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The effect of polishing and glazing on fibroblast cell adhesion and viability on zirconia frameworks

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ABSTRACT

Background: Dental implants face challenges such as bacterial infiltration and peri-implantitis, emphasizing the need for a robust mucosal seal to ensure long-term success. This study aimed to evaluate the impact of polishing and glazing zirconia on the adhesion and survival of human gingival fibroblasts, which are critical for establishing this protective barrier.

Materials and Methods: In this *in vitro* experimental study, 18 zirconia discs (2.5 mm thickness) were prepared, sintered, and divided into three groups: polishing, glaze, and simple. The polishing group underwent sequential polishing, whereas the glaze group was coated with glaze paste and heated in a vacuum oven; the simple group remained unaltered. Cell survival was assessed using direct and indirect 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, and cell adhesion was analyzed through fluorescence microscopy and quantitative fluorometry. Data analysis was performed using SPSS version 26, employing the Kolmogorov–Smirnov test for normality and one-way ANOVA ($P < 0.05$).

Results: Cell adhesion was significantly greater in the polished group compared to the simple group ($P = 0.001$) and the glazed group ($P = 0.002$). Cell survival did not significantly differ between the polished and plain groups ($P = 0.111$). However, the glazed group showed significantly higher cell survival compared to both the simple ($P < 0.001$) and polished groups ($P = 0.004$).

Conclusion: Within the limitations of this *in vitro* study, it can be concluded that polished zirconia surfaces promote greater gingival fibroblast adhesion. However, fibroblast cell viability was higher on glazed zirconia discs. These findings underscore the importance of zirconia surface treatments in improving gingival integration.

Key Words: Bacterial adhesion, cell survival, dental polishing/adverse effects, dental polishing/methods, dental porcelain, surface properties

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INTRODUCTION

Dental implants have demonstrated high success rates; however, challenges such as biofilm formation and peri-implantitis resulting from bacterial infiltration and proliferation in the peri-implant region remain significant concerns.[1] In natural teeth, the oral mucosa serves as a protective barrier for periodontal tissues and bone against bacteria and other disturbing stimuli. The placement of a dental implant disrupts this barrier, creating discontinuities. Establishing an

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effective seal between the implant surface and the surrounding soft tissue is critical for the long-term success of dental implants.[1,2]

Soft tissue growth around current dental implants often lacks specific orientation, resulting in an inadequate epithelial barrier that facilitates bacterial infiltration. Over time, this can lead to implant failure.[3] To mitigate these issues, it is crucial to establish a sufficient width of mucosa that adheres firmly to the implant surface and forms a robust epithelial seal. This reduces plaque accumulation, minimizes soft tissue recession, and decreases the risk of peri-implantitis.[1,4] Human gingival fibroblasts (HGFs), a dominant cell type in gingival tissue, play a critical role in forming the mucosal seal. Their proliferation is essential for developing and maintaining this protective barrier.[2,5]

The surface properties of materials, such as chemical composition, surface charge, material strength, and surface roughness, are crucial in influencing cell adhesion and proliferation in zirconia.[1,3,5] Surface roughness has been identified as a significant factor affecting cellular behavior.[6] Studies have demonstrated that rough surfaces promote greater cell adhesion than smooth surfaces. In addition, surface topography impacts various cellular activities, including adhesion, proliferation, differentiation, orientation, and migration.[1,7,8] Zirconia's high surface hardness necessitates using diamond burs for clinical adjustments, which can remove the glaze layer and compromise surface smoothness. Intraoral polishing systems, introduced as an alternative to reglazing, help prevent wear, enhance durability, and improve the aesthetics of restorations. Polishing and glazing can significantly modify the surface properties of materials, enhancing their functionality and interactions.[9-12]

Given the limited studies on the role of glazing and polishing in the survival and adhesion of HGFs, as well as comparisons between these techniques, this study aimed to compare the effects of polishing and glazing on the survival and adhesion of fibroblast cells to zirconia frameworks. It is hypothesized that there will be no significant difference in the survival and adhesion of fibroblast cells between polished and glazed zirconia frameworks.

MATERIALS AND METHODS

This experimental *in vitro* study was approved by the ethics committee of the university (Ethical code: IR.IAU.DENTAL.REC.1401.108).

