

Original Article

CORROSION INHIBITORY ABILITY OF OCIMUM SANCTUM LINN (TULSI) RINSE ON ION RELEASE FROM ORTHODONTIC BRACKETS IN SOME MOUTHWASHES: AN INVITRO STUDY

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ABSTRACT

Introduction: In the oral environment, orthodontic appliances are exposed to potentially damaging physical and chemical agents which may cause metallic corrosion. Corrosion will occur continuously in mouth, due to release of ions with abrasion by food, liquids etc. The purpose of this study was to measure the amount of metal ion release from orthodontic brackets when kept in different mouthwashes and effect of tulsi rinse on ion release when combined with different mouthwashes.

Methods: Three hundred and twenty stainless steel brackets (0.022") were divided randomly into 8 equal groups and immersed in Mouthwash-A and Mouthwash-B and Mouthwash-C and distilled deionized water. These mouthwashes and distilled deionized water were all separately mixed with tulsi rinse and incubated at 37°C for 45 days. Nickel, Chromium, iron, copper and manganese released from the orthodontic brackets were measured with an inductively coupled plasma spectrometer.

Results: Ion release in deionized water was higher ($p < 0.05$) than in the 3 mouthwashes. Higher ion release was also found with Mouthwash-B compared with other 2 mouthwashes. There is no difference ($p > 0.05$) in nickel, chromium, iron and copper ion release in the Mouthwash-A and Mouthwash-C. Tulsi rinse showed significant reduction ($p < 0.05$) in all ion release in 3 mouthwashes used along with distilled deionized water group.

Conclusion: If ion release is concerned, Mouthwash-C mixed with tulsi rinse might be better option than Mouthwash-A as well as Mouthwash-B for orthodontic patients with stainless steel brackets.

Keywords: orthodontic appliances, tulsi, Mouthwash, Ocimum Sanctum Linn

INTRODUCTION

During the last decade, there has been increased interest among dental and biomedical professionals in the side effects associated with the use of biomedical, especially the metallic materials. Fixed appliances in orthodontics involve brackets and arch wires that are metallic. These brackets are exposed to the oral cavity, which is a potentially hostile environment where electrochemical corrosion phenomena can occur. Thus, orthodontic brackets and other auxiliary components should be made of highly corrosion-resistant metals and metal alloys.¹ In recent years, it has been reported that bracket corrosion can occur in the oral environment. In an acidic environment and in the presence of fluoride ions, the corrosion resistance of certain materials, particularly titanium and titanium alloys, can

deteriorate.²⁻⁸ Practitioners can choose from a wide range of wires and brackets made from various alloys such as stainless steel (iron, chromium and nickel), titanium and cobalt-chromium alloy. Generally, stainless steel alloys that contain 8% to 12% nickel and 17% to 22% chromium are used for the metallic parts of orthodontic appliances.⁹

The harmful effects of nickel –its carcinogenicity, allergenicity and mutagenic substances have been systematically investigated at the cell, tissue, organ and organism level.¹⁰⁻¹² Approximately 10% of the general population has a hypertensive reaction to nickel. **Peltonen**⁵ reported that women are 10 times more sensitive to nickel than men. Nickel can cause hypersensitivity, contact dermatitis, asthma and cytotoxicity. In a

study in which cultured human cells were used, nickel was recently reported to be moderately cytotoxic, whereas chromium was considered to have little cytotoxicity. **Park and Shearer**¹⁴ measured the invitro average amounts of nickel and chromium released per day, and **Barrett et al**¹⁵ studied the corrosion rate of simulated orthodontic appliances. However, the ion release in only a short time was not enough to evaluate the biocompatibility of orthodontic appliances that are in the mouth for 2 to 3 years.¹³

During orthodontic treatment, practitioners recommend that their patients use mouthwashes, especially since most are adolescents who do not always follow a satisfactory oral-hygiene regimen and have a high risk of dental caries.⁷ Nowadays, the regular use of fluoride-containing products such as toothpastes and mouthwashes during orthodontic treatment is recommended to reduce the risk of the development of white spots around orthodontic brackets. Although fluoride ions in the prophylactic agents have been reported to cause corrosion and discoloration, little information is available regarding the effect of different mouthwashes in ion release of orthodontic brackets.^{2,4} The purpose of this study was to measure the levels of metal ions released from orthodontic brackets after immersion in several mouthwashes and corrosion inhibition ability of tulsi rinse on ion release from orthodontic brackets if immerse together with different mouthwashes. These results should help practitioners to decide which mouthwash to prescribe for their patients.

