Evaluation of *Candida albicans* formation on feldspathic porcelain subjected to four surface treatment methods

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Candida albicans, known for its adhesion on prosthetic materials and oral tissues, is the most frequently encountered fungal infection in dentistry. The aim of this study was to evaluate the effects of four different surface treatment methods and immersion in artificial saliva on the surface roughness of and *candida* adhesion on dental porcelains. The four surface treatment methods were namely: natural glaze, overglaze, dual ion exchange, and polishing. Surface roughness of porcelain was evaluated using a surface profilometer and by SEM. *Candida* adhesion was examined by culturing two *Candida* strains on porcelain specimens followed by a colorimetric method using XTT/Coenzyme Q0. It became evident that *Candida* adhesion was found more in the specimens treated with natural glaze and polishing. Further, by the visual inspection of SEM images and comparison of surface roughness, polished and natural-glazed specimens showed rougher surface characteristics than overglazed and dual-ion-exchanged specimens.

Keywords: *Candida albicans*, Feldspathic porcelain, Surface treatment

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INTRODUCTION

Feldspathic porcelain has been frequently used in fixed dental restorations because of its outstanding esthetic and biocompatible features¹. However, due to its brittle nature after firing, various surface treatment procedures need to be applied on the porcelain surface in order to strengthen the material and inhibit fractures, thereby increasing its long-term use. To date, several methods for surface treatment prior to final cementation have been introduced^{2,3}, and they are namely: natural glaze, overglaze, dual ion exchange, and polishing.

Autogenous or natural glaze results from vitrification of the surface when it is heated up to a glazing temperature. Low-fusing glaze powder is applied evenly on the veneer and fired up to its maturation temperature⁴⁻⁶. In overglaze procedure, overglaze powder is applied on the porcelain surface and heated up to a temperature close to the firing temperature^{6,7}. In dual ion exchange method, sodium ions are replaced by larger potassium ions on the porcelain surface. During the initial phase of this method, sodium and potassium ions are exchanged for smaller lithium ions at a temperature above the melting point of the ceramic, and then the smaller lithium ions are exchanged for sodium ions at a temperature below the melting point of the ceramic. The main advantage of this technique is the development of an ion-exchanged layer with greater strength^{1,6,8}. As for polishing, which is another alternative for the finishing procedure, it differs from the dual ion exchange method in that it does not affect the chemical structure of porcelain. This method reduces surface roughness by employing various finishing burs with different grit sizes.

Candida albicans (*C. albicans*), the most frequently encountered intraoral commensal is an opportunistic and toxic pathogen⁹. It accounts for 25% of the intraoral microorganisms in healthy individuals and 50–90% in individuals with immunosuppressive or various health problems¹⁰. On the adhesion mechanism of this microorganism on intraoral prosthetic materials, numerous research studies have been conducted to examine this concern and gain more insight¹¹⁻¹⁸. Piling in comparison, on the other hand, are the investigative efforts on the effects arising from the surface variations of porcelain materials, and the related long-term alterations, on *Candida* adhesion.

To quantitatively measure *C. albicans* adhesion, various methods have been proposed — such as colony counting, radiolabeling, and antibody assays. These procedures, however, are tedious, expensive, and time-consuming. With regard to assaying the adhesion of *C. albicans* on non-biological materials, the quantitative colorimetric method has emerged as one of the most efficient and cost-effective methods. In particular, XTT assay employs the oxidation of 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenyl-amino)carbonyl]-2H-tetrazolium hydroxide, which is a tetrazolium salt, to 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide. The XTT assay monitors and measures the metabolic activity, and it has been proved to be a simple, rapid, and reliable colorimetric method for quantitative determination of *C. albicans* adhesion on the surfaces of diverse materials^{12,19-23}.

Based on published literature, the porcelain surface presents the least potential for the adhesion and biofilm formation of *C. albicans*^{2,6,16,17}. However, to the best of the authors' knowledge, no research has been conducted to endorse the preferred choice of surface treatment vis-à-vis *Candida* adhesion and

formation. Therefore, the aim of this study was to develop an *in vitro* model to quantitatively determine the adhesion of two strains of *C. albicans* on feldspathic porcelain subjected to four different surface treatment methods. After aging was performed in artificial saliva, quantitative measurement was carried out using XTT assay. For better insight into surface characterization in the long term, surface roughness was also measured and the values compared with the SEM images.