Based on a similar previous study,[13] the minimum required sample size was estimated to be 6 samples per group (a total of 18). This was determined using one-way ANOVA in PASS 11 software, with $\alpha = 0.05$, $\beta = 0.2$, an average standard deviation of 0.15 for cell viability, and an effect size of 0.76.

Sample preparation

The precolored monolithic zirconia blocks (Zolid Gen-X Esthetic all-rounder, AmmanGirrbach, Austria) were used for this study. The monolithic zirconia (ø = 95 mm, thickness 16 mm) was initially sectioned into 2.5 mm and 1 mm thick plates using a low-speed diamond wet saw. The surfaces were then smoothed with 220-grit silicon carbide sandpaper (3M, Saint Paul, MN, USA) and further machined into discs (ø = 5 mm, thickness 2.5 mm and 1 mm) using a vertical milling unit (TRAK K2 SX, Southwestern Industries, Rancho Dominguez, CA, USA). The water from the machining process was removed by placing the discs in an oven at 78°C (Precision 658 Compact Oven, Thermo Fisher, Waltham, MA, USA) for at least 72 h. The samples were then sintered in a box furnace, following the manufacturer's specifications for ramp and hold time, with a final sintering temperature of 1480°C for 2 h (Lindberg/Blue M 1700°C Tube Furnace, Thermo Fisher, Waltham, MA, USA) using the corresponding control unit (Lindberg/Blue M CC59246PCOMC-1, Thermo Fisher, Waltham, MA, USA). After sintering, the discs were polished with 320-grit silicon carbide sandpaper (Norton, Worchester, WA, USA) to achieve a uniform thickness of 2.0 mm, resulting in final dimensions of ø =5 mm, thickness 2.0 mm and 1 mm.

The samples were divided into three groups based on the preparation method: (1) Glaze, (2) polish and (3) simple [Figure 1].

Glaze

The samples were coated with a thin layer of glaze paste (IPS e.max Ceram Glaze; Ivoclar Vivadent AG) and fired in a vacuum furnace for 30 s at 950°C.

Polish

The surface of the samples was initially shaped using a cylindrical diamond bur (Tizkavan–Iran) in a back-and-forth motion from right to left. The samples were then polished sequentially using coarse, medium, fine, and finally, x-fine polishers (OptraFine, Ivoclar Vivadent, Liechtenstein). Following the manufacturer's recommendations and previous

studies, a rotary tool (Dremel 4000, Racine, WI, USA) was mounted on a unit (Dremel 220, Racine, WI, USA) and set to 15,000 rpm. Polishing was performed manually for 15 s with an applied force of approximately 3 N, with the zirconia surface positioned perpendicular to the polishing head. A stroboscope (Strobotac Type 1531-A, General Radio Co., Boston, MA, USA) was used to confirm the rotation frequency.

Simple

The samples in this group were left untreated.

Fibroblast cell culture and preparation

HGFs were seeded in 96-well plates at a cell density of 10^4 cells/well using Dulbecco's Modified Eagle Medium (DMEM) culture medium (Idehizist, Iran) supplemented with 10% Fetal Bovine Serum) FBS((Burge Jady, Germany) and 1% penicillin/streptomycin (Biosera, France). The cells were incubated for 24 h at 37°C in a humidified atmosphere with 5% CO_2. To evaluate cytotoxicity, discs were subjected to indirect cytotoxicity testing according to the ISO 10993-2012 protocol. Briefly, extracts were prepared by incubating sterilized discs (autoclaved) in serum-containing medium with an extraction ratio of 6 cm^2/ml for 48 h. Unmodified cells were used as a control.

Cytotoxicity testing-indirect 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The effect of materials on cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After 48 h of incubation with the extracts, the solutions were removed from the wells, and 40 µL of MTT solution (5 mg/ml MTT, Sigma, Germany) in phosphate-buffered saline (PBS) was added. The plates were then incubated for 3–4 h at 37°C and 5% CO_2. Afterward, the MTT solution was removed, and 60 µL of DMSO solution was added to each well. The absorbance was measured at 570 nm using a microplate reader (BioTek, USA), and the cell viability was reported as a percentage relative to the control [Figure 2].

