

RESEARCH ARTICLE

Bacterial adhesion of *Streptococcus mutans* to cobalt chromium recast alloys

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ABSTRACT

Cobalt chromium (CoCr) alloys are utilized to make dental prostheses. Casting CoCr alloys is a common operation in dentistry laboratories due to its low cost. Casting surplus (metal remaining in the sprue and crucible former) is frequently reused by dental laboratories to reduce and recycle metal waste. However, the quality and safety of these recast alloys require further information. Microbial attachment to the surface of metal prostheses may affect its quality and safety. Biofilm formation on metal surface can cause biocorrosion and secondary infection. The effects of different proportions of recast Cobalt Chromium alloy on the bacterial adhesion are not clear. The purpose of this study was to investigate how recasting affects the *Streptococcus mutans* (*S. mutans*) adhesion. Twenty disk-shaped specimens (n= 20, allocated for 5 groups) were prepared from CoCr alloys (Remanium GM; Dentaurum) with different proportions (100% new alloy, 25% recast alloy, 50% recast alloy, 75% recast alloy, and 100% recast alloy). After the immersion of the specimens in bacterial suspension for 24 hours, the number of bacteria that adhere to the specimen's surface was counted using Colony Forming Units. Data were analyzed with one-way ANOVA ($\alpha = 0.05$). The bacterial adhesion was significantly affected by the recast alloys ($p < 0.05$). An increased proportion of CoCr recast alloys showed an increase in *S. mutans* adhesion to the specimen surface.

Keywords: bacterial adhesion; *Streptococcus mutans*; recasting; cobalt chromium alloys

INTRODUCTION

Cobalt chromium (CoCr) alloys are widely used in the fabrication of metal prostheses in dentistry. The casting of CoCr alloys has become a routine procedure in dental laboratories because it is relatively inexpensive compared with other fabrication methods.¹ Dental laboratories often reuse the casting surplus (metal remaining in the sprue and crucible former) to reduce cost and recycle metal waste. Adding a certain amount of previously cast alloy to new alloy is a common dental practice in many dental laboratories. However, the quality and safety of these recast alloys require further information. These recasting procedures change the chemical composition, grain dimension, and microstructure of alloys.² Some studies emphasized that repeated casting could interfere with the composition of alloys by reducing trace elements such as molybdenum, silicon, carbon, manganese, and beryllium, which could, in turn, affect the microstructure and surface roughness.³ This study tried to investigate whether

the adhesion of *Streptococcus mutans* (*S. mutans*) correlates with the change in surface roughness.

The oral cavity is a complex environment in which several substances gather, ranging from food, saliva, oral biofilms, and their metabolites. Oral biofilms are well-organized communities of microorganisms surrounded by a polysaccharide-based matrix containing nucleic acids, proteins, and H₂O, that adhere to tooth, dental prostheses structures, or oral soft tissues. As a result, the pH in the oral cavity frequently changes, reaching low values after the intake of acidic substances or acid release from oral microbial metabolism. Because of the acid condition, microbial attachment to the surface of metal prostheses may affect its quality and safety. Biofilm formation on metal surface can cause biocorrosion and secondary infection.⁴ The most common infection that occurs in prosthetic users is secondary carries that is caused by *S. mutans*.⁵ The growth of biofilms on dental materials varies, depending on the surface roughness which determines the early biofilm interlocking that helps

to the maturation process.⁶ The surface roughness, surface free energy, wettability, and chemical composition of metallic materials are dominant factors that influence bacterial adhesion.⁷

The effects of different proportions of recast Cobalt Chromium alloy on bacterial adhesion are not clear. Researchers have continued to examine the ratios of new to recast alloy needed to obtain an alloy with acceptable clinical properties. The purpose of this study was to investigate how recasting affects the *S. mutans* adhesion. The null hypothesis for this study was recasting of CoCr alloys would affect the microbial adhesion.

MATERIALS AND METHODS

Disk-shaped specimens (10x1 mm) were prepared from CoCr alloys (Remanium GM; Dentaurum) with different proportions of new alloys and recast alloys (%weight). The proportion of new alloys and recast alloys is shown in Table 1. The specimens were made from CoCr alloys with 100% new alloys and 0% recast alloys (Group 1, n=4), CoCr alloys with 75% new alloys and 25% recast alloys (Group 2, n=4), CoCr alloys with 50% new alloys and 50% recast alloys (Group 3, n=4), CoCr alloys with 25% new alloys and 75% recast alloys (Group 4, n=4), and CoCr alloys with 0% new alloys and 100% recast alloys (Group 5, n=4). All the specimens were fabricated using the lost wax casting technique and the casting procedures were performed according to the manufacturer's instructions. Phosphate-bonded investment material (Bellasun; Bego Germany) was used. The specimens were finished and polished with an abrasive rubber wheel using hand motor instrument rotating at 3500 rpm. After finished, the specimens were cleaned, rinsed, dried, and autoclaved at 121 °C for 15 minutes.

