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Influence of acidic solutions on surface roughness of polished and glazed CAD-CAM restorative materials

Kübra Nur Tad[1], Ayhan Gürbüz[2] and Perihan Oyar[3*]

Abstract

Background The purpose of this in vitro study was to compare the surface roughness (Ra) changes of different dental ceramic materials with different compositions, which were applied two different surface treatments after exposure to acidic pH. The purpose of this in vitro study was to compare the Ra changes of different CAD-CAM materials with different compositions, which were applied two different surface treatments, after exposure to acidic pH.

Methods A total of the 168 samples (12 × 14 × 2 mm) were obtained from ceramic blocks (IPS e.max CAD (LDS)), GC Cerasmart (RNC-C), Lava Ultimate (RNC-L), and Vita Enamic (PIC). Half of each group was subjected to mechanical polishing, and the other half was glazed. After the initial Ra evaluations were made, the samples classified with 7 in each subgroup were kept in three different solutions (citric acid, Coca-Cola, and artificial saliva-control group). After 168 h, surface roughness values of the specimens were measured again.

Results In the RNC-C samples, varying surface treatments and exposure to various solutions did not produce a statistically significant difference. Different acidic solutions did not affect the Ra values of LDS and RNC-C ceramics. The percentage change in Ra values in the glazed samples of PIC exposed to Coca-Cola and RNC-L exposed to artificial saliva were higher than those applied mechanical polishing.

Conclusion The Ra values of RNC-C ceramics were not affected by both surface treatment and acid exposure. The percentage change in Ra values was highest in PIC ceramics. In general, glazed samples had larger Ra change values and higher percentage change in Ra values than manually polished ones.

Keywords Ceramics, Surface properties

*Correspondence:
Perihan Oyar
poyar73@gmail.com
[1]Specialist Prosthodontist, Ankara, Turkey
[2]Department of Prosthodontics, Faculty of Dentistry, Ankara University, Ankara, Turkey
[3]Department of Dental Prostheses Technology, Vocational School of Health Services, Hacettepe University, D Block, 3. Floor, Sihhiye, Ankara 06100, Turkey

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Background

The use of computer-aided design and computer-aided manufacturing (CAD-CAM) devices in today's dentistry is becoming more and more common clinically. CAD-CAM systems have developed very rapidly, and this development has also led to an increase in material diversity. Block materials have a great range of qualities, structures, and compositions, and each has distinct physical qualities [1]. Resin-ceramic polymer-based materials and resin nanoceramics were presented as substitutes for machinable blocks in CAD-CAM systems [2]. The main component of CAD/CAM resin-based ceramics is an organic phase, which is reinforced by nano- or nano-hybrid ceramic fillers within a highly cross-linked polymeric matrix [1, 2]. Lava Ultimate and Cerasmart are defined as resin nanoceramics. Lava Ultimate, a CAD/CAM resin-based ceramic material, which is *composed* of silica and zirconia nano-fillers in the form of scattered or aggregated particles and urethane dimethacrylate (UDMA) as the resin matrix [1]. Ultrafine glass particles are present in Cerasmart because of a highly crosslinked resin matrix [3]. Nanohybrid fillers, which are made of a polymeric matrix reinforced with ceramic fillers, are present in Cerasmart. The material known as polymer-infiltrated ceramic network (PICN) primarily consists of an inorganic phase [1]. It is made up of two continuous interpenetrating networks, one made of ceramic material and the other of the polymer, as a result of a polymer infiltrating a porous feldspar ceramic network [4]. The PICN material that is currently offered for sale is called Vita Enamic. This material was created by capillary action infiltrating a pre-sintered glass-ceramic network (86 weight%) that was conditioned by a coupling agent with 14 weight% triethylene glycol dimethacrylate (TEGDMA) [5–7]. These composite blocks have a resin matrix based on UDMA and scattered filler materials [7]. High pressure and temperature during production greatly enhances the mechanical qualities of these blocks [8].

Due to their differing structures, CAD/CAM materials may be contributing factors to a range of surface roughness values [8]. Furthermore, variations in the surface roughness values of the materials can be attributed to the organic matrix's structure, inorganic fillers, and the material's size, ratio, type, and distribution [9]. Surface roughness and post-polishing surface chemistry differ in resin-based composites, which are commercially available in a range of filler and resin structures with different filler shapes and sizes [9]. Furthermore, the polishing process eliminates superficial matrix-rich resin-based composite layers, creating a surface that differs from its unpolished counterpart both chemically and physically; as a result, the surface's physicochemical characteristics are significantly altered [10].

