduction: Mouthwashes are regularly prescribed as a maintenance phase of treatment by the dentist. The pH of the mouthwash can enhance the elemental release from the cemented prosthesis.

Aim: To evaluate the effect of two types (Oral-B and Listerine) of mouthwashes on the release of elements from Nickel-Chromium (Ni-Cr) dental alloys.

Materials and Methods: An in-vitro comparative study was conducted in the Department of Prosthodontics, School of Dental Sciences, Karad, Maharashtra, India, from April 2018 to April 2022. A total of 90 crowns were fabricated using three different brands of dental casting alloys (DCA) using lost wax technique. The crowns were immersed into two different mouthwashes (Oral-B and Listerine) in polypropylene test tubes, and then incubated at 37°C to simulate the oral temperature for 1, 4, and 7 days. The leaching of the elements from the

fabricated crowns into the mouthwashes was analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Data analysis was performed using the t-test and repeated measure Analysis of Variance (ANOVA) followed by post hoc Dunnett Multiple Comparisons Test.

Results: The Ni and Cr levels in the mouthwashes were significantly elevated in brand I (Ruby, Japan Dentistry Products Pvt. Ltd.), brand II (Dentmark, R&D Impex International), and brand III (BEGO, GmbH & Co KG). Presence of Ni released from all the three brands of DCA in mouthwash over a period of 1, 4, and 7 days was detected to be in the range of 16 to 39.9 µg/L. Cr released from all the three brands of DCA in mouthwash over a period of 1, 4, and 7 days was detected to be in the range of 10.3 to 42.8 µg/L.

Conclusion: The Ni and Cr were released in the mouthwash and the release increased with time. The acidic pH of Listerine adds to the increased release of Ni and Cr.

Keywords: Biocompatibility, Corrosion, Fabricated crowns, Leaching, Plasma mass spectrometry

INTRODUCTION

Nickel Chromium castings are regularly used for crowns, bridges, and partial denture frameworks due to its low cost and superior properties (higher hardness values and substantially higher modulus of elasticity) in porcelain-fused-to-metal (PFM) [1]. All metal crowns, metal ceramic crowns, post and core, stainless steel crowns for primary teeth, orthodontic appliance, and implants in oral environment are permanently exposed to conditions such as variable (acidic) pH, which can be related with dietary intake, temperature, mechanical fatigue, and susceptibility of alloy to corrosion. Leaching of heavy metals from dental restorations in saliva is well-known [2].

The leaching of metals present in dental casting alloys is well documented in several studies [3-6] and it depends upon the oral pH, duration of restoration, corrosion of dental alloys, and wear of dental alloys due to heavy mastication load. Acidic pH and long duration of restoration of dental alloys increase the leaching of metals from dental casting alloys, which may increase the level of Ni, Cr, and Co in soft tissues and blood [2].

The permanent restorative prosthesis fabricated is made of alloys containing Ni, Co, Cr, and molybdenum in different percentages. Thermal, microbiological, and enzymatic properties of oral environment make the mouth an ideal place for biodegradation of these alloys [7]. Ni, Co, and Cr have been identified as cytogenic, mutagenic, and allergenic [8]. Ni is the most common cause of metal induced allergic contact dermatitis followed by Cr [9].

Mouthwashes that are regularly prescribed by dentist to maintain the oral hygiene generally contain chlorides and fluorides. These mouthwashes may add to the corrosion of the elements from the dental casting alloys. Mouthwash is regularly prescribed as a maintenance phase of treatment. The effects of the contents in the mouthwash on the restored metal crown are less known [10]. Therefore, the crowns fabricated from different brands of Ni-Cr

alloys were placed in two different types of mouthwash for a period of 1, 4, and 7 days. The present study aimed to estimate Ni and Cr metals leached from the three different brands of fabricated crowns after immersion in two different mouthwashes for different time period using ICP-MS.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Prosthodontics, School of Dental Sciences, KIMSUDU, Karad, Maharashtra, India, as a part of the PhD thesis during the April 2018-April 2022, after obtaining ethical clearance (KIMSUDU/IEC01/2018) from the Institutional Ethical Committee.

Inclusion criteria: The completely casted crowns without any casting defects were included in the study.

Exclusion criteria: Any of the incompletely casted crowns or crowns with defects were excluded from the study.

