Effect of different finishing techniques on the surface roughness and bacterial adhesion of cast Nickel-chromium alloy

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Abstract: The formation of biofilm and bacterial accumulation on dental materials may lead to the development of gingival inflammation and secondary caries. Finishing and Polishing constitutes an essential requisite after the fabrication of fixed partial dentures. Improper finishing and polishing of cast restoration will adversely affect the quality of functional of it. The roughness of intra oral hard surfaces plays an important role in bacterial adhesion and colonization. The adhesion of this bacterium to fixed partial denture materials can be responsible for secondary caries and periodontal disease around the restoration. Modification of dental alloy surfaces with new techniques of finishing and polishing in order to obtain highly polished surface may be beneficial for preventing these problems. **Aim of the study**: To develop new strategies to prevent bacterial biofilm formation. This study was carried out to evaluate the effectiveness of two different methods of surface finishing techniques (conventional method and electrochemical finishing) on the surface roughness and adhesion and colonization of *Streptococcus mutans.* **Material and Methods** Twenty Specimens from Ni Cr alloy were made for this study by using lost wax technique. Specimens were divided into 2 groups: *group A* in which the specimens were finished and polished by conventional methods. While in *group B* the specimens were polished by using electrochemical procedures using special equipment. The surface roughness for all specimens was evaluated using Rugosimeter .After that specimens were cleaned and autoclaved. The tested organism (*Streptococus mutans*) was isolated and counted .Data obtained was tabulated and analyzed statistically using one-way ANOVA. **Results**: Group A with conventional methods of finishing showed high surface roughness with total count of bacteria (9 x 10^4CFU/ml) which was greater than that of group B of electrochemical finishing with lower surface roughness associated with the absence of bacteria count. **Conclusion**: A positive correlation was found between the surface roughness value of specimen and the amount of *S. mutans* adhesion to these surfaces

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Key words : Surface roughness, electro-chemical polishing, *Streptococus mutans*, adhesion and colonization

1. Introduction

The surface of the casting should be smooth and highly polished. Such surfaces limit the accumulation and retention of plaque and facilitate maintenance of health of the supporting periodontal tissues. Although conventional polishing kits reduced the surface roughness and S. mutans adhesion, Ni Cr alloy restorations are still showing a considerable amount of surface roughness with *S. mutans* adhesion, **Shafagh (1986).**

There are an increasing number of patients requiring prosthetic devices made of metal alloys, ceramics, polymers and polymer composites and, in many times, replacing their entire teeth arch. These materials are exposed to the oral environment and, depending on their chemical composition and surface roughness, they are colonized by oral bacteria. Unlike natural oral tissues, prosthetic devices are made of a wide variety of materials and are manufactured by dentists or dental technicians. Hence, the extent of adhesion of oral bacteria onto these devices varies greatly in respect to their chemical composition, placement in the arch and the actual manufacturing technique used as well on the dentist or technician skills, **Brno (2008).**

The presence of restorative materials on tooth surfaces is perceived to be a contributing factor to periodontal disease. This observation is a result of the increased accumulation of plaque on restorations adjacent to the gingiva, which may lead to gingivitis. Plaque is believed to adhere better to restorations than to enamel. This may be due to the surface characteristics of restorative materials such as surface roughness and surface-free energy inherent in the materials, **Luis et al.,(2011).**

It is expected that difficulties will occur during the finishing and polishing of base metal alloys because of their high hardness. High macro hardness of some alloys has been associated previously with greater abrasion resistance and time required to polish restorations. Such alloys would be difficult to finish, **Bezzon et al.,(2001)**

A Chinese study was carried out to investigate the correlation between surface roughness of chromium-cobalt and bacteria adhesion. A Six kinds of chromium-cobalt pieces with different surface were determined by Talysurf/S5-CD topographer. Surface roughness (Ra) value were gathered. *S. mutans* were used in adhesion experiment. The results showing that, The surface roughness value and the amount of plaque adhesion decreased with the increase in polishing level. Statistical difference was observed between groups of bacteria adhesion and the same in groups of surface roughness besides the rubber wheel and polishing group. The Conclusions were, To minimize the amount of plaque adhesion, the surface of Co-Cr crowns should be polished as smooth as possible, **Cui Dong-pei et al.,(2008)**