In general, finishing and polishing processes are applied for restorations produced with ceramics and CAD-CAM systems; there are two types: mechanical polishing and glaze application. Finishing and polishing of ceramic restorations containing CAD-CAM resin can be performed in one session with mechanical polishing without the need for glazing [1, 11]. It has generally been demonstrated that hand polishing produces a smoother surface than oven glazing and is a preferable method to reglazing a glazed surface that needs to be adjusted [12]. According to a recent study, CAD/CAM ceramics with manual polishing have smoother surfaces than feldspathic ceramics with glazing [13] This could have to do with the filler particles, which not only give the material better mechanical and physical qualities and shield the organic matrix from the force being applied to the restoration, but also have a direct impact on surface characteristics like gloss and smoothness [14]. Therefore, using an abrasive tool to remove the resin matrix and cut the relatively harder filler particles results in smoother polishing outcomes for resin-based dental materials [15]. Literature indicates that consumption of cola drinks and fruit juices has been rising recently [16, 17].

Citric acid is a common component of sour fruits (such as mango and pineapple), and cola is one of the most popular beverages with its low pH and citric, carbonic and phosphoric acid [18]. Acids, like citric acid, exist in water as an amalgam of hydrogen ions, acid anions (citrate), and an undissociated acid molecule. The crystal surfaces of dental porcelain are directly impacted by the hydrogen ion. Citrate anion can react with metal ions on the surface of dental porcelain under the influence of hydrogen ions, removing these ions from the glass surface. Because of this, acids like citric acid have two opposing effects and are extremely harmful to dental porcelain [19]. Therefore, in order to see the comprehensive effect of acidic solutions on dental materials, it is necessary to examine these materials over a long immersion time. Although there are some studies assessing the mechanical and physical properties of these newly released materials, there are very few studies examining the behavioral properties of solutions with different acidic pH that mimic the variability of the oral environment [21–23]. After being exposed to an acid solution for 45 and 91 h, it was discovered that resin matrix ceramic displayed statistically significant increases in surface roughness, while lithium disilicate did not exhibit any statistically significant changes [20]. The surface roughness of polished and glazed lithium silicate glass ceramics reinforced with zirconia was found to be significantly elevated by orange juice and cola. After being submerged in various liquids, the specimens' surface roughness was not significantly impacted by the type of surface finishing [21]. Low-pH soft drinks have been found to affect the volumetric wear of materials made of

Table 1 Dental ceramics and manufacturers

Materials	Class/terminology	Contents	Manufacturer
IPS e.max CAD (LDS)	Lithium disilicate ceramic (LDS)	SiO_2(57–80%), Li_2O(11–19%), K_2O(0–13%), P_2O_5(0–11%), ZrO_2(0–8%), ZnO(0–8%), Al_2O_3(0–5%), MgO(0–5%), Coloring oxides (0–8%)	Ivoclar Vivadent AG
GC Cerasmart (RNC-C)	Resin nanohybrid ceramic	71% Barium (300 nm) and silicate glass ceramics (20 nm) Bis-MEPP, UDMA, DMA	GC Dental Products
Vita Enamic (PIC)	Polymer Infilter Glass Ceramic Mesh Structure	86% Ceramic Structure 14% Polymer Structure	VITA Zahnfabrik
Lava Ultimate (RNC-L)	Resin nanohybrid ceramic	80% nanoceramic, 20% resin matrix	3 M ESPE

Table 2 Application of mechanical polishing process

Ceramics	Mechanical and Chemical Surface Polishing Process /Materials (Manufacturer)	Mechanical Polishing /kits
LDS	IPS Ivocolor Glaze GZ/Glaze (Ivoclar Vivadent) Instrumente Diapro polishing set/LDS (Polishing Set G&Z Instrumente)	GZ Instrumente Diapro Polishing Set (30 s mechanical polishing with brown disc and yellow disc- medium and fine grain)
RNC-C	GZ Diamond Flexible Radial Disk / Polishing Set (G&Z Instrumente)	GZ Diamond Flexible Radial Disk (30 s mechanical polishing with blue disc, pink disc, and yellow disc- thick medium and fine grain)
RNC-L	GZ Diamond Flexible Radial Disk / Polishing Set (G&Z Instrumente)	GZ Diamond Flexible Radial Disk (30 s mechanical polishing with blue disc, pink disc, and yellow disc- thick medium and fine grain)
PIC	Optiglaze color clear / Optiglaze color clear (GC Corporation)	GZ Diamond Flexible Radial Disk (30 s mechanical polishing with blue disc, pink disc, and yellow disc- thick medium and fine grain)

