



COMPARISON OF THE BACTERIAL ADHESION AND COLOUR STABILITY OF POLISHED AND GLAZED MONOLITHIC ZIRCONIA CROWNS- AN IN-VITRO INVESTIGATION

Prosthodontics

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ABSTRACT

This article aims at comparing the two finishing techniques i.e glazing and polishing on the basis of bacterial adhesion and colour stability of monolithic zirconia crowns. 20 monolithic zirconia crowns were fabricated using CAD CAM Technology, 10 crowns were glazed using Multimat NT press and 10 crowns were polished using a polishing bur, a polishing paste and a polishing buff and divided into two groups. The baseline values of ΔE were taken using the spectrophotometer in comparison to $1^* a^* b^*$ values taken before finishing the crowns and all samples were dipped in saliva collected from 10 patients and incubated for quantitative assessment of microbial growth. After 1 week of incubation ΔE values were recorded. There was significant difference in the aerobic growth between the two groups ($p=0.009$), it was more for Group 1 (Glazed). Colour stability was better for Group 1 (Glazed) as significant differences were found in ΔE values for Group 2 (Polished) ($p=0.005$).

KEYWORDS

Surface Roughness, monolithic Zirconia, Glazing, Polishing, Bacterial Adhesion, colour Stability.

1. INTRODUCTION:

Ceramics have become an alternative material for the manufacture of dental prostheses due to their aesthetics and long-term proven resistance¹. Due to the good mechanical properties, biocompatibility, and excellent aesthetic properties, zirconia has been widely applied for the fabrication of crowns, bridges, and ceramic posts in dentistry.^(2,3) Monolithic ceramics were developed with the aim of achieving better translucency and mechanical properties to overcome failures due to chipping of veneers, decreasing time and cost¹.

Surface modifications are important for correcting occlusal interferences and contours, finishing the margins of ceramic restorations, and improving the appearance and surface smoothness of restorations. Before the final cementation it's common to adjust the glazed surface in the patient's mouth⁴. These adjustments break the glaze layer which creates a rough surface which causes plaque formation leading to gingival inflammation and soft tissue reactions^(4,5). Smooth surface is important for successful restoration. Rough surfaces in restoration cause increased plaque accumulation which can lead to the development of caries in adjacent teeth⁶. Increased roughness also can lead to discoloration of the restoration and wearing off the opposite dentition. The formation of oral biofilms has been closely correlated with the occurrence of oral diseases¹⁰. Thus, it is obvious that extensive plaque formation on dental restorations may contribute to the occurrence of secondary caries lesions or induce periodontal inflammation, which indicates the demand for dental materials with low susceptibility to biofilm formation.

Zirconia is known to be associated with lower plaque formation than other restorative materials⁷. However, when any adjustments are made, polishing should be performed to restore a smooth surface to the crown. Polishing the ceramic restoration can reduce plaque formation, discoloration, tooth wear, and improve oral health, function, patient comfort and esthetics.⁽⁸⁻¹⁰⁾ Bacteria present in the oral cavity naturally tend to adhere to the ceramic materials or to the interface between the tooth and the restoration¹¹, the cervical third of the proximal surface, along the gingival margin, where they are protected from mechanical action¹². Bacterial adhesion is influenced by many factors including the smoothness of the restoration¹³. Maeir et al reported to no correlation between surface roughness and bacterial adhesion¹⁴. Bollen et al reported that when surface roughness is lower than 0.2 micron meters, there is no significant effect on bacterial adhesion¹⁵. The correlation between the surface roughness and bacterial adhesion, however,

remains controversial.

Although many studies have investigated the surface roughness and bacterial adhesion in dental restorative materials, such as resins and metals, few have examined zirconia. It is well known that oral biofilms are complex and dynamic microbial structures¹⁶. The formation of biofilm in oral cavity has four stages: transport of bacteria, initial bacterial adhesion, attachment, and biofilm maturation¹⁷. This study tried focusing on quantifying the microbiological load in the stage of bacterial adhesion.