For bacterial adhesion, *S. mutans* ATCC 25175 were used. The strains were streaked onto brain heart infusion (BHI) agar plates and incubated for 48 h at 37 °C. Then a fresh colony was inoculated in appropriate growth medium, namely BHI broth, for making bacteria suspension using McFarland 0.5 Standard. Human saliva was collected and sterilized. The specimens were immersed in sterile

Table 1. Proportion of new alloys and recast alloys in the specimens

	new alloys		recast alloys	
	weight (grams)	% wt	weight (grams)	% wt
Group 1	48	100	0	0
Group 2	36	75	12	25
Group 3	24	50	24	50
Group 4	12	25	36	75
Group 5	0	0	48	100

saliva for 60 minutes at 37 °C, thus the specimen was covered by the salivary pellicle. Thereafter, the specimens were immersed individually in the bacteria suspension and incubated for 24 h at 37 °C. After the immersion of the specimens in the bacteria suspension, the specimens were moved into sodium chloride solution and vortex to detach the bacterial adhesion to the specimen surface. A total of 0.1 ml from the detached bacteria was collected and diluted in 0.9 ml of the sodium chloride solution until dilutions at 10⁻⁴. Then 0.1 ml of the diluted solution was inoculated on BHI agar plates and incubated for 48 h at 37 °C. Thereafter, the number of bacteria that adhered to the specimen's surface was counted using Colony Forming Units. The data were analyzed with one-way ANOVA ($\alpha = 0.05$).

RESULTS

This study showed that the recast alloy influenced the roughness property and the changes in the surface roughness could affect the *S. mutans* adhesion. The surface roughness of Cobalt Chromium alloys is shown in Table 2.

The *S. mutans* adhesion to the surface of Cobalt Chromium alloys was counted using colony forming units and all the data were collected and analyzed. The mean number of bacteria and the standard deviation of the measurements in each groups are shown in Table 3.

The bacterial adhesion was significantly affected by the recast alloys as shown by the P value of one-way ANOVA ($p < 0.05$). Post Hoc LSD

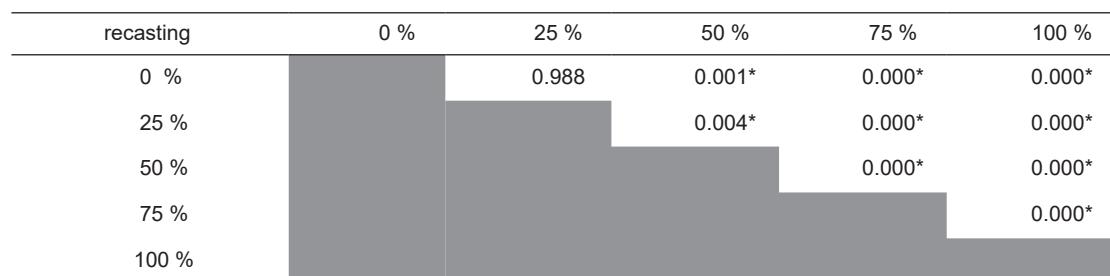
Table 2. Mean surface roughness and standard deviation of measurements

	surface roughness (μm)	
	mean	standard deviation
Group 1	1.3650	0.137
Group 2	1.7350	0.107
Group 3	1.8325	0.097
Group 4	2.1025	0.084
Group 5	2.2075	0.083

Table 3. Mean number of bacteria and standard deviation of measurements

	number of bacteria (10^4 CFU/ml)	
	mean	standard deviation
Group 1	23.50	2.646
Group 2	25.75	3.403
Group 3	46.75	7.365
Group 4	75.75	6.652
Group 5	108.50	10.247

Table 4. Post Hoc LSD result



showed the differences among the groups.⁸ The results of the Post Hoc LSD in this research are shown in Table 4.

There were significant differences among the groups ($p<0.05$). An increased proportion of CoCr recast alloys showed an increase in *S. mutans* adhesion to the specimen surface.

DISCUSSION

The hypothesis was accepted because the recasting of CoCr alloys affected the *S. mutans* adhesion. In the present study, the amount of microbial adhesion increased significantly with the addition of recast alloys. This study verified previous reports indicating that surface roughness was affected by addition of recast alloys. Recast alloys had statistically significant differences in the surface roughness (maximum and mean surface roughness) as compared to new alloys. Repeated casting procedures increase element loss including trace elements, compositional change, and microporosity, yet decrease the oxide layer formation.⁹ Some studies explained that loss of elements can cause the hard-agglomerate

particles with high porosity to be formed in the microstructure. Thus, the roughness can be seen all over the surface of alloys. This is because two end members (Co and Cr) require different melting temperatures and the trace elements used as the temperature gradient controller may diffuse into the alloy surface during firing. The reduction in the grain size is also caused by inhibition of grain growth by chromium particle.¹⁰