Sample size calculation: The sample size of study groups for in-vitro studies were calculated, by using mean and SD of Ni, and Cr leaching level as per earlier study [11] and using the formula below.

$$n = \frac{4SD^2}{(M \times \epsilon)^2}$$

SD is Standard Deviation, M is mean, and ϵ is precision=3%

As per 95% confidence interval and 90% power of earlier studies [11] maximum sample size was 15 in each group.

A total of 90 crowns were fabricated from three different brands (30 crowns from each brand) of dental casting alloys and were immersed in two different mouthwashes (Oral-B and Listerine) in polypropylene test tubes, and then incubated at 37°C to simulate the oral temperature for 1, 4, and 7 days.

Three commercially available Ni-Cr alloys were selected with the following percentages. Brand I-Ruby, Japan Dentistry Products Pvt. Ltd.

(Ni 72.8%, Cr 4.9%, Cu 12.3%, others 10%) [12], Brand II-Dentmark, R and D Impex International (Ni 74.7%, Cr 4.8%, Cu 13%, others 7%) [13], and; Brand III-BEGO, GmbH and Co KG (Ni 65%, Cr 22.5%, Mo 9.5%, No 1%, Si 1%, Fe 0.5%, Ce 0.5%, C 0.02%) [14].

Study Procedure

Preparation of Standard Metal Model through CAD/CAM: An Ivorine mandibular first molar was prepared using Diamond points for receiving full veneer metal restorations. The prepared ivorine teeth was scanned using 3M Lava Optical Scanner and contra spray and chrome cobalt (SP2:BEGO) models of chamfer margins were milled such that distance from the base to the finish line for each of the model was 10 mm.

The model so prepared were welded to a metal base that had specific orientation grooves to fit into the corresponding projections of custom tray.

Preparation of custom tray: A custom tray was designed using Magics software, the data was exported to ProJet 7000 SLA 3D printer series, SLS/SLA light curable resin was the material of choice for fabrication of resin trays. The trays had projections corresponding to the grooves of the metal base. This ensures accurate and precise fit of the trays during impression making. The surface of the tray facing the standard metal model was perforated to provide an escape for impression material.

Impression making and fabrication of die in die material: A tray adhesive (Dentsply Caulk) was applied to custom trays, medium-bodied elastomeric impression material (Aquasil Monophase, Denstply™) was manipulated and loaded onto the custom tray to make an accurate fitting impressions of the standard models. The impressions were poured in Type IV gypsum (Kalrock-Kalabhai Karso, Mumbai Pvt. Ltd.), having a water powder ratio of 0.23 according to the manufacturer's instructions. A single coat of die spacer (Sigmadent) 25 µm in thickness was applied to all the samples, this relief was kept short of margins by 1 mm by brush [15].

Casting, finishing and polishing: The wax pattern were sprued to a crucible former, invested in Phosphate Bonded Investment (Wirovest) manipulated according to product catalogue. Castings were made using the centrifugal induction casting machine (BEGO Germany). The sprue attachment was cut from the metal copings and white aluminous oxide stones were used for trimming. Ni-Cr pellets from each of the 03 brands were used to fabricate 90 crowns as mentioned [Table/Fig-1]. Oral-B (Procter and Gamble, Weybridge, London, UK) and Listerine (Johnson and Johnson Healthcare Products, USA) mouthwashes were used in the present study as the immersion medium. Each Ni-Cr crown was immersed in the solutions in polypropylene test tubes and then incubated at 37°C to simulate the oral temperature. Five crowns were immersed for one day, another five crowns were immersed for four days, and

five crowns were immersed for seven days in each mouthwash. After the immersion period, the samples were tested for elemental release using inductively coupled plasma mass spectroscopy (Shimadzu ICPMS 2030). The incubated blank solution without the fabricated crown for 1, 4, and 7 days was considered as the control group. ICP-MS is a highly sensitive analytical technique for the determination of elements in the biological fluids. It also has advantages of wide linear dynamic range, wide elemental coverage, multi-element capability, and simple sample preparations. Plasma is essentially an ionised gas, consisting of positively-charged ions and free electrons that help in ionising the samples [16].

STATISTICAL ANALYSIS

Comparison between two groups was performed by using t-test. Comparison of the release over period of time was assessed using repeated measures of One-way Analysis of Variance (ANOVA), followed by post hoc Dunnett Multiple Comparisons Test for comparing duration with control. A p-value less than 0.05 were considered as significant. Data analysis was performed by using statistical software Statistical Package for the Social Sciences (SPSS), version 20.0. Percentage change of the release of Ni, and Cr in Oral-B and Listerine mouthwashes at different duration as compared to blank test was performed by mathematical calculations.

