ISSN: 03875547
Volume 45, Issue 03, May, 2022

The Effect of Saliva pH and Protective Silver Coatings on Denture Base Materials (Cobalt Chromium Alloys) Ion Release

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Keywords:
Denture Base Cobalt Chromium Alloys, Saliva pH, Silver Coating, Cobalt ion, Chromium ion

ABSTRACT

Cobalt chromium alloys is an essential alloys in dentistry and mostly used to fabricate rigid and strong denture base. Yet, dynamic oral saliva pH may cause the alloys to corrode and release toxic cobalt and chromium ions in the oral cavity. Surface modifications like silver coating is known to reduce the ion release in physiological environment. But its effect on different saliva pH is still yet unknown. This research examine the effect of protective silver coating and saliva pH towards the toxic cobalt and chromium ion release from cobalt chromium alloys. Total of 24 pieces of cobalt cromium alloys samples were casted. Half were coated with 5 µm silver, and the remaining were not coated. Samples were grouped and immersed in artificial saliva (pH Value: 5, 7 and 9), incubated in 37^0C for 14 days. Data were analyzed using two ways ANOVA and LSD post hoc test ($p<0.05$). The highest amount of cobalt (0.480 ppm) and chromium (49.109 ppb) ion were released from uncoated alloys in pH 5. Yet, comparatively, the lowest amount of cobalt (0.240 ppm) and chromium (17.865 ppb) ion were released from silver coated alloys in pH 7. Statistics analysis showed a significant results ($p<0.05$) of silver coating and saliva pH effects on the release of cobalt and chromium ion of cobalt chromium alloys.

1. INTRODUCTION

Edentulous is an irreversible condition that may disturb oral and systemic health [1- 3]. Prosthodontics treatment such as removable partial dentures (RPD) may prevent any side effect of edentulous. The denture base of RPD is mostly casted from cobalt chromium alloys (CoCr) which has superior physical properties [4]. Yet, CoCr alloy is known to corrode and release cobalt (Co) and chromium (Cr) ion in the oral cavity, especially in acidic pH [5- 8]. Saliva pH is known to be unstable in several conditions. It tends to be acidic in *gastropharyngeal reflux disease* (GERD) patient or those who consume acidic food or drinks. In the other hands, local inflammations like chronic general periodontitis may turn saliva to be more alkalic. It is known that either acidic or alkalic environments may cause alloys to corrode and release metal ions, which may

affect its biocompatibility, cytotoxicity, mutagenicity and even cause allergic [9- 12]. Released metal ion will be swallowed and circulate into the body. An evidence of increasing blood's nickel (Ni) and chromium (Cr) ion are founded in patients with nickel chromium dental restorations [13], [14].

Released Co and Cr ions may have side effect on oral and systemic health such as allergy, oral lesions, metallic taste and burning sensations in the oral cavity [15], [16]. Research on animal shows that CoCr alloys causes oral tissues inflammations and toxicity, which getting worse over time [17]. Similar reactions also occured in human beings, which happen under the supporting mucosa of cast denture framework mayor connetor that is fabricated from CoCr alloys [18]. Allergic patch test also revealed that Co and Cr ion may cause 15,9 – 16,7 % allergic events. In vitro test shows that it poses cytotoxicity on fibroblast and osteoblast. It also harms DNA and cause genotoxicity. As a mostly used dental alloys, CoCr alloys seems to poses some biocompatibility issues [5], [17- 20].

It seems like CoCr alloys of denture frameworks may cause further effect on the body through the release of Co and Cr ions. Some attempts such as surface modifications of CoCr alloys have to be carried out to reduce ion release [10]. Silver may be used as a coating material since its antibacterials property and biocompatibility towards fibroblast and osteoblast. It has also been used to modify the surface of dental implants [21- 23].