MATERIALS AND METHODS

Specimen preparation

A total of 88 feldspathic porcelain disks (VMK 95, shade A2, Vita Zahnfabrik, Bad Sackingen, Germany), of 5 mm diameter and 2 mm thickness, were prepared for this study. The test disks were fabricated according to the manufacturer's recommendations by mixing porcelain powder (1.3 g) with a modeling fluid (0.3 cc). The slurry was vibrated and condensed into a resin mold, and excess fluid was absorbed using a paper tissue.

The specimens were vacuum-fired in a porcelain furnace (Vacumat 200, Vita Zahnfabrik, Bad Sackingen, Germany) at an initial temperature of 600°C for 5 minutes. The temperature was gradually increased at a rate of 100 °C/minute until it reached 940°C, then rapid air cooling was applied.

One side of each disk was ground with a 70-µm diamond abrasive wheel using a 300-rpm grinding machine. After polishing, the disks were cleaned with distilled water in an ultrasonic cleaner for 10 minutes. A single investigator was assigned to prepare the specimens in the same manner to ensure standardization.

The prepared specimens were assigned into four experimental groups according to their surface treatment methods — namely, natural glaze, overglaze, dual ion exchange, and polishing.

1. Natural glaze method

Natural glaze (Vita Zahnfabrik, Bad Sackingen, Germany) was performed in a porcelain furnace (Vacumat 200, Vita Zahnfabrik, Bad Sackingen, Germany) according to the manufacturer's recommendations at an initial temperature of 600°C for 5 minutes. The temperature was gradually increased at a rate of 100 °C/minute until it reached 940°C, then rapid air cooling was applied.

2. Overglaze method

Overglaze powder (Vita Zahnfabrik, Bad Sackingen, Germany) was mixed with deionized water and applied on the surfaces of the porcelain disks to create a layer of approximately 0.3 mm thickness. Glaze sintering was performed in a porcelain furnace (Vacumat 200, Vita Zahnfabrik, Bad Sackingen, Germany) at 600°C with a dwell time of 3 minutes. The temperature was then increased at a rate of 100 °C/minute until it reached 920°C. Dwell time for the latter sintering temperature was 1 minute, and then rapid air cooling was applied.

3. Dual ion exchange method

Disk specimens were coated with slurries ranging from 10 mol% lithium chloride (LiCl) to 90 mol% sodium chloride (NaCl) in distilled water. The coated disks were heat-treated at 750°C for 30 minutes and then at 450°C for 30 minutes.

4. Polishing method

For the initial polishing of the porcelain disks, it was performed using a 50-µm diamond wheel (ECOMET III, Buehler Ltd., Lake Bluff, IL, USA). This was followed by a 35-µm diamond wheel, and then 15-µm diamond paste (Cosmedent Inc., Chicago, IL, USA) on a cloth wheel (ECOMET III, Buehler Ltd., Lake Bluff, IL, USA). A polisher (ECOMET, Buehler Ltd., Lake Bluff, IL, USA) was used to ensure the reproducibility of the polishing procedure. A pressure of 66.72 N for 15 seconds at a speed of 350 rpm was applied to the porcelain surfaces for each of the two diamond grit sizes. The disks were cleaned in a soap solution (Evyap Tic AS., Levent, Istanbul, Turkey) in an ultrasonic device, and then finally in deionized water.

Artificial saliva immersion

All specimens were rinsed twice with sterile water, then placed in an artificial saliva solution (1.0 mL/well; 4.1 mM KH₂PO₄, 4.0 mM Na₂HPO₄, 24.8 mM KHCO₃, 16.5 mM NaCl, 0.25 mM MgCl₂, 4.1 mM citric acid, and 2.5 mM CaCl₂; pH 6.7) at 37°C. The specimens were disinfected and cleaned as previously described, then tested for adhesion after 2 weeks of immersion in artificial saliva.

One specimen from each experimental group was used for SEM analysis and surface roughness evaluation. The remaining specimens of each experimental group were used for *C. albicans* adhesion investigation.

Surface topography

After immersion in saliva, the effects of four surface treatment methods were evaluated using a scanning electron microscope (SEM) (JSM 6400, JEOL Ltd., Tokyo, Japan). The disks were sputter-coated with 25-nm gold, and surface images of ×500 magnification were used for topographical evaluation and comparison.