MATERIAL AND METHODS

Three hundred twenty premolar stainless steel orthodontic brackets (0.022") were used for this study. All brackets were used in as-received condition. The brackets were divided randomly into 8 equal groups and immersed in Mouthwash-A, Mouthwash-B and Mouthwash-C and distilled deionized water. These mouthwashes were chosen because of their commercial availability and identical methods of application.

- Group 1 used mouthwash-A containing water, glycerin, alcohol, aroma, methyl paraben, poloxamer 407, cetyl pyridinium chloride, sodium fluoride, sodium saccharin and propylparaben.
- Group 2 used Tulsi (*Ocimum sanctum* Linn.) rinse containing aqueous extract of Tulsi leaves, in addition to Group 1.
- Group 3 containing mouthwash-B containing Mouthwash-B gluconate 0.2% W/V.
- Group 4 used Tulsi rinse+ Mouthwash-B,
- group 5 containing Mouthwash-C [Bibhitaka (*Terminalia hellica*) 10 mg, Nagavalli (*Piper betle*) 10 mg, Pilu (*Salvadora persica*) 5 mg, oils of Gandhapura Taila (*Gaultheria fragrantissima*) 1.2mg, Ela (*Elettaria cardamomum*) 0.2 mg, powders of Peppermint satva (*Mentha spp.*) 1.6 mg and Yavani satva (*Trachyspermum ammi*) 0.4mg]
- Group 6 containing Tulsi rinse+Mouthwash-C,

- Group 7 containing Deionized water,
- Group 8 containing Tulsi rinse+ Deionized water.

Each bracket was incubated in an oven set at constant temperature of 37°C in individual 20-mL plastic capped vials containing 15mL of 1 mouthwash solution or distilled deionized water for 45 days.

After incubation for 45 days, the immersion solution was tested with an inductively coupled plasma (ICP) spectrometer (ICP-OES, Varian, Vista-Pro model, Mulgrave, Victoria, Australia; 1400W applied power). Unlike other methods such as atomic emission spectrometry, ICP has the advantage of extracting each ion simultaneously and detecting the metals without the interference of other ions.^{3,8}

Standard stock solutions (100mg mL⁻¹) of chromium, copper, iron, manganese and nickel were prepared by dissolving their appropriate salts in distilled deionized water. More dilute solution (0.1-10 mg mL⁻¹) of each ion was freshly prepared daily by appropriate dilutions of their stock solutions. To minimize the matrix effect in ICP measurements, the stock solutions of each ion was diluted with the appropriate mouthwash. Each solution was analyzed for chromium, copper, iron, manganese and nickel ions. Measurements of pH for each mouthwash and the distilled deionized water were made with pH meter (model 781, Metrohm AG, Hensau, Switzerland) by using a combined glass electrode.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to analyze the difference among mean ion concentration in the 4 groups. The Duncan multiple range test was applied to show the differences between groups.

RESULTS

Mean levels of the ions released in the groups are showed in the table. The results of the kolmogorov-Smirnov test showed that, except for copper and iron ions, all other ions had normal distributions. Therefore, a nonparametric test (Kruskal-Wallis) showed that the release of copper and iron in the 4 mouthwashes was significantly different. (p=0.00) Also, the Mann-Whitney test with a significance level of less than 0.008 showed no significant difference in iron release between Mouthwash-A and Mouthwash-C (p=0.032) and was significantly lower than what was observed for water. Copper release between Mouthwash-B and Mouthwash-C and also between Mouthwash-C and distilled water was not significantly different. (p=0.383 and p=0.008 respectively).