The roughness of intraoral hard surfaces plays an important role in bacterial adhesion and colonization. Earlier studies have shown that rough surfaces accumulate up to 25 times more subgingival plaque than do smooth sites, **Quirymen et al.,(1996).**

The oral cavity is inhabited by microflora composed of a very wide spectrum of organisms, bacteria comprising the dominant part (approx. 70% Streptococci), there are also smaller numbers of virae, mycoplasmates, yeasts and protozoa. Microorganisms present in the oral cavity can be both pathogenic as well as symbiotic, **Kagermeier-Callaway et al.,(2000).**.

Streptococcus mutans is the most cariogenic bacteria in the mouth and play a role as an early colonizer on formation of dental plaque. The adhesion of this bacterium to fixed partial denture materials can be responsible for secondary caries and periodontal disease around the restoration. Modification of dental alloy surfaces with surface coating materials may be beneficial for preventing these problems, **Kesim et al.,(2011).**

Electro-chemical polishing is a process for improving micro-smoothness, micro-topology, and material brightness by anodic dissolving of the substrate in an electrolyte with an external source of electricity. The resulting surface improvements depend on the uniformity of the material microstructure, the lack of surface inclusions, and the consistency of the surface finish all over the target area. **Dobrev et al.,(2007**).

Electro-chemical machining is a process for removing material by electrochemical means from metallic material without direct contact .It is basically a depleting processes in which the tool is a cathode and the work piece is the anode both must be electrically conductive, the electrolyte which can be pumped rapidly between the cathode and the anode sweeps away the waste product and captures it by setting in filter. The material to be treated is exposed to neither mechanical nor thermal stresses, consequently there is absolutely no change in the physical or chemical properties of the materials, **Fahmy(1999).**

Basically, the process mechanism involves immersing a metal target in a chemical solution, and making it the anode in a direct current circuit. Electrochemical Polishing (ECP) is highly dependent on the ability of the solution to polish uniformly the surface of the material without the occurrence of corrosion pits that penetrate the substrate as a result of the etching process **,Dobrev et al.,(2007).**

The equipment setup for ECP could be used for electroplating by reversing the polarity. In particular, the components are made the anode in the circuit in the case of electro-polishing, while in electroplating they are the cathode. The setup is relatively simple and requires a tank, a solution, and a low voltage direct current provided by a rectifier **Dobrev et al.,(2007).**

Several techniques can be used to clean and smoothen the titanium substrate surface, namely chemical, mechanical and thermal polishing. Chemical polishing requires HF-containing acid solutions and it does not provide as smooth a surface as the other methods; mechanical polishing can provide a very smooth and flat surface topography, but it is work-intensive and time consuming, furthermore, polishing media can become embedded in the surface. Thermal polishing requires heat-treatment in a high vacuum and specimens must be very clean to start with. Compared to the other polishing processes, electro polishing is an effective method to clean, smoothen and polish, the alloy surface. It removes impurities from the metal surface and gives the surface a high luster, **Chau et al.,(2011).**

The “as-cast” fit of metal-ceramic restorations has been reported to deteriorate during the high-temperature firing cycles used for the application of porcelain veneer. In this study, thermo cycling and surface finishing or cold working were examined for their effects on marginal adaptation of metal-ceramic castings. Methods for minimizing the loss of marginal adaptation were evaluated, and casting variables were eliminated by construction of acrylic resin measuring dies directly in the restorations. Thermo cycling of metal-ceramic restorations resulted in the increasing of marginal openings, and all of the loss of marginal fit occurred during the first thermo cycling of the alloy .The restorations that were cold worked and then oxidized by conventional manipulation had substantially more marginal opening than any other group. A fourfold, statistically significant improvement ($p<0.001$) in the marginal adaptation of a metal-ceramic restoration was observed when the initial thermal cycle was completed before the specimens were finished, **Campbell et al.,(1995).**

Techniques to minimize the thermal cycling distortion were also studied. It was found that all of the significant distortion occurred during the first thermal cycling of the alloy (oxidation) and that no distortion resulted from the application of body porcelain. The specimens that were cold worked and then oxidized had significantly more distortion than any other group. A significant reduction in distortion was observed when the initial thermal cycling was completed before the specimens were cold worked. It was determined that the release of casting- and cold working-induced stresses had a synergistic effect **,Capbell and Pelletier(1992)**.