The optical behaviour of zirconia after being submitted to aging in a humid environment is also important to draw a conclusion about its long term success. The normal pH of saliva is 6.7 to 7.4 but as bacteria break down the environment changes. The effects of polishing and finishing techniques on colour and surface texture of different porcelain systems have been studied but there are no studies on evaluating the colour change in ceramics (monolithic/layered zirconia) after finishing using various techniques & exposure to saliva. This study aimed to evaluate the influence of two finishing techniques (polishing or glazing) on the surface properties of monolithic zirconia crowns, as well as initial heterotypic biofilm formation in vitro and evaluate the colour stability after salivary exposure. The null hypothesis was that surfaces resulting from polishing or glazing do not influence bacterial adhesion. Colorimetric parameters of the zirconia (polished or glazed) would not be significantly affected after exposure to saliva in vitro.

2. MATERIALS AND METHODS

10 Patients undergoing fixed prosthodontic treatment with monolithic zirconia crowns in the age group of 20–45 years were selected for the study. After obtaining a detailed case history and thorough clinical examination patients were advised to rinse mouth with water at least 10 minutes before the saliva collection. Subjects were allowed to accumulate saliva in mouth and then expectorate into collection tubes for every 60 seconds, for 2-5 minutes till sufficient sample is collected i.e. 2-3 ml. The samples were stored between 2-8°C and transported to laboratory within 3 hours of sample collection.

A total of 20 monolithic zirconia crowns (Fig. 2) were fabricated in lab using CAD CAM Technology, 10 crowns were glazed in the laboratory using Multimat NT press and 10 crowns were polished using Zilmaster –Shofu polishing bur (grit-medium, shank-HP, shape-assort, pn-0650), renfert polishing paste (fig. 1) and polishing buff.

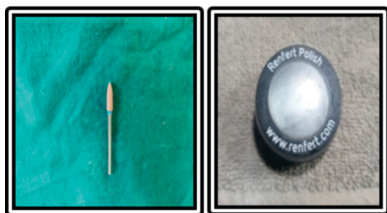


Figure 1- Zilmaster polishing bur and polishing paste

The crowns were divided into two groups based on the type of finishing technique used i.e. glazing & polishing.

The crowns were washed in Phosphate buffered saline. After all the samples were properly labelled according to two groups i.e. group A- Glazed and group B – Polished. The spectrophotometer baseline values of ΔE were taken using the spectrophotometer (Fig.3) in comparison to standard $L^* a^* b^*$ values in the software and the 20 samples were dipped into the saliva collected from 10 patients and incubated in incubator at 37°C till the quantitative assessment of microbial growth was done for all the crowns. For the microbial count, samples were vortexed for 10s and a series of three 10 fold (10^{-3} dilution) of each samples were prepared. Ten millilitres of each dilution was seeded on to the Brain Heart Infusion broth and trypticase soya agar plates for aerobic and anaerobic bacterial growth respectively. Plates for the aerobic bacteria estimation were incubated in an incubator at 37°C with 5-10% CO_2 for 24-48 hours. For anaerobic bacterial growth the plates were incubated at 37°C for 48-72 hours at 5% CO_2 in the vacuum anaerobic jar. (Fig. 4).

2.1 Colony Counting

Plates were removed from the jar, growth was noted and the number of colonies were counted with a colony counter (Fig.5) (Fig.6). The data was expressed as number of colonies per sample/CFU (colony forming units) and tabulated and sent for statistical analysis.

2.2 Colour Assessment

To study the nature of the bacterial growth on the glazed and polished zirconia crowns and to analyse whether it can cause any colour change in the zirconia crowns, colour assessment was done in the spectrophotometer before salivary exposure and 1 week after the exposure. This study basically focuses on the fact that the colour change or discolouration of the crowns in intraoral environment is also due to bacterial adherence apart from primary factors that cause extrinsic staining or discolouration example residue from food, beverages or any habits.