resin, but lithium disilicate has shown greater resistance to abrasive mechanisms [22]. Therefore, the purpose of the present study was to investigate the Ra of four different dental ceramic materials with polished and glazed surfaces before and after they are exposed to acidic solutions (Coca-Cola, artificial saliva, and citric acid) for 168 h [23], and to determine which acidic solution causes a rougher surface on glazed and polished ceramic surfaces. The first null hypothesis was that the acidic solutions would not affect the Ra of dental ceramic materials, and the second null hypothesis was that two different surface treatments would not affect percentage changes in Ra values of dental ceramic materials.

Methods

Preparation of samples

LDS, PIC, RNC-C, and RNC-L blocks were used in the study ($n = 7$). The blocks were cut using a Micracut 201 linear precision cutting device (Metkon, Bursa, Turkey) with dimensions of 12×14 mm and a thickness of 2 mm (± 0.1 mm) under water cooling. LDS samples were crystallized in a Programat P 510 furnace (Ivoclar Vivadent AG, Shaan, Liechtenstein) according to the manufacturer's instructions for complete crystallization. The blocks used and the manufacturers are shown in Table 1, and the glaze and polishing kits used in the finishing processes and the manufacturers are shown in Table 2. The 168 samples obtained were randomly divided into groups after being cleaned with distilled water for 15 min in an ultrasonic cleaning device (Whaledent Biosonic, Coltène/Whaledent Inc.). Of the samples obtained from ceramic blocks, half of each group (21 samples) was mechanically polished, and the other half was glazed.

Mechanical polishing process

The mechanical polishing process was carried out in the laboratory with the help of a micromotor at constant speed (8000 rpm) and for 30 s for each tire [24]. The polishing process was applied to a single surface of the samples by means of a mechanical polishing kit in accordance with the manufacturer's suggestions. Mechanical polishing was performed by the same researcher in all groups.

Glaze process

The glaze process was determined according to the block type in line with the recommendations of the manufacturer. IPS Ivocolor Glaze (Ivoclar Vivadent) in powder-liquid form was prepared according to company instructions and applied with a brush in a single layer on only one surface of the samples, and the Programat P 510 (Ivoclar Vivadent) Glazing process was performed in the oven in accordance with the manufacturer's instructions. Before the glaze process, the surfaces of the PIC, RNC-L and RNC-C samples were sandblasted for 10 s from a

Table 3 Solutions

Solutions	Manufacturer	Chemical composition	pH
Citric acid	Atsc chemistry	6 g citric acid in 100 ml distilled water	4.0
Coca-cola	Coca-cola	Water, sugar, carbondioxide, colorant (caramel), natural flavorings, acidity regulators (phosphoric acid, potassium citrate), preservative (sodium benzoate), caffeine	2.52
Artificial saliva (control group)	was done in a laboratory environment	5 mmol HEPES, 2.5 mmol $CaCl_2$, 0.05 mmol $ZnCl_2$, 0.68 mmol/L KH_2PO_4, 30 mmol/L KCl and 120 mm NaCl	7

distance of 10 mm using 50 µm Al_2O_3 powder under two bar pressure with a sandblasting device (Heraeus Combilabor CL-FSG 3, Germany) in accordance with the manufacturer's recommendations. After sandblasting, the samples were washed with air-water spray for 10 s, cleaned in pure water in an ultrasonic bath for 5 min, and then air-dried. After sandblasting, GC Optiglaze (GC Corporation) material was applied to each sample surface in accordance with the manufacturer's suggestions. First, GC G-Multi Primer (GC Corporation) was applied for 30 s and air dried. The second stage of glazing, Optiglaze Color Clear, was applied to the sandblasted and silaned surfaces in a thin layer with the help of a brush and polymerized with LED (SDI radii) light devices for 40 s [24].