The test of homogeneity (Levene statistics) showed that chromium, manganese and nickel releases were not homogenous; a post-hoc test (Tamhane) was used to compare the groups. Only nickel release between Mouthwash-A and Mouthwash-C was not significantly different (p=0.238). Nickel release was similar in Mouthwash-A and Mouthwash-C (p>0.05) (Table) and

this was significantly lower than in Mouthwash-B and water.

Nickel release in other mouthwashes (Mouthwash-B and distilled deionized water) and chromium and manganese release in all the mouthwashes were significantly different ($p=0.00$).

Also, the level of chromium in Mouthwash-B was significantly lower than water. The level of manganese release was in the following order: Mouthwash-B < Mouthwash-C < Mouthwash-A < Water. Except for copper release, which was similar in Mouthwash-B and distilled water, the amounts of all ions released in deionized water were significantly higher than the amounts released in the 3 mouthwashes. Compared

with the other ions, the level of copper release was least among all the studied. For further elucidation of the reasons for ion release in the different solutions, the pH values of the 3 mouthwashes and distilled deionized water were measured. The values were 7.5 for deionized water and 5.4, 5.2 and 5.9 for Mouthwash-A, Mouthwash-B and Mouthwash-respectively.

When combined with Tulsi rinse, different mouthwashes (Table:1) comprised of Group 2, Group 4, Group 6 and Group 8, the release of metal ion concentrations ($\mu\text{g/L}$) is significantly reduced ($p>0.05$) (Table), and pH value of these mouthwashes reached to the controlled level according to specifications laid down by Federal USA, when combined with the Tulsi rinse.

Table: 1. Metal ion concentrations ($\mu\text{g/L}$) in the solutions at 37°C after 45 days: mean concentration levels, standard deviations, ranges and pH

Solutions with pH (n=40)	Chromium	Copper	Iron	Manganese	Nickel
Mouthwash-A (group1) (pH-5.4)	90.7 \pm 6.8 (0.0-295.0)	0.0 \pm 0.0 (0.0-0.0)	53.2 \pm 6.1 (0.0-218.0)	1064.6 \pm 16.9 (710.0-1800)	170.5 \pm 17.0 (0.0-535)
Tulsi rinse+ Mouthwash-A (group 2) (pH- 6.6)	40.0 \pm 5.0 (0.0-801.0)	0.0 \pm 0.0 (0.0-0.0)	25.0 \pm 4.0 (0.0-100.0)	800.0 \pm 15.0 (600.0-1500)	90.0 \pm 10.0 (0.0-200.0)
Mouthwash-B (group 3) (pH-5.2)	483.9 \pm 38.5 (0.0-1350.5)	19.0 \pm 5.7 (0.0-308.0)	60.0 \pm 27.0 (0.0-170.0)	205.0 \pm 8.1 (0.0 \pm 308.0)	1196.0 \pm 35.0 (540.0 \pm 1950.0)
Tulsi rinse+ Mouthwash-B (group 4) (pH-6.2)	300.0 \pm 30.0 (0.0-700.0)	10.0 \pm 3.0 (0.0-100.0)	40.0 \pm 20.0 (0.0-110.0)	120.0 \pm 5.0 (0.0-150.0)	606.0 \pm 10.0 (200.0-700.0)
Mouthwash-C (group 5) (pH-5.9)	23.1 \pm 2.2 (0.0-70.0)	0.0 \pm 0.0 (0.0-0.0)	70.0 \pm 4.3 (0.0-150.0)	740.0 \pm 30.0 (0.0-830.0)	100.0 \pm 4.0 (4.0-150.0)
Tulsi rinse+ Mouthwash-C (group 6) (pH-6.9)	15.0 \pm 2.0 (0.0-30.0)	0.0 \pm 0.0 (0.0-0.0)	20.0 \pm 2.3 (0.0-70.0)	500.0 \pm 10.0 (0.0-602.0)	50.0 \pm 2.8 (4.0-110.0)
Deionized water (group 7) (pH-7.5)	837.9 \pm 31.8 (0.0-1618)	80.2 \pm 11.7 (0.0-370.0)	882.0 \pm 112 (4.0-228.0)	1860.0 \pm 60.0 (550.0-4150)	2625.0 \pm 150.0 (0.0-7010.0)
Tulsi rinse+ Deionized water (group 8) (pH-7.1)	500.0 \pm 20.0 (0.0-980.0)	50.0 \pm 7.2 (0.0-110.0)	500.0 \pm 60.0 (4.0-230.0)	600.0 \pm 21.8 (300.0-1000)	1000.0 \pm 90.0 (0.0-1100.0)