ΔE values were taken for all the samples after 1 week of incubation at 37°C and the colour change was noted for each sample from its baseline value. Colour assessment of zirconia crowns was done before and after exposure to saliva. Colour evaluation was done in accordance with the CIE (Commission Internationale de l'Eclairage) $L^* a^* b^*$ colour system (1931) that uses three parameters to define colour: L^* coordinate represents degree of lightness and darkness and ranges from 0 (black) to white (100), a^* and b^* coordinates represent chroma and positions on red(+)/green(-) and yellow(+)/blue(-) axes respectively. The differences between the two colours can be calculated using the following equation (eq)

$$\Delta E (L^*a^*b^*) = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]^{1/2}$$

The software calculated the ΔE values for both glazed and polished groups from the corresponding ($L^*a^*b^*$) values using the above formula.

ΔE baseline values were taken for all the crowns after glazing and polishing and second ΔE value was taken after exposing the crowns to saliva for 1 week.



Figure 2- Monolithic Crowns



Figure 3-spectrophotometer

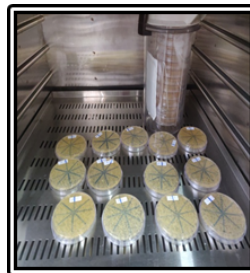


Figure 4- Incubation Of Plates And Anaerobic Jar With Plates In Incubator



Figure 5 – Colony Counter



Figure 6 - Counting of bacterial colonies

4.RESULTS

4.1 Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences computer software (SPSS version 20.0) to analyze the data. Test was carried out to assess the normality of variables in the study.

Descriptive statistics -The mean, standard deviation and standard error was calculated for each group.

- Comparison between the groups (glazed vs polished) for all the four parameters was done using Mann-Whitney U test (non-parametric tests)
- Intergroup comparison was performed for pre-treatment, 2nd week and 4th week with the help of Mann Whitney U test.
- And the comparison between baseline and after exposure ΔE values was done using Wilcoxon Signed Ranks Test.

Bar graph for each comparison was plotted. Significance for all the tests was predetermined at $P \leq 0.05$.

4.2 Tables

Table 1- Aerobic and anaerobic bacterial count for glazed crowns in colony forming units (CFU)

Aerobic	Anaerobic
$776 \times 10^3 \text{ CFU}$	$17 \times 10^3 \text{ CFU}$

1448x10 ⁻³ CFU	7x10 ⁻³ CFU
976x10 ⁻³ CFU	81x10 ⁻³ CFU
720x10 ⁻³ CFU	10x10 ⁻³ CFU
1208x10 ⁻³ CFU	76x10 ⁻³ CFU
996x10 ⁻³ CFU	39x10 ⁻³ CFU
816x10 ⁻³ CFU	44x10 ⁻³ CFU
1520x10 ⁻³ CFU	10x10 ⁻³ CFU
960x10 ⁻³ CFU	21x10 ⁻³ CFU
880x10 ⁻³ CFU	13x10 ⁻³ CFU

Table 2- Aerobic And Anaerobic Bacterial Count For Polished Crowns In Colony Forming Unit (cfu)

Aerobic	Anaerobic
576x10 ⁻³ CFU	37x10 ⁻³ CFU
400x10 ⁻³ CFU	28x10 ⁻³ CFU
1280x10 ⁻³ CFU	30x10 ⁻³ CFU
736x10 ⁻³ CFU	5x10 ⁻³ CFU
680x10 ⁻³ CFU	8x10 ⁻³ CFU
800x10 ⁻³ CFU	10x10 ⁻³ CFU
536x10 ⁻³ CFU	6x10 ⁻³ CFU
816x10 ⁻³ CFU	1x10 ⁻³ CFU
752x10 ⁻³ CFU	19x10 ⁻³ CFU
768x10 ⁻³ CFU	5x10 ⁻³ CFU