Surface roughness

A profilometer device (Perthometer M2, Mahr) was used for Ra measurements of the samples. After mechanical polishing or glaze processes were applied to the sample surfaces, the initial Ra values of all samples were recorded. Parameters of the device, measuring length (Lt); 5.6 mm, cutoff value (λc); 0.25, sampling length (n); It is set to be 5. The instrument was calibrated before measurement of each group. Each specimen's average profile depth (Ra), maximum profile deviation (Rz), and Rmax were measured. Each measurement was taken three times, and the average value was recorded. Ra (µm) measures average roughness by identifying maximum peak-to-valley distances on a surface profile. The average Ra value was calculated by taking the averages of the obtained data (Ra, Rz, Rmax) with 3 parallel measurements from each sample in the center of the samples.

After the initial Ra measurements were made, the samples were kept in distilled water at 37 °C for 24 h. The solutions used in the study and their pH are shown in Table 3. Artificial saliva solution with a pH of 7 was prepared using 5 mmol HEPES, 2.5 mmol $CaCl_2$, 0.05 mmol $ZnCl_2$, 0.68 mmol/L KH_2PO_4, 30 mmol/L KCl, and 120 mm NaCl [25]. The artificial saliva group was used as the control group. The citric acid solution was prepared with a pH value of 4 by dissolving 6 g of citric acid in 100 ml of distilled water [19]. After three different solutions were prepared, the samples with different surface treatments were classified with 7 samples in each subgroup ($n=7$), in glass containers and in an oven at 37 ± 1°C (Köttermann Labortechnik) for 168 h. Citric acid, coke (Coca-Cola Company), and artificial saliva solutions. The amount of solution used was arranged to 5 ml for each sample, and the solutions were changed every 24 h during the study. The samples were taken out of the solutions after 168 h, cleaned with distilled water for 10 s, dried, and the Ra readings were taken once again [23].

Table 4 Ra (µm) values in different solutions according to two different surface treatments of ceramics

Ceramics	Solution	Mechanical Polishing	Glazing
LDS	Citric acid	0.20 ± 0.06^{bA}	0.39 ± 0.07^{aA}
	Cola-cola	0.23 ± 0.04^{bA}	0.37 ± 0.10^{aA}
	Artificial saliva	0.20 ± 0.03^{bA}	0.30 ± 0.08^{aA}
RNC-C	Citric acid	0.28 ± 0.08^{aA}	0.25 ± 0.12^{aA}
	Cola-cola	0.25 ± 0.06^{aA}	0.32 ± 0.12^{aA}
	Artificial saliva	0.21 ± 0.05^{aA}	0.23 ± 0.06^{aA}
PIC	Citric acid	0.35 ± 0.05^{bAB}	0.50 ± 0.13^{aA}
	Cola-cola	0.37 ± 0.05^{aA}	0.49 ± 0.16^{aA}
	Artificial saliva	0.30 ± 0.04^{aB}	0.24 ± 0.05^{bB}
RNC-L	Citric acid	0.29 ± 0.05^{aA}	0.30 ± 0.07^{aAB}
	Cola-cola	0.28 ± 0.05^{bA}	0.41 ± 0.13^{aA}
	Artificial saliva	0.21 ± 0.04^{aB}	0.22 ± 0.03^{aB}

Different lowercase letters in rows shows the difference between surface treatments (a, b,ab), Different capital letters in columns shows the difference between solutions (A, B,AB) ($P<.05$)

Statistical analysis

The effect of the interaction of ceramic, solution, and surface treatment on the Ra was evaluated with the three-way ANOVA test. The Tukey HSD test was used in post hoc analyses, $P<.05$ was accepted for significance.

The Mann-Whitney U test was used to evaluate the surface roughness change values in the samples in two-group comparisons. For three-group comparisons, the Kruskall-Wallis H test was used. 0.05 was used as the significance level.

Results

A comparison of the Ra of the ceramics with two different surface treatments and exposure to different solutions is given in Table 4.