DISCUSSION

Mouthwashes or rinses are aimed to reduce oral microbes, remove food particles, temporarily reduce bad breath and provide a pleasant taste. Usually, mouthwash must be used twice a week for about 1 minute. But it is recommended that after mouthwash the patient must not eat, drink and rinse so the components of mouthwash are present for a longtime, and it is difficult to determine the extract duration of contact between brackets and mouthwashes. We assumed that each time the mouthwash was present for 6 hours in a patient's mouth (24 months, twice a week=about 69,000 minutes), so for this study the brackets were immersed in mouthwashes and incubated at 37°C for 45 days (45 days=about 64,000 minutes). Also, several studies have release from fixed orthodontic appliances peak at day 7, and that all release is completed within 4 weeks.

In our study, the distinct increase in the level of the release of all ions in deionized water might be attri-

buted to its corrosive nature. The corrosion of different metals and alloys as a result of immersion in deionized water has been studied.²⁶ Many parameters affect the corrosion of metals in a water environment, including pH level, oxygen content, water temperature and duration of immersion. It has also been reported that the corrosion rates of steel increase with aeration of pure water, and dissolved oxygen in pure water is 5 to 10 times more aggressive than carbonic acid. The deionized distilled water used in this study had a pH of 7.5; therefore the deionized water was not acidic and was not responsible for its corrosiveness. The reason might be because deionized water has an extremely low concentration of ions, and the lack of ions makes this solvent one of the most aggressive solvents known.

A comparison of nickel release from the brackets in the various solutions showed that the maximum release was in deionized water and the next highest was in Mouthwash-B mouthwash. Mouthwash-B mouthwash released greater amounts of metal ions (except

manganese) than did the Mouthwash-A and Mouthwash-C mouthwashes. Mouthwash-B not only caused the release of significantly higher amounts of nickel and chromium ions among the 3 mouthwashes studied, but it also caused not significantly higher release of copper than did Mouthwash-C (Table). Since the pH values for mouthwashes had no significant difference in the acidity of the 3 mouthwashes, this could be attributed to the corrosiveness of Mouthwash-B compared with the other 2 mouthwashes; this agrees with previous reports about the irrigating effects of Mouthwash-B.⁹ But the corrosiveness of Mouthwash-B is not the sole parameter in the release of all metallic ions from the brackets, because high releases of manganese in Mouthwash-A and Mouthwash-C solutions were observed. The level of manganese release was significantly different in all 4 groups and interestingly, was lowest in Mouthwash-B mouthwash. This might be the result of the fluoride anion in the Mouthwash-A mouthwash; under acidic conditions, the fluoride anion increases the dissolution of manganese. In an acidic environment, corrosion could easily occur even with low fluoride concentrations.⁵ There was no significant difference between the nickel and iron ion release abilities of Mouthwash-A and Mouthwash-C.

From a clinical viewpoint, the corrosion of brackets might affect how they slide on the archwire,^{29,30} and the final result of orthodontic treatment could be compromised.⁸

Certain ions such as nickel and chromium can result in symptoms of toxicity and allergic reactions.³¹ These symptoms can be short-lived and intense or longer lasting and moderate, and some might be resolved, whereas others can become a chronic problem. Since the toxicity of nickel is a concern and the natural capacity to eliminate nickel exceeds the accumulation capacity, the risks are minimal.³² However, clinicians should be aware that the release of metal ions might cause a local hypersensitivity reaction at oral soft-tissue sites, such as mild erythematous or redness with or without edema.¹⁶ Also, severe gingivitis can be related not only to poor oral hygiene but also to a hypersensitivity reaction to nickel or chromium ions released from stainless steel.⁹ We also need to determine whether these ion releases have clinical significance in sensitized patients with a history of hypersensitivity.