Silver coatings on orthodontics implants (Remanium GM®800 CoCr alloys) has shown to reduce Ni and Cr ion release in neutral pH saliva solutions. The thickness of 5 µm dan 10 µm silver coating were shown to be effective on reducing Ni and Cr release [24]. According to the author knowledge so far, there is still no futher studies conducting the effect of silver coatings on cast denture framework materials and various saliva pH on Co and Cr ion release. Thus, this research aims to evaluate the 5 µm thickness of silver coating on cast denture framework CoCr alloys surface and its effect on Co and Cr ion release when immersed in various artificial saliva pH.

2. Materials and methods

This is a laboratory experimental research on prosthodontics denture base materials. The samples used in this research are cast denture framework materials (Remanium® GM380+) with the independent variables are 5 µm silver coated CoCr alloys, uncoated CoCr alloys and saliva pH variations (pH 5, 7 and 9). The dependent variables are amount of Co and Cr ion released. Sample size were determined with the fomula of (n-1) (t-1) ≥ 15. The t value is 6, considering there will be six groups of treatment. Hence total of the samples were 4 each group. This research has been approved by ethical commision of Gadjah Mada University Faculty of Dentistry No. 00460/KKEP/FKG-UGM/EC/2020.

a. CoCr alloys sample preparations

Samples were casted from CoCr alloys (Remanium® GM380+) (Fig.1) to form a 10 mm x 10 mm x 2 mm rectangular shape. The components of this alloys were 64,6% Co, 29% Cr and 4,5% Molybdenum (Mo). Total of casted alloys were 24 pieces. Casted samples were polished and cleaned with ultrasonic cleaner and alcohol (Fig. 2).

ISSN: 03875547
Volume 45, Issue 03, May, 2022

Figure 1. CoCr Alloys (Remanium ® GM380+)

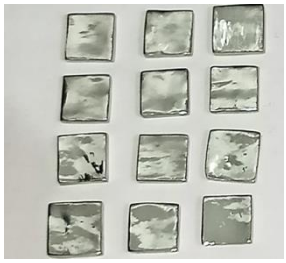

Figure 2. Casted CoCr Alloys

b. Silver coating of alloys with 5 µm thickness

Twelve casted CoCr alloys were randomly chosen and then silver coated with electroplating for 5 minutes to produce 5 µm of thickness (Fig. 3). Electrolyte that was used were s*ilver* nitrat, natrium fosfat dan amonium fosfat. Silver was connected with positif pole and act as anode, meanwhile CoCr alloys was connected wuth negative pole and act as cathode. Electrons from silver will move from anode to cathode which causes the silver ion released from the silver. The silver ion will travel through the solutions and adhere to the surface of CoCr alloys. During the process of electroplating 0,25 Amperes of currents was used [25].

Figure 3. Coated CoCr Alloys

c. Artificial saliva preparations

In this research saliva was prepared with pH 5, 7 and 9. The composition of pH 7 artificial saliva was K2HPO4 0,2 g/L, Ca3PO4 0,3 g/L, KSCN 0,33 g/L, NaCl 0,7 g/L, KCl 1,2 g/L, urea 0,13 g/L, NaHCO3 1,5 g/L. To obtain pH 5 saliva, 0,1 M HCl was added. Meanwhile to pH 9 artificial saliva 0,1 M NaOH was added.

d. Samples immersion

Samples were immersed with artificial saliva in polyethylene bottles for 14 days (Fig. 4) and kept in 370C incubators. All the bottles were labeled with spesific code.