Table 3- ΔE baseline and after exposure to saliva for glazed crowns

ΔE (BASELINE)	ΔE (AFTER EXPOSURE)
0.053	0.155
12.551	23.53
15.258	15.295
9.793	9.222
11.287	11.617
10.848	11.217
0.089	0.196
9.661	9.698
8.761	8.988
9.123	9.324

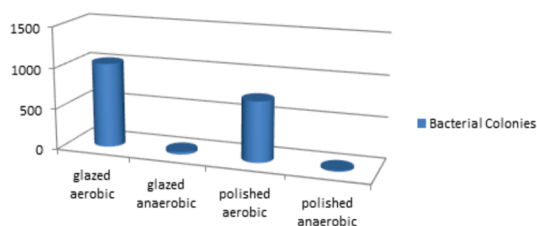
Table 4- ΔE baseline and after exposure to saliva for polished crowns

ΔE (BASELINE)	ΔE (AFTER EXPOSURE)
11.583	13.782
10.198	10.232
3.741	4.112
3.523	4.004
9.556	9.789
8.776	9.001
10.238	10.312
3.779	3.89
6.576	6.668
5.098	5.432

4.3 Bacterial adhesion assessment

Bacterial adhesion was assessed according to the number of colonies formed in BHI and soyaagra plates after 48 hours of incubation in the incubator at 37° for all the 20 samples. The mean values and standard deviation values for the aerobic and anaerobic bacterial counts were obtained for both groups A (Glazed) and B (Polished). Mann-Whitney test indicated that there was no statistical significant difference between the anaerobic bacterial counts for group A and B (p=0.069).

Whereas the aerobic bacterial counts showed significant difference. (p=0.009)



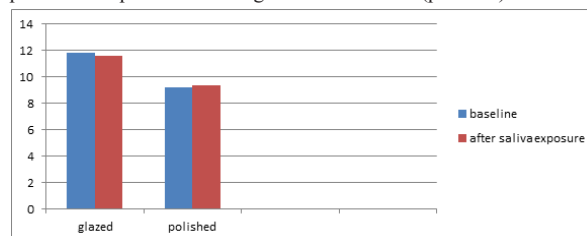
Graph 1 shows the intergroup comparison of bacterial growth for glazed and polished zirconia crowns

4.4 Spectrophotometric evaluation

ΔE values for each sample was obtained in comparison to the values of

1* a* b* taken before any finishing technique was employed using the spectrophotometer.

For glazed samples there was no statistical difference (p=0.059) between the baseline and after saliva exposure ΔE values whereas for polished samples there was significant difference (p=0.005).



Graph 2 – shows the intergroup comparison of ΔE Values (baseline and after exposure) for glazed and polished zirconia crowns.

ΔE values for all the polished and glazed samples were compared using Mann-Whitney Test and Wilcoxon Signed Ranks test.

4. Discussion

Ceramics have multiple flaws due to inhomogenous crystalline structure in the glass matrix. During the ceramic processing defects caused in the structure reduce the strength of ceramic and increase the wear of opposing tooth or restoration. Sealing of these defects with glaze or polish not only increase the strength of ceramics but also reduce the abrasiveness²⁰

It is well-known that less plaque accumulates on ceramic or porcelain restorations; a rough surface accelerates plaque accumulation⁵. Increased amount of plaque (ie more bacterial adhesion) on the rough surfaces of ceramics will exert not only caries-causing virulence, but also a harmful influence on periodontal tissue. The results of this short study are in accordance with Kawai et al., who concluded that more plaque was adhered over glazed surfaces of ceramics as compared with their polished surfaces. The aerobic bacterial growth was statistically significant in the glazed zirconia crowns whereas there was no significant difference with respect to anaerobic growth in polished and glazed zirconia crowns. This means that a glazed surface would not be clinically acceptable from a biologic point of view. Glazing can produce an undulating and rough surface that, usually, has irregularities, inducing more adhesion of bacteria and other substances. Rashid et al also concluded that glazed surfaces are rougher as compared to the polished surfaces²¹. Although polished surfaces have been reported to have voids and micro cracks on the subsurface of porcelain²² these superficial defects did not contribute to the Average Roughness (Ra) values or the amount of plaque adhesion. Contrary to other reports, polishing with diamond paste is helpful for obtaining a smoother surface that will prevent plaque from accumulating.