RESULTS

The release of Ni in Oral-B mouthwash from all 3 brands at 1st, 4th, and 7th day was significantly ($p < 0.001$) increased in the range (125.35% to 438.02%) as compared to control. Leaching of Ni in Listerine mouthwash at 1st, 4th, and 7th day was significantly ($p < 0.001$) increased in the range (123.37% to 418.18%) from all three brands as compared to control [Table/Fig-2]. Statistically significant ($p < 0.001$) difference was found on comparing different durations for all brands with control [Table/Fig-3]. There was statistical significant difference of release of Ni between Oral-B and Listerine mouthwashes at 4 days ($p < 0.001$), 7 days ($p < 0.001$) for brand I, II, and ($p = 0.08$) for brand III at 4 days, whereas there was no statistical significant difference of release of Ni between Oral-B and Listerine mouthwashes at 1 day for all three brands [Table/Fig-4].

The release of Cr in Oral-B mouthwash from all three brands at 1st, 4th, and 7th day was significantly ($p < 0.0001$) increased in the range (30.37% to 367.08%) as compared to control. The release of Cr in Oral-B mouthwash was found to be statistically significant ($p < 0.0001$) for brands I, II, and III. Leaching of Cr in Listerine mouthwash at 1st, 4th, and 7th day was significantly increased in the range (26% to 415.66%) from all three brands as compared to control. There was statistically significant ($p < 0.0001$) release of Cr in Listerine mouthwash for brands I, II, and III [Table/Fig-5]. On comparing duration with control, it was observed that there was statistical significant difference at different duration for all brands [Table/Fig-6]. There was statistical significant difference of release of Cr between Oral-B and Listerine mouthwashes at 1 day ($p < 0.001$), 4 day ($p < 0.001$), and 7 days ($p < 0.001$) for brands I, II, and III. For brand I, at 4 days p-value was 0.003 and for brand II, p-value was 0.02 [Table/Fig-7].

These observations confirmed that the release of Ni and Cr was evident in both the mouthwashes. The release of Ni was more in Oral-B mouthwashes. Release of Ni from brand II crowns was more in both the mouthwashes and release of Ni from brand III crowns was the

| Immersion media | Fabricated crowns (n=90) | | | | | | | | |
|-----------------|--------------------------|--------|--------|----------|--------|--------|-----------|--------|--------|
| | Brand I | | | Brand II | | | Brand III | | |
| | 1 day | 4 days | 7 days | 1 day | 4 days | 7 days | 1 day | 4 days | 7 days |
| Listerine | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Oral-B | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

[Table/Fig-1]: Number of fabricated crowns estimated of metal released after immersion in mouthwash.

| Brands | Oral-B mouthwash (µg/L) | | | | | Listerine mouthwash (µg/L) | | | | |
|--------|-------------------------|----------|----------|----------|---------|----------------------------|----------|----------|----------|---------|
| | Control | 1 day | 4 days | 7 days | p-value | Control | 1 day | 4 days | 7 days | p-value |
| I | 7.1±1.3 | 19±1.7 | 22.6±1.1 | 26.8±0.9 | <0.0001 | 7.7±1.3 | 20.4±1.5 | 25.9±1.1 | 33.8±1.0 | <0.0001 |
| II | 7.1±1.3 | 20.6±1.3 | 25.9±0.8 | 38.2±0.7 | <0.0001 | 7.7±1.3 | 20.6±1.2 | 34.8±0.9 | 39.9±1.1 | <0.0001 |
| III | 7.1±1.3 | 16±1.9 | 20.2±2.5 | 24.6±2.2 | <0.0001 | 7.7±1.3 | 17.2±2.2 | 22±1.7 | 28.6±1.9 | <0.0001 |

[Table/Fig-2]: Estimation of the release of Ni in different mouthwashes at different durations and compared to the blank test. Figure indicate mean±SD values and Significance level as compared to elemental release of the respective mouthwash recorded using unpaired t-test