Surface roughness (*Ra*) was measured using a profilometer (Mitutoyo Surftest 402 Analyzer, Mitutoyo Corp., Kawasaki, Japan). The profilometer was calibrated with a standard reference specimen, and then set to travel at a speed of 0.100 mm/s with a range of 600 µm during testing. To measure the *Ra* value, the diamond stylus (5-µm tip radius) was moved across the surface under a constant load of 3.9 mN. The surface analyzer was used to determine the roughness profile of each specimen. This procedure was repeated three times at different locations to obtain the general characteristic of the surface.

Quantitative measurement of Candida adhesion

Quantitative measurements were made using ELISA readings (EL800, Bio-Tek Instruments, ABD) before

and after immersion in artificial saliva. Adhesion measurement was performed using a modified technique as introduced by Sen *et al.*²⁰⁾ and Ramage *et al.*²⁴⁾.

Two *Candida* strains were used in this study: GDH 2346 and a clinical strain known for its adhesion to YPD medium (1% yeast extract, 2% peptone, 2% dextrose). Five identical colonies of each strain were inoculated in YPD liquid medium and grown overnight at 37°C in a shaking incubator (100 rpm). Pellets which were obtained after 10-minute centrifugation at 5,000 rpm were washed twice with PBS (8.0 g NaCl, 0.2 g KCl, 1.15 g Na₂HPO₄, 0.2 g KH₂PO₄, 1000 ml dH₂O) adjusted to pH 7.2. Cell concentration was standardized to 1.0×10⁶ cells/ml. These cells were suspended in RPMI 1640 medium (ICN-Flow, Aurora, OH, USA) and buffered with 3-(N-morpholino) propanesulphonic acid (MOPS; ICN-Flow, Aurora, OH, USA). Standard yeast cell suspensions of 4 ml were added into six cell culture vials containing sterile porcelain disks and incubated at 37°C in a shaking incubator (60 rpm) for 2 weeks.

After incubation, the porcelain disks were removed and washed twice with sterile PBS solution. The disks were then placed in 24 sterile cell culture vials, to which were added a mixture of 1 ml of sterile PBS and 500 µl of XTT/Coenzyme Q₀ (0.5 mg/ml XTT in 40 µg/ml CoQ₀/PBS). After incubation at 37°C for 2 hours, the liquid part was transferred into microcentrifuge tubes and centrifuged at 10,000 rpm for 2 minutes. Liquid specimens of 100 µl each were collected and transferred into microplates, and then their optical densities (OD) measured at 490 nm with an ELISA reader. The OD value of XTT/Coenzyme Q₀ mixture was excluded from all the ELISA readings. Each quantitative measurement was done three times.

Statistical analysis

Results obtained in this study were statistically analyzed using SPSS (Statistical Package for Social Sciences SPSS, Chicago, IL, USA) for Windows 13.0. Definitive statistical methods (mean, standard deviation, median) were employed in the determination of the results. Mann–Whitney U tests were used to compare the differences within groups and between groups before and after immersion in artificial saliva. The relation of XTT/CoQ₀ variable with the other variants was investigated using Pearson's correlation

coefficient. For surface roughness, the mean *Ra* values were analyzed using one-way analysis of variance and Tukey's HSD test at 5% significance level.

RESULTS

Surface topography

Surface roughness (*Ra*) measurements before and after immersion in artificial saliva were compared, and no statistically significant changes ($p>0.05$) were observed for the overglazed and dual-ion-exchanged specimens. On the contrary, statistically significant differences ($p<0.05$) were observed for the specimens treated with polishing and natural glaze (Table 1).

SEM analysis revealed that overglaze and dual ion exchange treatments rendered a smoother, slightly pitted surface than the specimens treated with natural glaze and polishing. For overglazed and dual-ion-exchanged specimens (Figs. 2 and 3 respectively), their surface topographies became similar after immersion in artificial saliva. As for the natural-glazed and polished specimens (Figs. 1 and 4 respectively), their surfaces were observed to be slightly rougher after immersion, whereby the roughness of the polished surface seemed to be greater than that of the natural-glazed surface.

In summary, evidently rough surfaces were seen for natural-glazed and polished specimens through SEM observation with accompanying high *Ra* values. On the contrary, smoother surfaces were observed for overglazed and dual-ion-exchanged specimens with accompanying lower *Ra* values.