Metal is released into the oral cavity with saliva as the medium, and this could be influenced by a high chloride mixture, and this could be influenced by a high chloride mixture in the saliva or the intake of various foods and drinks with a low pH. Also, the characteristics of saliva change according to the patients' health and the time of day.^{2, 7} We used mouthwashes in a static condition, but more metal release could occur in life because oxide layers are removed by tooth brushing.¹³ Kerosuo et al.¹⁰ found a great amount of release after using an oral functioning simulator apparatus to stimulate the dynamic conditions of the mouth.

Adhesive resins were not used on the base of brackets in this study to prevent other sources of ion re-

lease.^{11,12} Therefore the exposed surface for ion releases was approximately twice that of clinical conditions because the brackets bases would be covered with a bonding material in clinical use.

Daily amounts of chromium and nickel intake from foods are 5 to 100 micrograms and 300 to 500 micrograms, respectively.¹³ Nickel concentrations in drinking water generally measure below 20 micrograms per litre, a average chromium levels in drinking water are 0.43 microorganisms per liter. The ions released from orthodontic appliances in this study were insignificant when compared with the amount from daily food and water intake. Although there are different study designs, this result was also reported in other studies.¹⁴

However, even such a small amount of release might produce sensitivity when the orthodontic appliances are in place for 2 to 3 years. But, for an allergic reaction in the oral mucosa, an antigen must be 5 to 12 times greater than that needed for a skin allergy.

The general mechanism for the corrosion and subsequent release of metal ions from stainless steel involves the loss of the passive layer consisting of chromium oxide and chromium hydroxide that forms on contact with oxygen on the surface of stainless steel. Crevice corrosion, which is an intense local attack in shielded areas on a metallic surface, is the mechanism involved in the corrosion of orthodontic brackets.⁷

In our study the total amount of nickel and chromium released during 45 days in deionized water were greater than the results of Barrett et al.¹⁵ Also the differences have been found in the metal release between corresponding products of different manufacturers.⁴⁰ Surface area is a relevant factor in the corrosion of metals, but determine the surface areas of orthodontic bands and brackets with their complex geometry was beyond the scope of this study.

The amount of chromium and nickel released in Mouthwash-B and deionized water during 45 days were more than those found by Kerosuo et al.,¹⁰ although they studied ion releases of different appliances in NaCl under the dynamic conditions of an oral simulator. Since they used different appliances comparisons between studies must be done with due consideration of the problem in measuring surface areas with complex geometry.

Tulsi (*Ocimum sanctum*) rinse is known for its differential medicinal uses such as cardiopathy, homeopathy, leucoderma, asthma, bronchitis, vomiting, hiccups, gastropathy, genitourinary disorders, skin diseases etc.¹² Our study revealed that the pH values of various dentifrices reached to the control level (according to federal USA specifications) when combined with *Ocimum sanctum* extract.

However, from our results it can be concluded that the corrosiveness of the mouthwashes which in turn depends on their chemical structure, pH and their combination with Tulsi rinse having adaptogenic property

are the main factors responsible for the release of metal ions from dental brackets.

CONCLUSIONS

The orthodontic brackets released the most ions in the presence of Mouthwash-B mouthwashes. It might be recommended to avoid prolonged application of Mouthwash-B in patients who have allergies. If at all it is used it should be combined with Tulsi rinse to protect from allergy. In the presence of Mouthwash-A and Mouthwash-C, ions are released from orthodontic brackets. Mouthwash is less as compared to ion release in Mouthwash-B. Mouthwash-C seems to be safe mouthwash in this respect. Even Mouthwash-C also showed better response if compared with Tulsi rinse owing to its corrosion inhibitory ability, adaptogenic properties, anti allergic and anti plaque properties and its eco-friendly nature.

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