| Mouthwash | Brands | (I) | (J) | Mean difference (I-J) (µg/L) | p-value |
|-----------|--------|---------|--------|------------------------------|---------|
| Oral-B | I | Control | 1 day | 11.90 | <0.001 |
| | | | 4 days | 15.50 | <0.001 |
| | | | 7 days | 19.70 | <0.001 |
| | II | Control | 1 day | 13.50 | <0.001 |
| | | | 4 days | 18.80 | <0.001 |
| | | | 7 days | 31.10 | <0.001 |
| | III | Control | 1 day | 8.90 | <0.001 |
| | | | 4 days | 13.10 | <0.001 |
| | | | 7 days | 17.50 | <0.001 |
| Listerine | I | Control | 1 day | 12.70 | <0.001 |
| | | | 4 days | 18.20 | <0.001 |
| | | | 7 days | 26.10 | <0.001 |
| | II | Control | 1 day | 12.90 | <0.001 |
| | | | 4 days | 27.10 | <0.001 |
| | | | 7 days | 32.20 | <0.001 |
| | III | Control | 1 day | 9.50 | <0.001 |
| | | | 4 days | 14.30 | <0.001 |
| | | | 7 days | 20.90 | <0.001 |

[Table/Fig-3]: Post hoc Dunnett Multiple Comparisons Test for comparing duration with control-Ni. Significance level as compared to elemental composition of the respective As- Received pellet recorded using Post hoc Dunnett Multiple Comparisons

| Timeline | Brand I | | | Brand II | | | Brand III | | |
|----------|------------------|---------------------|---------|------------------|---------------------|---------|------------------|---------------------|---------|
| | Oral-B mouthwash | Listerine mouthwash | p-value | Oral-B mouthwash | Listerine mouthwash | p-value | Oral-B mouthwash | Listerine mouthwash | p-value |
| 1 day | 19±1.7 | 20.4±1.5 | 0.07 | 20.6±1.3 | 20.6±1.2 | 0.99 | 16±1.9 | 17.2±2.2 | 0.2 |
| 4 days | 22.6±1.3 | 25.9±1.2 | <0.0001 | 25.9±0.8 | 34.8±0.9 | <0.0001 | 20.2±2.5 | 22±1.7 | 0.08 |
| 7 days | 26.8±0.9 | 33.8±1.0 | <0.0001 | 38.2±0.7 | 39.9±1.1 | 0.0009 | 24.6±2.2 | 28.6±1.9 | 0.0005 |

[Table/Fig-4]: Comparison of the release of Ni between Oral-B and Listerine mouthwashes at different duration. Figure indicate mean±SD values and significance level as compared to elemental release of the respective mouthwash recorded using unpaired t-test

| Brands | Oral-B mouthwash (µg/L) | | | | | Listerine mouthwash (µg/L) | | | | |
|--------|-------------------------|----------|----------|----------|---------|----------------------------|----------|----------|----------|---------|
| | Control | 1 day | 4 days | 7 days | p-value | Control | 1 day | 4 days | 7 days | p-value |
| I | 7.9±1.4 | 15.7±1.3 | 19.5±1.2 | 21.6±0.9 | <0.0001 | 8.3±1.3 | 18.5±1.5 | 22.4±2.3 | 30.4±1.8 | <0.0001 |
| II | 7.9±1.4 | 31.6±1.4 | 35.7±1.7 | 36.9±1.9 | <0.0001 | 8.3±1.3 | 21.8±1.6 | 37.3±0.9 | 41.7±1.1 | <0.0001 |
| III | 7.9±1.4 | 10.3±2.6 | 13.5±3.1 | 19.4±2.4 | <0.0001 | 8.3±1.3 | 17.2±2.2 | 27.7±0.9 | 42.8±1.6 | <0.0001 |

[Table/Fig-5]: Estimation of the release of Cr in different mouthwashes at different duration and compared to the blank test. Figure indicate mean±SD values and significance level as compared to elemental release of the respective mouthwash recorded using unpaired t-test

| Mouthwash | Brands | (I) | (J) | Mean difference (I-J) (µg/L) | p-value |
|-----------|--------|---------|--------|------------------------------|---------|
| Oral-B | I | Control | 1 day | 8.60 | <0.001 |
| | | | 4 days | 12.40 | <0.001 |
| | | | 7 days | 14.50 | <0.001 |
| | II | Control | 1 day | 24.50 | <0.001 |
| | | | 4 days | 28.60 | <0.001 |
| | | | 7 days | 29.80 | <0.001 |
| | III | Control | 1 day | 3.20 | <0.001 |
| | | | 4 days | 6.40 | <0.001 |
| | | | 7 days | 12.30 | <0.001 |
| Listerine | I | Control | 1 day | 10.80 | <0.001 |
| | | | 4 days | 14.70 | <0.001 |
| | | | 7 days | 22.70 | <0.001 |
| | II | Control | 1 day | 14.10 | <0.001 |
| | | | 4 days | 29.60 | <0.001 |
| | | | 7 days | 34.00 | <0.001 |
| | III | Control | 1 day | 9.50 | <0.001 |
| | | | 4 days | 20.00 | <0.001 |
| | | | 7 days | 35.10 | <0.001 |