Cell proliferation assay-direct 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

For the direct MTT assay, the discs were sterilized and placed in each well of the 96-well plates. HGFs were directly seeded onto the discs in the 96-well plates. The cells were incubated for 48 h at 37°C in a humidified atmosphere with 5% CO_2 in DMEM culture medium containing 10% FBS. After 48 h, the wells were washed twice with PBS to remove nonadherent cells. The discs were then transferred to another plate containing 200 µL of MTT solution (5 mg/ml MTT, Sigma, Germany) in PBS and incubated for 3–4 h at 37°C with 5% CO_2. The MTT solution was then removed, and 120 µL of DMSO was added to each well. The plates were agitated for 30 min, and the absorbance was measured at 570 nm using a microplate reader (BioTek, USA) [Figure 3].

Cell adhesion-fluorescent staining

For assessing cell adhesion, the discs were sterilized and placed in each well of the 96-well plates. HGFs were directly seeded onto the discs in the 96-well plates. The cells were incubated for 4 h at 37°C in a humidified atmosphere with 5% CO_2 in DMEM culture medium containing 10% FBS. After 4 h, the wells were washed twice with PBS to remove nonadherent cells, and the discs were transferred to new 96-well plates. The cells were then permeabilized with 0.2% Triton-X 100 in 1X PBS for 3 min and stained with SYBR Green (1:5000 dilution) as a DNA stain. The cells were observed under a fluorescence microscope (Leica, USA) and analyzed quantitatively using fluorometry [Figures 4 and 5].

Data analysis

Data analysis was performed using SPSS (version 26.0; IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was employed to assess the normality of the data. One-way ANOVA was conducted for statistical analysis, with a $P < 0.05$ considered statistically significant.

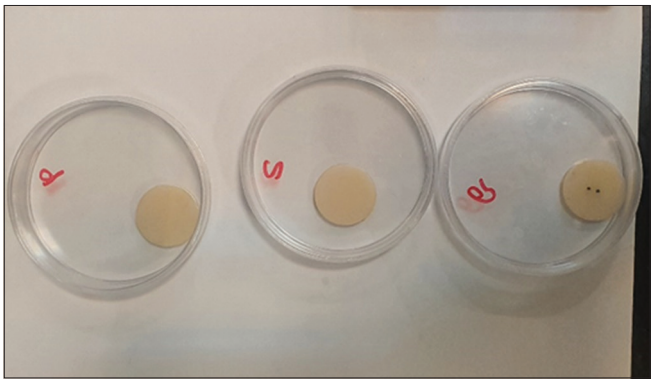

Figure 1: Polished, glazed, and simple zirconia discs.

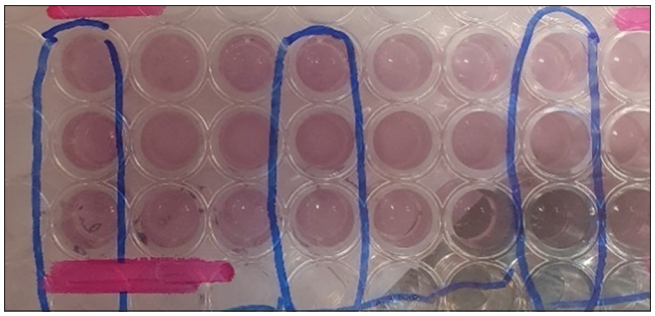

Figure 2: Indirect 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell assay. The samples are simple, glazed, and polished from left to right.

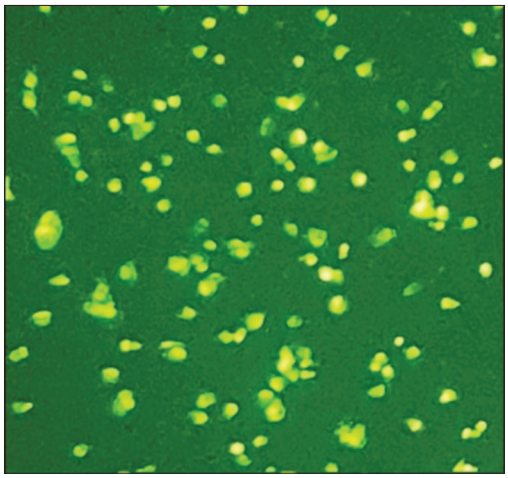

Figure 4: Fluorometric images of cells adhered to polished samples.