From the previous review, it is apparent that, the conventional method of polishing (cold working) has certain disadvantages. So in this study, we applied the electrochemical methodology as a polishing technique, as well as, the conventional method for aiming of comparison.

2.Materials and Methods

2.1.Materials:

2.1.1. Alloy Specimens

This in vitro study was undertaken with Ni–Cr alloy, Wiron light (Bego-Germany).An aluminum metal die with a circular space of 5 mm radius and 3 mm depth was milled in the centre portion of 1 cm thick aluminum block using milling machine. Wax patterns 10 mm in diameter &3 mm thickness were prepared with green inlay casting wax (Bego-Germany) using the metal die. A total of 20 wax patterns were made .The wax patterns were sprued and invested in phosphate bonded investment (Bella vest SH, Bego, Germany) and casted by lost wax technique in an induction casting machine (Bego-Germany) following the manufacturer's instructions. After bench cooling the casting ring was divested and sandblasted using 250 lm Aluminum Oxide to remove the remnant investment material. Sprues were cut off and specimens were divided into 2 groups A & B.

Group A: 10 Specimens were finished and polished using carborundum discs, metal trimmers, rubber wheels, sandpapers and polishing cake using hand motor instruments.

Group B: 10 Specimens were finished and polished using electrochemical polishing, **El-Hakim and Abdel Mohsen(1989).**

2.1.2. Microorganism

The tested organisms was Gram-positive *Streptococcus mutans* .it was isolated from staff members of microbiology department, Faculty of science, Ain Shams University, Cairo, Egypt. The bacteria was maintained on nutrient agar (Oxoid) slants and stored at 4°C.The isolated strain was identified according to Bergy̓s manual of systematic bacteriology.

2.2.Methods:

2.2.1.The equipment used for electrochemical finishing: (Fig.1), is composed of:

1- Glass tank
2- Electrolyte solution (13% aqueous solution of Sodium Chloride).The function of the electrolyte is to complete the electrical circuit between the specimen (anode) and the cathode offering a medium for electrochemical reactions and carries away the heat generated and the products of the reactions.
3- Pan containing the work piece (specimen) and fixture.
4- Fixture to support the specimen and connected to the anode, the fixture composed of 2 acrylic plates, base & top. Base measuring 10cmx10cmx5cm with central groove 1cm x1cm x3mm to support the specimen .Top measuring 10cmx 10cm x2cm with central hole 1cm x 1cm to allow the cathode to pass through it. Base and top were connected together with acrylic plate 10cm x 10 cm x 2cm.
5- Cathode made from stainless steel rode measuring 10mm x 10mm and 10cm length.
6- Switch to conduct the electric power.
7- DC power supply composed of step-down power transformer 15, 18, 21, 24, 27 and 30 volt, together with a full bridge metal rectifier to convert the AC current to D C.
8- The specimen supported on the fixture and connected to the anode.
9- Electric Pump and tubes to pump and move the electrolyte from the glass tank to the specimen.
10- Filter, to capture the waste product from the circulating electrolyte.

Parameters of the experiment were obtained after many attempts of trial and error carried out on a disc specimens made from the same alloy that used in the experiment to obtain a suitable surface finish.

Each casting was supported by the fixture of the apparatus so that working gap between the casing and the cathode was 2 mm, the electric pump and tubes pump and move the electrolyte between the cathode and the specimen anode, the electric current (60 ampere, 30 volt) from the DC power supply well allowed to pass through the circuit by closing the switch, the time needed, was 40 seconds.

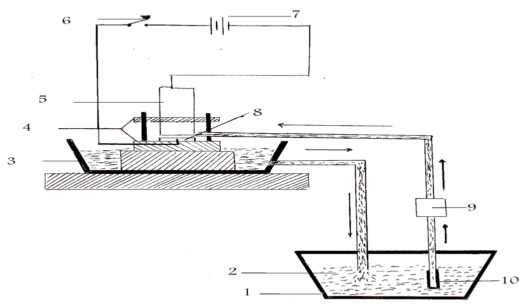

Fig 1-: Schematic diagram of electrochemical polishing equipment.