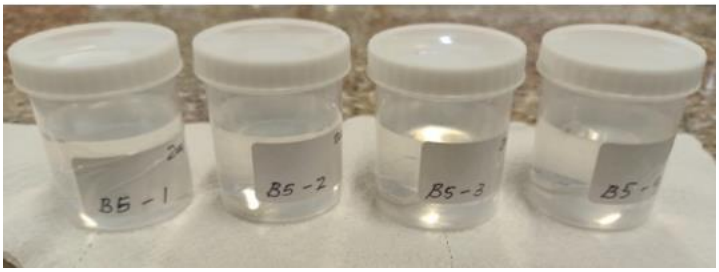

Figure 4. Immersing samples

e. Analysing amount of released Co and Cr ion

After 14 days of immersion, all CoCr alloys were took out from the artificial saliva solutions. The solutions were analysed with AAS (PinAAcle 900T Perkin Elmer) in UII Intergrated Laboratory to detect the amount of Co and Cr ion released for the alloys. Atomisation of Co ion using flame AAS, meanwhile Cr ion using graphite furnace AAS (GFAAS). Before analysing, Co and Cr ion detection limit was set using standard Co and Cr solutions. The detection limit of Co ion was 0,05 mg/L (ppm) dan Cr ion were 0,034 □g/L (ppb). During analysis, argon flow was set to be 200 mL/minute. Since different atomization is used, the final results of Co will be read in ppm, meanwhile Cr ion in ppb.

2.1 Statistical Analysis

Data were analyzed using SPSS version 23 (Chicago, IL, USA). All data normality and homogenity were tested with Shapiro Wilk and Levene test. Then, tests were proceeded to two ways ANOVA and post hoc LSD test, with significane level of 95% ($p<0,05$), to analyze the amount of cobalt and chromium ion release.

3. Results and Statistical Analysis

As shown in table 1 and 2, the highest Co (0.480 ± 0.086 ppm) and Cr (49.109 ± 2.405 ppb) ion were released from uncoated CoCr alloys immeresed in pH 5 artificial saliva. Meanwhile the lowest Co (0.240 ± 0.017 ppm) and Cr (17.865 ± 1.508 ppb) ion amount was released from 5 µm silver coated CoCr alloys immersed in pH 7 arificial saliva. Slightly increased amount of Co and Cr ion were released in pH 9 saliva than that of pH 7. Comparatively, the amount of ion released seems to be lower at silver coated CoCr alloys than from the uncoated one. Figure 5 and figure 6 shows that the amount of Cr ion released is lower than amount of Co ion released both from silver coated and uncoated CoCr alloys.

Table 1. Mean and standard deviations of released Co ion from 5 µm thicknes silver coated and uncoated CoCr alloys in ppm

Group	n	pH		
		5	7	9
CoCr alloys coated with 5 µm silver	4	0.352±0.066	0.240±0.017	0.263±0.031
Uncoated CoCr alloys	4	0.480±0.086	0.322±0.013	0.337±0.021

Table 2. Mean and standard deviations of released Cr ion from 5 µm thicknes silver coated and uncoated CoCr alloys in ppb

Group	n	pH		
		5	7	9
CoCr alloys coated with 5 µm silver	4	31.397 ± 1.703	17.865 ± 1.508	18.604 ± 1.283

Uncoated CoCr alloys	4	49.109 ± 2.405	20.766 ± 1.321	21.175 ± 1.688

Table 3. Two Ways ANOVA Test of Co Ion

Variable	F	p
Silver coating	23.840	0,000*
Saliva pH	18.908	0,000*
Silver* Saliva pH	0.737	0,492

Table 4. Two Ways ANOVA Test of Cr Ion

Variable	F	p
Silver coating	125.036	0.000*
Saliva pH	397.062	0.000*
Silver * Saliva pH	52.196	0.000*

Table 5. Post hoc LSD test of Co ion

	A1	A2	A3	B1	B2	B3
A1	-	0.157*	0.142*	0.127*	0.240*	0.217*
A2		-	-0.015	-0.030	0.082*	0.059
A3			-	-0.015	0.097*	0.074*
B1				-	0.112*	0.089*
B2					-	-0.023
B3						-

Notes to table 5:

1. A= Uncoated CoCr alloys immersed in artificial saliva with pH 5 (A1); pH 7 (A2) dan pH 9 (A3).
2. B = 5 µm silver coated CoCr alloys immersed in artificial saliva with pH 5 (B1), pH 7 (B2) dan pH 9 (B3).
3. *) shows significant difference ($p<0.05$).