Adhesion of the clinical strain of *Candida*

For the clinical strain specimens (Table 2; Fig. 5), the natural glaze treatment resulted in significant differences ($p=0.04$) before and after immersion in artificial saliva. Similarly, significant differences ($p=0.04$) were observed for the polished specimens before and after immersion in saliva. In contrast, no statistically significant differences were observed for the overglazed specimens ($p=0.16$) and dual-ion-exchanged specimens ($p=0.39$) before and after immersion.

Upon comparing among the four different surface treatment methods (Table 4), statistically significant differences were observed between the natural-glazed and dual-ion-exchanged specimens and between the natural-glazed and polished specimens before

Table 1 Surface roughness values (µm) of the specimens before and after immersion in artificial saliva

Surface Treatment	Before Immersion		After Immersion		Sig.
	Mean	SD	Mean	SD	
Natural glaze	0.67	0.34	1.17	0.43	<0.05
Overglaze	0.58	0.36	0.59	0.28	>0.05
Dual ion exchange	0.61	0.24	0.67	0.23	>0.05
Polishing	0.74	0.39	1.26	0.48	<0.05

Fig. 1 Surface topography of natural-glazed specimens before (A) and after (B) immersion in saliva ($\times 500$ magnification).

Fig. 3 Surface topography of dual-ion-exchanged specimens before (A) and after (B) immersion in saliva ($\times 500$ magnification).

Fig. 2 Surface topography of overlazed specimens before (A) and after (B) immersion in saliva ($\times 500$ magnification).

Fig. 4 Surface topography of polished specimens before (A) and after (B) immersion in saliva ($\times 500$ magnification).

Table 2 Mann–Whitney U test results for the clinical strain before and after immersion in artificial saliva

Clinical strain		Minimum	Maximum	Mean	SD	Median	Z	p-value
Natural glaze	Before immersion	0.12	0.14	0.134	0.007	0.135	-2.02	0.04
	After immersion	0.12	0.173	0.1493	0.019	0.155		
Overglaze	Before immersion	0.12	0.15	0.135	0.010	0.14	-1.39	0.16
	After immersion	0.12	0.16	0.14	0.013	0.14		
Dual ion exchange	Before immersion	0.12	0.15	0.138	0.009	0.14	-0.85	0.39
	After immersion	0.12	0.18	0.143	0.022	0.14		
Polishing	Before immersion	0.13	0.15	0.141	0.006	0.14	-2.05	0.04
	After immersion	0.13	0.18	0.153	0.016	0.15		

Table 3 Mann–Whitney U test results for the GDH 2346 strain before and after immersion in artificial saliva

GDH 2346 strain		Minimum	Maximum	Mean	SD	Median	Z	p-value
Natural glaze	Before immersion	0.12	0.14	0.135	0.007	0.14	-1.97	0.04
	After immersion	0.12	0.5	0.184	0.112	0.15		
Overglaze	Before immersion	0.12	0.15	0.136	0.010	0.135	-0.45	0.65
	After immersion	0.13	0.16	0.137	0.009	0.135		
Dual ion exchange	Before immersion	0.12	0.15	0.137	0.008	0.14	-0.43	0.67
	After immersion	0.12	0.16	0.139	0.012	0.14		
Polishing	Before immersion	0.14	0.15	0.141	0.003	0.14	-1.99	0.04
	After immersion	0.13	0.18	0.155	0.019	0.15		

Table 4 Mann–Whitney U test results for the binary comparison of the different surface treatment methods

	Clinical strain		GDH 2346	
	Before Immersion (Z; p-value)	After Immersion (Z; p-value)	Before Immersion (Z; p-value)	After Immersion (Z; p-value)
Natural glaze - Overglaze	-0.37; 0.71	-1.27; 0.2	-0.12; 0.9	-2.18; 0.03
Natural glaze - Dual ion exchange	-2.2; 0.03	-0.35; 0.73	-0.65; 0.52	-1.7; 0.09
Natural glaze - Polishing	-2.2; 0.03	-0.35; 0.73	-2.29; 0.02	-0.08; 0.94
Overglaze - Dual ion exchange	-0.69; 0.49	0; 0.99	-0.36; 0.72	-0.48; 0.63
Overglaze - Polishing	-1.5; 0.13	-1.78; 0.08	-1.5; 0.13	-2.43; 0.01
Dual ion exchange - Polishing	-0.73; 0.47	-1.35; 0.18	-0.36; 0.72	-0.48; 0.63

Fig. 5 Adhesion of the clinical strain on porcelain disks subjected to four surface treatments with and without aging in artificial saliva.