[Table/Fig-6]: Post hoc Dunnett Multiple Comparisons Test for comparing duration with control-Cr. Significance level as compared to elemental composition of the respective As- Received pellet recorded using Post hoc Dunnett Multiple Comparisons

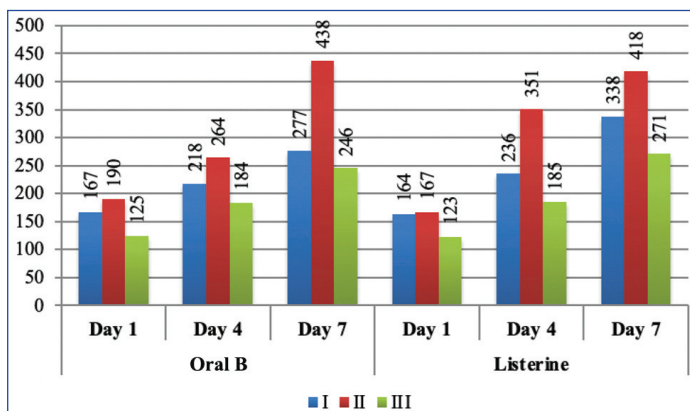
least among the compared samples [Table/Fig-8]. This may be due to the difference in composition of the alloy. The release of Cr was more in Listerine mouthwash. Release of Cr from brand II crowns was more

in both the mouthwashes and release of Cr from brand III crowns was the least among the compared samples in Oral-B mouthwash [Table/Fig-9]. Ni and Cr levels in the mouthwash were significantly elevated.

| | Brand I | | | Brand II | | | Brand II | | |
|--------|------------------|---------------------|---------|------------------|---------------------|---------|------------------|---------------------|---------|
| | Oral-B mouthwash | Listerine mouthwash | p-value | Oral-B mouthwash | Listerine mouthwash | p-value | Oral-B mouthwash | Listerine mouthwash | p-value |
| 1 day | 15.7±1.3 | 18.5±1.5 | <0.0001 | 31.6±1.4 | 21.8±1.6 | <0.0001 | 10.3±2.6 | 17.2±2.2 | <0.0001 |
| 4 days | 19.5±1.2 | 22.4±2.3 | 0.003 | 35.7±1.7 | 37.3±0.9 | 0.02 | 13.5±3.1 | 27.7±0.9 | <0.0001 |
| 7 days | 21.6±0.9 | 30.4±1.8 | <0.0001 | 36.9±1.9 | 41.7±1.1 | <0.0001 | 19.4±2.4 | 42.8±1.6 | <0.0001 |

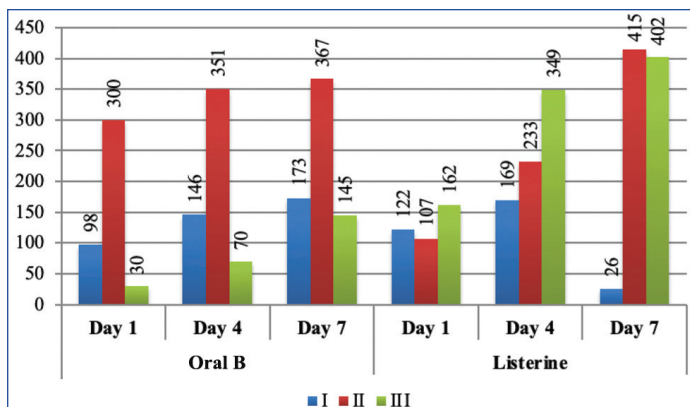
[Table/Fig-7]: Comparison of the release of Cr between Oral-B and Listerine mouthwashes at different durations.

Figure indicate mean±SD values and significance level as compared to elemental release of the respective mouthwash recorded using unpaired t-test



[Table/Fig-8]: Percentage change of the release of Ni in different mouthwashes at different durations as compared to the blank test.

X axis- duration of immersion; Y axis- percentage released



[Table/Fig-9]: Percentage change of the release of Cr in different mouthwashes at different durations as compared to the blank test.