The effect of surface glazing and polishing of ceramics on early dental biofilm formation was evaluated and found that glazed surfaces tended to accumulate more biofilm compared to polished surfaces¹⁸. However, Bremer et al. mentioned that Biofilm formation on various types of dental ceramics differed significantly; and found zirconia to exhibit low plaque accumulation.

Previous studies tested the different properties affected by finishing techniques. However, the basic property of surface roughness is of utmost importance for bacterial and plaque adhesion. More is the surface roughness more will be the plaque accumulation, lesser fracture strength and poor final aesthetics. This is in consensus with various studies⁽²³⁻²⁶⁾

This study hypothesized that polished and glazed zirconia crowns would have similar bacterial adhesion and color stability after saliva exposure. However, the research hypothesis was not corroborated by the results because significant changes were found in ΔE for polished zirconia crowns before and after saliva exposure for 36 hours. On the other hand there was no significant difference in ΔE values for glazed crowns. These results were in accordance to the studies that proved glazing is better than polishing and mechanical polishing was unable to re-establish sufficient surface smoothness for ceramics^(22,26,27,28,29)

Various factors can be responsible for the color change of polished crowns that can either be attributed to the surface irregularities that still persist after manual polishing³⁰ or the bacteria present in the oral biofilm & plaque that show inherent capability of changing color of the surface it grows on proving that the bacteria has chromogenic behavior.

The primary strength of this study is that it is an investigation that is giving an idea of the type of bacterial adhesion that is seen on zirconia surface with two most common finishing techniques. It also proves that the color stability of polished zirconia surface is inferior the that of glazed zirconia surface which is in accordance to the results obtained in SEM studies that concluded that glazed surfaces showed uniform surface texture and homogenous surfaces without any defect whereas polished surfaces had fine ridges and grooves¹⁹

This study is one of its own kind as no where in literature it has been proved that bacterial adherence on monolithic zirconia can also cause discolouration apart from other factors like food residues, beverages, aerated drinks ,tobacco and smoking habits. Further studies are required with larger sample size in order to find out the specifications about this aerobic and anaerobic growth and also prove the chromogenic nature of bacteria.

Reasons for variation in results can be explained by-

- Manual grinding and polishing is always difficult to standardize. However, in this study the same operator finished all the specimens in order to reduce this variation to an extent.
- The techniques in various studies for polishing are different that are being compared to glazing for effectiveness. The method used here is a manual method of using finishing bur (Zilmaster), polishing paste (Renfert) and buff.
- The study done was an *in vitro* study that may differ from actual oral environment.
- Many factors that affect the bacterial adhesion such as hydrophobicity, surface free energy and polarity were not considered and it was assessed solely on the basis of finishing techniques and the resultant surface roughness.

5. Conclusion

Within the limitations of this *in vitro* study, it can be concluded that with different finishing techniques the bacterial adherence and colour stability varies for monolithic zirconia. There was significant difference only in the aerobic growth between the two groups, it was more on Group 1 (Glazed) Colour stability was better for Group 1 (Glazed) as significant differences were found in ΔE values (before and after) for Group 2 (Polished). These findings concur with previous studies thus, further analysis is necessary to clarify the factors influencing microbial adhesion and colour stability of monolithic zirconia in oral environment in order to prove the better finishing technique for zirconia (considering all aspects), which holds a major role in restorative dentistry as one of the best restorative materials.

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