RESULTS

Table 1 presents the descriptive data of cell adhesion in different groups.

Based on the one-way ANOVA analysis (TOMHANE), the adhesion level in the polish group was significantly higher than in the simple group ($P = 0.001$). Furthermore, when comparing the glaze and polish groups, the polish group exhibited higher cell adhesion ($P = 0.002$). However, no significant changes were observed between the glaze and simple groups ($P = 0.968$).

Tables 2 and 3 indicate descriptive statistics of cell viability in direct and indirect MTT assay. According to the one-way ANOVA, no significant difference was found in cell viability between the polish and simple disk groups ($P = 0.111$). The glaze group showed significantly higher cell viability than the simple disk group ($P < 0.001$). Moreover, the glaze group exhibited significantly higher cell viability than the polish group ($P = 0.004$).

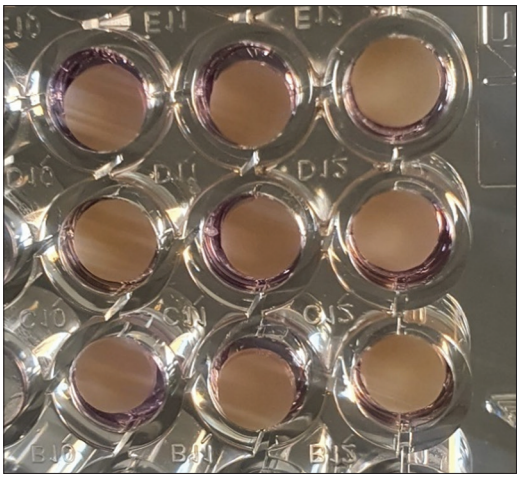

Figure 3: Direct 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell assay. The samples are simple, glazed, and polished from left to right.

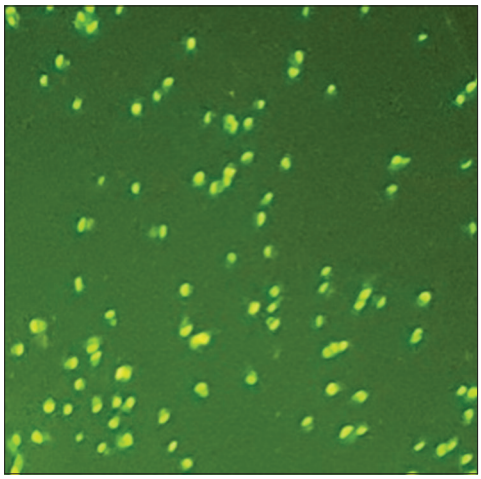

Figure 5: Fluorometric images of cells adhered to glazed samples.

DISCUSSION

Gingival growth and soft tissue integration around implants are crucial for stability and infection prevention. The surface properties of implant abutments affect the attachment and growth of HGFs, which is essential for adequate soft tissue integration.[14] Several surface preparation methods that can be applied to zirconia, altering their surface roughness, include airborne particle abrasion, rotary tool grinding, polishing, and glazing.[15] This study aimed to evaluate the differences between polishing and glazing regarding adhesion and viability of gingival fibroblast cells on zirconia. According to the results of this study, our initial hypothesis was rejected in both cases of the survival and adhesion of fibroblast cells. Based on the study's results, the polished zirconia

Table 1: Descriptive statistics of cell adhesion in three groups

Group	*n*	Mean	SD	95% CI for mean	Minimum	Maximum
S	6	330.00	37.34	290.81–369.19	288.00	393.00
G	6	336.83	11.14	325.14–348.53	327.00	357.00
P	6	483.33	52.71	428.01–538.65	426.00	559.00
Total	18	383.39	81.00	343.11–423.67	288.00	559.00

SD: Standard deviation; CI: Confidence interval

Table 2: Descriptive statistics of cell viability in direct 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Group	*n*	Mean	SD	95% CI for mean	Minimum	Maximum
S	6	96.04	3.01	92.88–99.19	92.38	100.61
P	6	100.31	4.69	95.39–105.23	94.21	105.19
G	6	108.08	2.04	105.94–110.22	105.19	110.67
Total	18	101.48	6.06	98.46–104.49	92.38	110.67