There was no statistically significant difference in the Ra of the LDS and RNC-C between different acid

Table 5 Ra change values (percentages) in different solutions according to two different surface treatments of ceramics

Materials	Solutions	Surface treatments	*n*	Mean	Median	Min	Max	SE	*P* value
LDS	Citric acid	Mechanical Polishing	7	12.42	24.69	-32.51	59.81	36.64	0.097
		Glazing	7	63.94	56.77	-37.87	189.13	67.04	
	Cola-cola	Mechanical Polishing	7	-1.18	-1.06	-29.44	49.45	25.65	0.165
		Glazing	7	19.44	30.95	-36.33	45.97	28.77	
	Artificial saliva	Mechanical Polishing	7	54.21	53.37	-48.81	160.77	73.50	0.383
		Glazing	7	26.04	20.74	-12.40	87.12	30.56	
RNC-C	Citric acid	Mechanical Polishing	7	29.09	12.32	-19.21	93.44	43.21	0.209
		Glazing	7	79.54	46.67	-2.67	286.61	99.84	
	Cola-cola	Mechanical Polishing	7	42.39	30.67	-2.67	88.39	32.32	0.71
		Glazing	7	73.93	71.53	-50.60	202.40	91.06	
	Artificial saliva	Mechanical Polishing	7	12.54	13.95	-38.44	81.03	41.14	0.165
		Glazing	7	163.67	81.16	-30.66	649.82	234.86	
PIC	Citric acid	Mechanical Polishing	7	15.19	6.02	-1.76	52.00	18.51	0.073
		Glazing	7	94.41	82.06	1.53	260.54	89.99	
	Cola-cola	Mechanical Polishing	7	21.73	14.34	7.65	46.88	14.75	0.002*
		Glazing	7	225.43	277.65	31.14	401.57	150.17	
	Artificial saliva	Mechanical Polishing	7	13.89	7.37	-3.55	55.79	20.21	0.71
		Glazing	7	91.12	79.20	-34.82	390.67	149.41	
RNC-L	Citric acid	Mechanical Polishing	7	38.10	28.44	-11.11	119.54	48.84	0.902
		Glazing	7	35.11	6.84	-34.65	197.55	76.38	
	Cola-cola	Mechanical Polishing	7	44.23	46.85	-8.78	93.68	34.07	0.165
		Glazing	7	105.80	105.00	-24.36	325.61	111.08	
	Artificial saliva	Mechanical Polishing	7	36.23	31.41	-12.67	103.07	40.17	0.026*
		Glazing	7	111.88	116.13	14.86	195.93	59.13	

* $P<.05$

solutions ($P>.05$). Different surface treatments caused a statistically significant variation in LDS, PIC, and RNC-L ($P<.05$). (Table 4). There were no statistically significant variations in the Ra of the RNC-C caused by either surface treatments or distinct acidic solutions ($P>.05$).

The percentage changes in Ra values of the LDS and RNC-C samples were not statistically different after mechanical polishing and glazing; however, there were significant differences in the PIC exposed to Coca-Cola solution and the RNC-L exposed to artificial saliva ($P>.05$). (Table 5).

The glazed PIC samples that were exposed to Coca-Cola solution showed the highest percentage Ra change values.

Following surface treatments, the PIC samples had the highest Ra values of all the seramics.

Discussion

In the present study, Ra values of 4 different dental ceramic materials (LDS, RNC-C, RNC-L and, PIC) with different compositions, which were applied to two different surface treatments, mechanical polishing and glaze, after 168 h [23] exposed to different acidic solutions, were evaluated, and exposure to different acidic solutions caused a statistically significant differences in the Ra values of PIC and RNC-L. Therefore the first null hypothesis was rejected. The present study also found that different surface treatments (glazed and polished surfaces) had an effect on the percentage changes in Ra values of dental ceramic specimens exposed to acidic solutions. Therefore, the second null hypothesis was partially accepted. The tested polishing techniques had an impact on the surface roughness. Reports claim that glazed surfaces are not always the smoothest [26, 27]. Gathering the smoothest restoration surface is always the main goal of surface treatments for dental restorations in order to achieve ceramic restoration durability and long-term clinical success [28].

The size, ratio, type, and distribution of the inorganic fillers in the material, as well as the kind of resin matrix, may affect how the Ra values of the materials differ from one another [29]. GC Cerasmart, 71% inorganic fraction and 29% polymer by weight; Lava Ultimate, 80% by weight inorganic fractions and 20% polymer; Vita Enamic contains 86% inorganic part and 14% polymer [29]. In the present study, among resin-based ceramics, the RNC-C ceramic with the highest polymer content was not affected by surface treatment and acid exposure. PIC ceramics had the highest Ra values and the largest percentage change in Ra values. PIC ceramics can be thought of as the ceramics whose surface roughness is most impacted because they have the highest inorganic substance content among resin ceramics.