Table 6. Post hoc LSD test of Cr ion

	A1	A2	A3	B1	B2	B3
A1	-	28.343*	27.934*	17.712*	31.244*	30.505*
A2		-	-0.408	-10.631*	2.901*	2.161
A3			-	-10.222*	3.309*	2.570*
B1				-	13.532*	12.793*
B2					-	-0.739
B3						-

Notes to table 6:

1. A= Uncoated CoCr alloys immersed in artificial saliva with pH 5 (A1); pH 7 (A2) dan pH 9 (A3).
2. B = 5 µm silver coated CoCr alloys immersed in artificial saliva with pH 5 (B1), pH 7 (B2) dan pH 9 (B3).
3. *) shows significant difference ($p<0.05$).

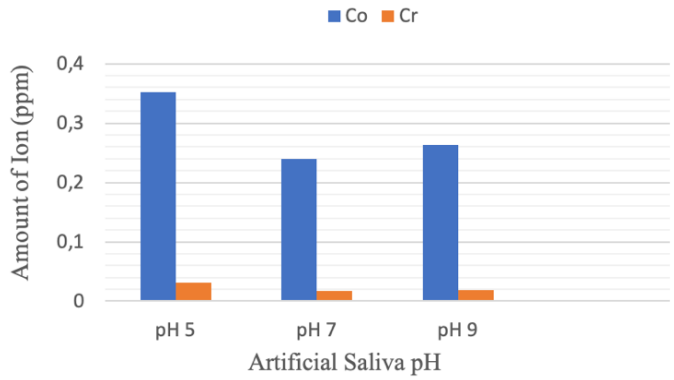

Figure 5. Comparison of released Co and Cr ion from 5 □m silver coated CoCr alloys immersed in artificial saliva with various pH

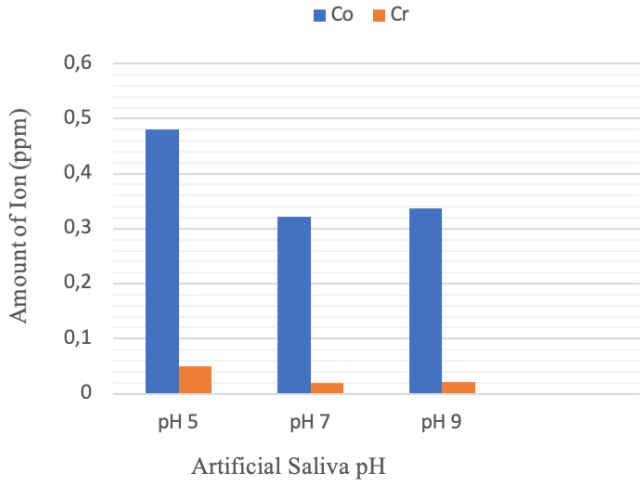

Figure 6. Comparison of released Co and Cr ion from uncoated CoCr alloys immersed in artificial saliva with various pH

Two ways ANOVA results (table 3 and 4) showed significant results of silver coating and saliva pH to Co and Cr ion release ($p<0.05$). Post hoc LSD test (table 5 and 6) showed siginificant results of Co and Cr ion released between silver coated and uncoated CoCr alloys immersed in saliva of pH 5, 7 and 9. It also showed significant results of Co and Cr ion released between pH 5 and 7, pH 5 and 9 ($p<0.05$) both in silver coated and uncoated CoCr alloys. Yet, no significant difference between pH 7 and pH 9 ($p>0.05$).

4. Discussion

This study shows that there are significant differences ($p<0.05$) of Co and Cr ion being released in artificial saliva between pH 5 and 7, pH 5 and 9 both from uncoated and silver coated dental CoCr alloys. The highest amount of Co (0.480 ± 0.086 ppm) dan Cr ion (49.109 ± 2.405 ppb) were detected in pH 5 artificial saliva from the uncoated CoCr alloys. Meanwhile the lowest amount of Co (0.240 ± 0.017 ppm) and Cr ion (17.865 ± 1.508 ppb) is released from silver coated CoCr alloys in pH 7 artificial saliva. Those results show that variations of saliva pH affect the amount of Co and Cr ion release from CoCr alloys. The more acidic the saliva, the more Co and Cr ion released.