SD: Standard deviation; CI: Confidence interval

Table 3: Descriptive statistics of cell viability in indirect 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Group	*n*	Mean	SD	SE	95% CI for mean	Minimum	Maximum
Control	3	99.40	1.30	0.75	96.17–102.63	98.65	100.90
S	3	104.96	4.70	2.72	93.27–116.64	99.55	108.11
P	3	101.35	5.46	3.15	87.78–114.92	98.20	107.66
G	3	112.63	2.71	1.57	105.89–119.37	109.50	114.41
Total	12	104.58	6.24	1.80	100.62–108.55	98.20	114.41

SD: Standard deviation; SE: Standard error; CI: Confidence interval

surfaces showed significantly higher cell adhesion than the simple and glazed groups. Moreover, the glazed zirconia surfaces exhibited significantly higher cell viability than the control and polished groups.

Irving *et al*.[16] found that polishing significantly enhanced cell attachment and migration, with results nearly equivalent to laser processing. They concluded that polishing provides a cost-effective method for creating functional surfaces at the nanoscale, potentially enhancing cell adhesion and proliferation. In addition, they observed that cells growing on polished surfaces moved less freely, suggesting that the restriction on cell migration direction may reduce the distance of cellular movement. Hamilton *et al*.[17] also concluded that since the extracellular matrix is randomly organized in three-dimensional space within the body, it resembles a polished surface more closely than surfaces with a more uniform topography. Therefore, the direction of surface modifications may be more important than surface roughness in promoting cell adhesion. This proposed effect aligns with previous work by Biggs *et al*.[18] It can be inferred that the increased cell adhesion on the polished surfaces is likely due to better alignment of cells on these surfaces and reduced migration rather than increased wettability and surface energy.

Dal Piva *et al*.[19] aimed to investigate the effects of different finishing techniques on the surface characteristics, bacterial adhesion, and fibroblast survival of two monolithic ceramics. In their study, 92 zirconia blocks were fabricated and divided into two groups: polished and glazed. The survival of HGFs (FMM-1) was assessed using the MTT assay.

The results showed that both materials were cytotoxic to fibroblasts when subjected to polishing and glazing techniques, with cell survival ranging from 50% to 79%. However, the polished groups showed initial cytotoxicity, which decreased over time, suggesting that the release of substances during polishing might have contributed to this effect. Similar to the current study, their findings indicated increased cell survival on glazed surfaces compared to polished ones, which could be due to the protective barrier created by the glazed surface. The glazing process altered the chemical properties of the surface, enhancing cell attachment and proliferation by creating a more biocompatible environment. These findings align with previous research that emphasizes the role of surface modifications, such as glazing, in improving cellular survival on zirconia materials.[19-21]

Brunot-Gohin *et al*.[22] conducted a study to evaluate the biological response of surface changes, specifically comparing the effects of polishing and glazing on lithium disilicate dental ceramics. Their study assessed cell adhesion based on surface roughness and wettability. Their results showed that polishing and glazing did not significantly alter surface roughness, but the contact angle of water differed significantly between polished and glazed surfaces. Cell culture on these surfaces revealed that polished samples enhanced cell adhesion and proliferation compared to glazed samples. Their study attributed better cell adhesion on polished surfaces to improved wettability, noting that higher surface energy leads to better wettability.

This study highlights the distinct impact of surface preparation techniques on zirconia's biocompatibility, offering new insights into cell adhesion and viability differences between polished and glazed surfaces. However, the study's limitation of evaluating only

monolithic zirconia restricts its generalizability to other ceramics or materials. Future research could expand the scope by including different materials and varying preparation methods to better understand their influence on soft tissue integration and long-term implant success.

CONCLUSION

Within the limitations of this *in vitro* study, it could be concluded that gingival fibroblast adhesion is higher on polished zirconia compared to glazed and unmodified zirconia. On the other hand, fibroblast cell viability was greater on glazed zirconia discs than on the other types of samples used in this research. These findings suggest that the surface treatment of zirconia may influence its biocompatibility, which is clinically significant for optimizing gingival tissue integration in dental restorations.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial, in this article.

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