The polished disks were soaked in a detergent solution for 5 min , then scrubbed using a soft bristle brush and rinsed under tap water for 5 min before roughness measurements. Surface roughness (Ra parameter in micrometer) was evaluated using a Rugosimeter (Mitutoyo-Surf Test 301,Kanagawa, Japan). The diamond stylus (5-mm tip radius) moved across a 600mm-range at 0.100mm/s under a constant load of 3.9mN during testing. This procedure was repeated five times at a different location for each specimen, and the measurements were averaged. Data were analyzed by one-way ANOVA, with the level of significance set at 5% table 1 &chart 1. Specimens were then replaced in sterile distilled water and cleaned in an ultrasonic bath for 6 min at room temperature ,then packed and autoclaved at a temperature of 150°C for 60 min.

2.2.2.Biofilm production

Bacteria was grown overnight on Tryptone Soy Broth (TSB), Difco Laboratories in a shaker incubator at 35°C. The inoculums used for biofilm formation contained 10^7–10^8 colony-forming units (CFU)/ml broth. Each alloy slide was suspended in a sterile flask containing 50 ml TSB, which was inoculated with 1 ml of an overnight culture of the test organism. About 25 ml of the culture broth was replaced with the equivalent volume of fresh medium every 2 days, for a total of 6 days .All slides were removed from the broth on day 6 (cleaning procedures repeated every 6 days) and the surface rinsed with 10 ml sterile distilled water. Each alloy was washed 4 times with sterile phosphate buffer saline.

2.2.3. Enumeration of surface-attached bacteria

Alloy slides were shaken for 15 min, with 5 g sterile glass beads (3 mm) in 20 ml phosphate buffer saline, to dislodge attached cells. The suspension was serially diluted and the cells enumerated on nutrient agar using duplicate spread plates. Plates were incubated for 24 h at 35°C prior to counting. The processes were repeated twice.

3.Results:

3.1.Surface roughness of test specimen

Table 1 displays the comparison of Ra mean values of the tested groups.

Ra was significantly affected by surface treatment (P<0.001).

Conventionally handled samples Group A had the greater Ra mean (1.7µm) and was statistically different from the electrochemical finishing group B (0.2µm).

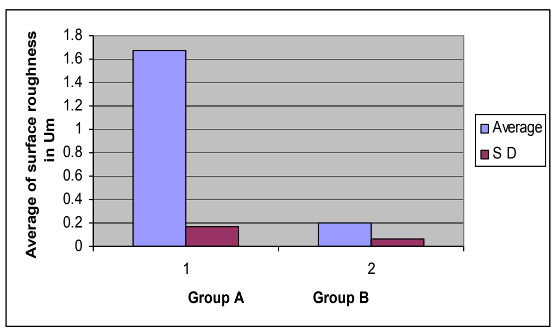

Chart 1: Surface roughness (mean and standard deviation µm) for group A& B

Table 1: Surface roughness Ra (mean and standard deviation µm) for group A& B

	Average of five readings In µm										Av	S.D
Samples	1	2	3	4	5	6	7	8	9	10		
Group A	1.36	1.72	1.98	1.66	1.62	1.62	1.52	1.9	1.78	1.62	1.7	0.17
Group B	0.15	0.27	0.19	0.32	0.25	0.15	0.17	0.2	0.13	0.12	0.2	0.06

3.2. Counting of bacteria adhering to casting surface

Table 2 displayed the amount of *Streptococcus mutans* adhered to specimens. Total count of bacteria in group A = 9 x 10^4CFU/ml.

Statistically, group B electrochemical finishing exhibited significantly lower bacterial adhesion than group A conventional finishing ($p<0.05$) (Chart 2).