According to studies by Trussi et al. [30] and Marcela et al. [31], exposure to acid may hydrolyze methacrylate ester bonds, which would degrade the polymer matrix of resin composites. When resin composites are exposed to acids, the material's water sorption may increase, causing the resin matrix to expand and pore spaces to form between the molecules. This might cause the inorganic fillers to leach out, causing surface roughness and general material degradation [32].

In the present study, the Ra values of PIC with mechanically pollishing and glazing were higher than those of other ceramics. Statistically, the percentage change in Ra values were in PIC and RNC-L samples. This may be explained by the fact that, among resin ceramics, PIC and RNC-L have the highest inorganic content.

Although the same polishing process was applied to the materials, there were still noticeable variances in their roughness before and after acid contact [33]. In the current study, Ra values of LDS ceramic samples were found to be lower than other resin-based ceramics. The results of the present study and the studies stated above can be explained by the fact that the material structure LDS does not contain any resin matrix content and that alkali oxides are much more stable in the crystal phase of ceramics than in the glass phase.

The potential of the acid to soften the polymer matrix was also addressed in other studies [34, 35] that examined the impact of simulated gastric juice or citric acid on various composite resins. A low-viscosity copolymer is infused throughout the feldspathic ceramic matrix (86% weight) in Vita Enamic (urethane dimethacrylate and triethylene glycol dimethacrylate) [34]. The dissolution of the ceramic component, which makes up the majority of this material, may have contributed to the reduction in its roughness.

The three acids that are most frequently found in Coca-Cola are citric, carbonic, and phosphoric [36]. The chelating impact of these acids can result in ionic dissolution, release of alkaline lithium and aluminum ions, and deterioration of the ceramic silicate network, all of which can be hazardous [37]. For all acid solutions in the current investigation, the Ra of the glazed LDS group was found to be statistically significantly higher than that of the mechanical polished group ($P<.05$). In RNC-C, there was no statistically significant difference in terms of Ra between mechanical polishing and glazing processes ($P>.05$).

In the current study, the Ra of PIC ceramic with glaze, which was exposed to citric acid, and RNC-L, which was exposed to Coca-cola, was found to be significantly higher than the mechanically polished groups. As a result, higher Ra values were found in the glazed samples. When evaluated from this point of view, it can be said that mechanical polishing is more advantageous.

According to the findings obtained from the present study, chemical composition and finishing techniques (surface treatment) are important in the selection of ceramic materials to be used in restoration for people who consume acidic foods and beverages a lot. The surfaces of glazed ceramics are more affected by acidic solutions.

According to the results of this present study, dentists may be advised to prefer GC Cerasmart ceramic (resin nanohybrid ceramic) and mechanical polishing processes when selecting ceramic restorations for patients who consume excessive acidic foods.

Limitations of the present study include that the complexity of the oral environment, including changes in ambient temperature, the role of saliva's buffering system, the dilution effect on beverages and foods, and the pH level were not taken into account. The scope of the present study is restricted to three distinct approaches and four distinct dental ceramic materials. It is recommended to conduct additional research to examine the effects of acidic substances with various pH levels on various ceramic materials. The measurement of Ra was made with a mechanical profilometer. With the use of optical profilometers or 3D instruments such as SEM and atomic force microscopy, highly detailed data can be obtained. The lack of simulation of chewing forces is considered a limitation of this study, and if chewing is simulated, the roughness of the tested materials may be affected. In the present study, only Ra was evaluated. The effect of acidic solutions on the mechanical properties of the material, such as surface hardness, optical properties, fracture strength, and abrasion resistance, can also be investigated in future studies. The duration of exposure to the substances differs slightly from the duration of contact with the oral environment (i.e., they are not exposed in the mouth directly for the entire duration). This situation could not be fully imitated. In order to reach definite conclusions about the effect of acidic pH on the physical, chemical, and mechanical properties of dental ceramics, it needs more in vitro studies, and these should be supported by further studies in vivo.

Conclusions

The following conclusions were reached in light of the findings of this in vitro study:

- The Ra values of RNC-C ceramics were not affected by both surface treatment and acid exposure.
- The percentage change in Ra values was highest in PIC ceramics.
- In general, glazed samples had higher Ra values and, larger percentage change in Ra values than manually polished ones.

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Author contributions
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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent
For this type of study, formal consent is not required.

Competing interests
The authors declare no competing interests.

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