X axis- duration of immersion; Y axis- percentage released

DISCUSSION

The mouthwashes with contents like fluoride, chloride, and essential oils are commonly prescribed by the dentist as an additional treatment to reduce the risk of caries and plaque formation and overcome periodontal conditions, such as gingivitis, maintenance of oral hygiene; however, the effects of these mouthwash on the regularly used prosthesis fabricated using casting alloys such as Ni-Cr, Co-Cr alloys is not well-known [10]. Information regarding the release of metal ion is needed to avoid adverse effects of mouthwashes on these fixed prosthesis fabricated using Ni-Cr alloys.

Corrosion leads to the release of metal ions, which can occur as a result of internal (metal composition and structure of the alloy) and external factors (biological environment, pH, and temperature) [17]. Therefore, in the present study effect of two types of mouthwashes on the release of elements from Ni-Cr dental alloys have been evaluated.

Mouthwashes are regularly recommended by dentist to be used twice a week for about 1 minute and the patients are asked not to eat, drink or rinse after using a mouthwash. This causes the components of mouthwash to be in contact with the Ni-Cr crowns for a long time. In the current study, we assumed that the mouthwash was present for 6 hours in a patient's mouth each time [18]. Hence, the metal crowns were immersed in mouthwashes and incubated for 1, 4, and 7 days.

Ni and Cr levels in the mouthwash were significantly elevated from brands I, II, and III dental casting alloys as compared to blank test. Ni was detected to be 7.1 and 7.7 µg/L in the blank Oral-B and Listerine mouthwash, respectively. Presence of Ni released from all the three brands of DCA in mouthwash over a period of 1, 4, and 7 days was detected to be in the range of 16 to 39.9 µg/L. Cr was detected to be 7.9 and 8.3 µg/L in the blank Oral-B and Listerine mouthwashes, respectively. Cr released from all the three brands of DCA in mouthwash over a period of 1, 4, and 7 days was detected to be in the range of 10.3 to 42.8 µg/L. This may be due to the difference in composition of the alloy.

In conjunction to the present study, Jafari K et al., (2019) reported similar results that Ni ion release from the fabricated Ni-Cr alloy disks was higher with the immersion in Listerine mouthwash than that with the Oral-B mouthwash [18]. In the present study, crowns were fabricated instead of disc to standardisation of the area and weight of the alloy to be tested. Another study by Erdogana reported that the Listerine that is an alcohol-based mouthwash exhibited the highest amount of metal ion release from silver soldering and laser [19]. Mihardjanti M et al., (2017) [20] and Mandaurwala M et al., (2015), also reported that Listerine mouthwash caused the release of the highest amounts of Ni and Cr ions from stainless steel brackets [21], which is in agreement with the present study conducted for the Ni-Cr alloy crowns.

Many mouthwashes are alcohol-based and may have a low pH, which affects the ion release from the alloys into the oral cavity where saliva acts as the medium. The release of metals could be influenced by high chloride content in the saliva or the intake of various foods and drinks with low pH levels [22]. The pH of Oral-B is 5.1 and Listerine 4.33 as mentioned by the manufacturer and is the reason for increased release in Listerine mouthwash [18].

Keosuo H et al., (1995) reported that the release of the elements from mouthwashes after using an oral simulator apparatus [23]. However, in present study, the authors have not used any oral simulator apparatus, still we had observed the release of elements from mouthwashes in a static condition. This observation indicates that the component present in mouthwash may be stimulating leaching of heavy metals from DCA. Mouthwash generally consist of alcohol, fluoride, chloride, and essential oils, which are useful for oral hygiene maintenance and plaque removal. Mouthwash content may react with high chloride of saliva or different foods with different pH may stimulate the release of heavy metals from DCA [17]. In the present study, mainly Ni and Cr release at different periods were significantly elevated in both Oral-B and Listerine mouthwashes; hence, it is difficult to suggest the better mouthwash. However, it is good to avoid both the mouthwashes in case of Ni and Cr allergies.

Limitation(s)

The limitation of the study was that, it reported only the static release.

CONCLUSION(S)

Within the limitations of the study, it was evident that Ni and Cr were released in the mouthwash and the release increased with time. The acidic pH (4.33) of Listerine adds to the increased release of Ni and Cr; hence, the authors recommend to prescribe mouthwash having pH >5 as evident in the present study, especially in patients allergic

to Ni or Cr. Further studies using simulations and clinical trials would be helpful.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Apr 01, 2022
- Manual Googling: May 31, 2022
- iThenticate Software: Aug 15, 2022 (18%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 26, 2022**

Date of Peer Review: **Apr 18, 2022**

Date of Acceptance: **Jun 01, 2022**

Date of Publishing: **Sep 01, 2022**