Table 2: Total count of bacteria

Alloy Samples	Average viable count of *S. mutans* corresponding to different bacterial dilutions cfu/ml		
	10^{-1}	10^{-2}	10^{-3}
Group A	230	46	9
Group B	82	11	-

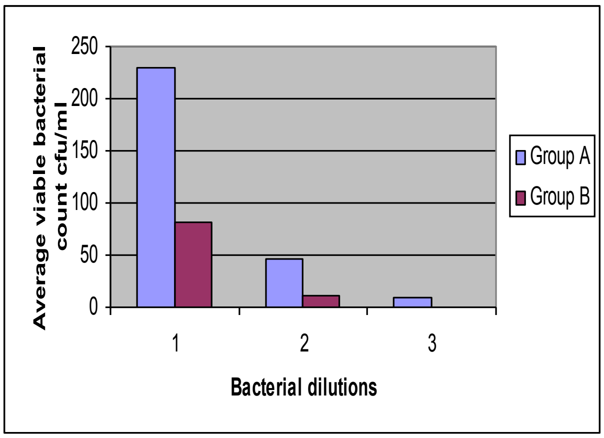

Chart 2: Total count of bacteria

4. Discussion:

The discovery of novel electrochemical treatment, is a promising methodology which disrupt the establishment and virulence of biofilm e.g. secondary caries and periodontal disease around restorations, **Takahashi et al.,(2012).** Dental caries is among the most prevalent chronic human infection diseases affecting both children and adults worldwide, **Petersen et al.,(2005); Dye et al.,(2008)**. Colonization of tooth and restoration surfaces by *mutans Streptococci* is associated with the etiology and pathogenesis of dental caries, as well as, periodontal disease in human, **Beighton(2005); Ramiro et al.,(2010)**.The ability of these organisms, particularly *Streptococcus mutans*, to synthesize extracellular glucans from sucrose using glucosyltransferases (Gtfs) is a major virulence factor, **Aykent et al.,(2010)**.

The results obtained from this study proved that, the electrochemical treated surface was the smoother Ra (0.2 μm) and showed a significant difference in roughness value compared to the conventional polishing method Ra (1.7 μm). This results were in agreement with the results obtained by **Dobrev et al.,(2007)** who concluded that electrochemical polishing operation improved the surface quality to Ra 0.14μm.

The surface roughness values in the conventional finishing specimens were related to miss use of abrasives as proved by **Rosenstiel et al.,(2006)**who mentioned that when abrasives are used, light pressure should be applied and the instrument must be kept rotating ;otherwise, the surface of the casting is ground into a series of facets that ultimately impede plaque control.

The electrochemical polishing eliminate various defects and flaws from the treated cast surface, causing an increase in smoothness of the surface. The conventional method of cast finishing in the study failed in obtaining smooth surface, **Albakry et al.,(2004).**

Results of the study reflect a genuine relationship between the surface roughness of the specimen and bacterial adhesion. Our resent study revealed that the electrochemical treatment of group B alloy greatly reduced the viable count of adhesion bacteria to a very low significant amount than the other treated group A alloy. This can be explained by the fact that a rough surface has irregularities inducing adhesion of bacteria and other substances, **Albakry et al.,(2004).** Also initial colonization of bacteria can easily occur in the depth of surface irregularities and it is difficult to completely remove plaque from these grooves that facilitate re-accumulation, **Kawai et al.,(2000).**

Another explanation to the effect of surface roughness on the attachment of biofilm bacteria is that more or less "surface" is available for bacterial attachment and more or less protection is provided for colonizing bacteria, **Quirynen et al.,(1993).**

From **Konishi et al.,(2003)** study which concluded that , the early dental biofilm was evaluated after one and four hours; these experimental periods were selected to evaluate the relationship between bacterial adhesion and surface roughness. The roughness seems to be particularly important in early bacterial colonization. These findings support our results.

Surface roughness is an important factor influencing the amount of plaque accumulation because bacterial cell attachment is expected to increase with an increase of the porcelain surface roughness, **Pereira da Silva et al.,(2005)**. Less plaque adhesion might suggest a clinically smoother surface. Therefore, measuring the amount of plaque accumulation on the metal restoration could be a good index for judging whether the respective polishing method can achieve less plaque adhesion.

Conclusions

Under the conditions of this in vitro study, the following conclusions can be drawn:

1- The surface-finishing procedures adopted proportioned favorable characteristics for clinical application of the cast metal restorations.

2- A positive correlation was found between the surface roughness value of specimen and the amount of *S. mutans* adhesion to these surfaces.

3- Electrochemical finishing specimens were the most smooth surface and exhibited the least amount of *S. mutans* adhesion.

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