Fig. 6 Adhesion of GDH 2346 on porcelain disks subjected to four surface treatments with and without aging in artificial saliva.

immersion in saliva ($p<0.05$). After immersion, there were no statistically significant differences among the four surface treatment methods.

Adhesion of GDH 2346 strain

For the GDH 2346 strain (Table 3; Fig. 6), statistically significant differences were likewise observed for the natural-glazed and polished specimens before and after immersion in artificial saliva ($p=0.04$). However, no statistically significant differences were observed for the overglazed ($p=0.65$) and dual-ion-exchanged ($p=0.67$) specimens before and after immersion.

Upon comparing among the four different surface treatment methods (Table 4), a significant difference ($p<0.05$) was observed between the natural-glazed and polished specimens before immersion. After immersion, significant differences ($p<0.05$) were observed between the natural-glazed and overglazed specimens and between the overglazed and polished specimens.

DISCUSSION

The effects of different surface treatments — namely, natural glaze, overglaze, ion exchange, and polishing — on strength have been widely mentioned in published literature^{1-4,6-8,23,25}, but not so with their

effects on *Candida* adhesion on porcelain surfaces^{16,17}. Against this backdrop of information scarcity, this study was undertaken to investigate the effects of different surface treatments on the adhesion and formation of *C. albicans*.

In the evaluation of *Candida* formation on several non-porcelain dental materials, surface roughness and aging in saliva were considered to be pivotal factors to the long-term success of these dental materials^{14,16,17,25}. However, similar research efforts pale in comparison for the porcelain surface^{13,15-17,22,23}. Therefore, in this study, both surface roughness and aging in saliva were included as primary determinants in the evaluation of *C. albicans* adhesion on the porcelain material.

For the quantitative measurement of *Candida* adhesion, XTT assay has been shown by Sen *et al.*²⁰ and Ramage *et al.*²⁴ to be a more advantageous microbiologic tool. Therefore, the XTT method was used in this study to assess *Candida* adhesion on porcelain surfaces with different surface characteristics.

On the preferred surface treatment for dental porcelains, discrepant conclusions abound in the published literature. In studies that have compared polishing and glazing, differing results were obtained^{1,3,5-8}. Nonetheless, since glazed porcelain

provides a dense and smoother surface, some studies supported that polishing can serve as an alternative to glazing^{1,3)}.

On *Candida* adhesion, Radford *et al.*¹⁵⁾ demonstrated that there was no direct relationship between surface roughness and adhesion, irrespective of the material. On the other hand, Wang *et al.*¹⁶⁾ demonstrated that more bacteria adhered on a rougher porcelain surface. In the present study, higher surface roughness (*Ra*) values were obtained for natural-glazed and polished specimens after immersion. In parallel with these results, a higher level of *Candida* adhesion was observed for natural-glazed and polished specimens. Based on these results, it could be said that as the surface became rougher, a higher level of *C. adhesion* was observed. Therefore, to eliminate *Candida* formation, the porcelain surface should not be subjected to polishing or natural glaze treatment.

In a study by Fuzzi *et al.*³⁾, SEM images of porcelain specimens revealed considerable changes to the surface characteristics. It was also found that overglazing produced a better surface than the polishing methods³⁾. In the present study, SEM observation likewise showed that polished and natural-glazed specimens presented rougher surfaces when compared with overglazed and dual-ion-exchanged specimens.

At this juncture, it must be pointed out that the dual ion exchange technique used in this study is an *in vitro* method rather than a routine laboratory procedure. Although this technique seemed to provide a smoother surface for preventing *Candida* adhesion, it did not carry a strong significance as it is not used in clinical practice. For the other three surface treatment methods investigated in this study, results showed that both polishing and natural glaze resulted in higher *Candida* formation. Therefore, overglaze should be the preferred method in preventing *C. albicans* adhesion. Moreover, both the *Ra* values and SEM images obtained supported the result obtained with the XTT test.

CONCLUSIONS

Within the limitation of this study, the following conclusions were drawn:

1. Surface treatment had an effect on *C. albicans* formation. In particular, a higher level of *Candida* adhesion was observed on natural-glazed and polished surfaces than on overglazed and dual-ion-exchanged surfaces.
2. After immersion in saliva, natural-glazed and polished surfaces became rougher, hence resulting in a higher level of *Candida* adhesion on these surfaces.
3. Statistically similar results were obtained between the clinical strain and the GDH 2346 strain before and after immersion in artificial saliva.

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