This study showed that the changes in the surface roughness could affect the *S. mutans* adhesion. Group 1, with 100% new alloys and 0% recast alloys, showed low surface roughness of Cobalt Chromium and low *S. mutans* adhesion. On the other hand, Group 5 with 100% recast alloys showed high surface roughness of Cobalt Chromium and high *S. mutans* adhesion. Recast alloy could affect the surface roughness of the alloy which then increased the contact areas, thus promoting *S. mutans* adhesion. Among the surface properties, surface roughness and topography have been the primary topics in dental biofilm research. An increase in surface roughness promotes bacterial attachment due to

the increase in contact area between the material surface and bacterial cells.¹¹ The factors of material properties such as surface charge, hydrophobicity, roughness, topography, stiffness, and chemistry can affect bacterial adhesion. The salivary pellicle has an important role among bacteria and material surface. Surface properties, saliva, and acquired pellicle affect the bacterial adhesion strength, the physicochemical and mechanical interactions between cell wall components (e.g. adhesins) and pellicle receptors. *Streptococcus mutans* are regarded as the main offending bacteria that have an important role in the first stage of caries development because they promote co-aggregation and colonization of cariogenic bacteria in biofilms. Two major *S. mutans* virulence factors are acidogenicity (ability to produce acid via glycolysis) and aciduricity (ability to survive in a low pH environment).¹² *Streptococcus mutans* adhesion to recast alloy may affect the alloy's properties. This study revealed that recasting procedure with an addition of recast alloy less than 50% is still considered to have a good quality as a prosthetic material but for the safety in patients, further studies are needed to investigate the virulence of *S. mutans*.

CONCLUSIONS

Cobalt chromium recast alloys have an effect on the bacterial adhesion of *S. mutans*. An increased proportion of CoCr recast alloys shows an increase in *S. mutans* adhesion.

REFERENCES

- Tuna SH, Pekmez NO, Kurkcuoglu I. Corrosion resistance assessment of Co-Cr alloy frameworks fabricated by CAD/CAM milling, laser sintering, and casting methods. *J Prosthet Dent.* 2015; 114(5): 725-734. doi: 10.1016/j.prosdent.2015.02.031
- Vaillant-Corroy A-S, Corne P, March PD, Fleutot S, Cleymand F. Influence of recasting on the quality of dental alloys: a systematic review. *J Prosthet Dent.* 2015; 114(2): 205-211. doi: 10.1016/j.prosdent.2015.02.004
- Oyar P, Can G, Atakol O. Effects of environment on the release of Ni, Cr, Fe, and Co from new and Recast Ni-Cr Alloy. *The Journal of Prosthetic Dentistry.* 2014; 112(1): 64-69. doi: 10.1016/j.jprosdent.2013.09.022
- Marsh PD, Martin MV. *Oral Microbiology* 4th ed. Oxford: Wright; 1999. 11-32.
- Featherstone JDB, Singh S, Curtis DA. Caries risk assessment and management for the prosthodontic patient. *J Prosthodont.* 2011; 20(1): 2-9. doi: 10.1111/j.1532-849X.2010.00596.x
- Gharechahi M, Moosavi H, Forghani M. Effect of surface roughness and materials composition on biofilm formation. *Journal of Biomaterials and Nanobiotechnology.* 2012; 3(4A): 541-546. doi: 10.4236/jbnb.2012.324056
- Teughels W, Van-Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Implants Res.* 2006; 17(2): 68-81. doi: 10.1111/j.1600-0501.2006.01353.x
- Daniel WW, Cross CL. *Biostatistic: A Foundation for Analysis in The Health Science.* 10th ed. John Wiley and Sons Inc New York; 2013. 207-210.
- Agrawal A, Hashmi SW, Rao Y, Garg A. Evaluation of surface roughness and tensile strength of base metal alloys used for crown and bridge on recasting (recycling). *J Clin Diagn Res.* 2015; 9(7): ZC01-ZC04. doi: 10.7860/JCDR/2015/11535.6141
- Poolphol N, Sakkaew T, Kachin K, Jantaratana P, Vittayakorn W. Physical, Mechanical, and Magnetic Properties of Cobalt Chromium Alloys Prepared by Conventional Processing. *Materials Today: Proceedings* 4; 2017. 6358-6364.
- Souza JCM, Mota RRC, Sordi MB, Passoni BB, Benfatti CAM, Magini RS. Biofilm formation on different materials used in oral rehabilitation. *Braz Dent J.* 2016; 27(2): 141-147. doi: 10.1590/0103-6440201600625
- Song F, Koo H, Ren D. Effects of material properties on bacterial adhesion and biofilm formation. *J Dent Res.* 2015; 94(8): 1027-1034. doi: 10.1177/0022034515587690