It was in agreement with the previous research which stated that dental CoCr alloys released significant amount of the Co and Cr ion in acidic saliva pH [6], [26]. The corrosiveness of saliva depends on pH and

chloride ions concentrations. The lower the pH and the higher the chloride ion, then the more corrosive it is to disrupt the protective oxide layers and dissolve alloys surface / mechanical disintegrations. It causes cathode reactions and increase the corrosion rate of the alloys. Hence, it releases more metal ion [12], [27-31]. In this study, the released ion both in coated and uncoated CoCr alloys, seems to be higher in pH 9 saliva compared to that of pH 7. Yet it did not show significant differences ($p>0.05$). It shows that dental CoCr alloy corrode more in alkalic than neutral pH artificial saliva. It might be caused by the concentrations of NaOH in pH 9 artificial saliva. The NaOH may dissolve the protective oxide layers and cause corrosion, which in turn produce porousity / pitting of alloys surface. But, corrosion of alloys in alkalic solutions seems to be lower because of the more stable protective oxide layers [12], [32].

It also shows that in each artifisial saliva pH, there were significant difference ($p<0.05$) of Co and Cr ion released between uncoated and silver coated CoCr alloys. Comparatively, there was lower amount of Co and Cr ion released in each saliva pH from silver coated dental CoCr alloys. It implied that silver coating reduced CoCr alloys ion release in various saliva pH conditions. It may be due to the protective layers of silver which hinder the direct contact of saliva to the CoCr alloys. Surface coating of CoCr alloys is one of the ways to reduce the corrosion process [5], [27], [33]. In agreement with the previous findings, the CoCr alloys ion releasing in neutral saliva pH may be reduced by coating it with silver with 5µm thickness [24]. Silver poses good corrosion resistance properties. The presence of silver on CoCr alloy surface may act as a barrier to the direct exposure of saliva. Silver coating with electroplating technique may results with even and solid silver layers on CoCr alloys and may be effective to protect alloys from corrosive media [34-36]. Silver prevents corrosion of the coated alloys by oxidation of silver itself. It gives passive effect to prevent cathode reaction of the underlying alloys [37].

In this study, the amount of released Cr ion is still within the safety range. The safety dose of Cr ion allowed in water is 100 ppb, and several studies shows that the ingestions 20mg/L (which is equivalent to 20×10^3 ppb) will start shows disturbance in body organs. The symptoms range from dermatological and gastrointestinal disturbances [38].

Meanwhile the highest amount of Co ion released in this study seems to exceed the safety dose of allowed Co concentration in the human blood. Yet the lowest amount released is still in the range of safety Co dose. The safety Co ion dose in the blood is 0,3 ppm [39]. But, not all Co ion was absorbed into blood, most of them is absorbed in another body organs and excreted through feces and urine. About 0% - 70% of it will be excreted between 24 hours to one week. Only 10% of it will remain after one years [40]. To the limitation of this study, it was total amount of Co ions released for 14 days, so there is a possibility that the number of ions released per day is lower.

The results from this research showed that in denture base CoCr alloy is prone to toxic ion release in variations of saliva pH conditions, especially in acidic saliva. But, the number of released Co and Cr ions were significantly reduced and affected with the modifications of its surface with 5 µm silver coatings, which is known to be biocampatible and is widely used to coat medical devices. However, this study is limited to the total ion obsevations after 14 days immersions. Dental CoCr alloys may release the amount of Co and Cr ion differently with the variations of time [7]. Hence, futher research should be conducted to evaluate the ion released in the variations of time.

5. Conclusions

Acidic saliva pH caused denture base CoCr Alloys, either silver coated or not coated, to release significant amount of cobalt and chromium ion than neutral or alkalic saliva. Yet, amount of ion released by Silver

Coated CoCr Alloys is significantly reduced in each saliva pH than that of uncoated alloys.

List of Abbreviations :
CoCr : Cobalt Chromium
Co : Cobalt
Cr : Chromium
Ppm : Parts Per Million
Ppb: Parts Per Billion
GERD : Gastroesophageal reflux disease
RPD : Removable partial denture

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