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Evaluating the effect of repeated use of milling burs on surface roughness and adaptation of digitally fabricated ceramic veneers



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ABSTRACT

Objectives: This study aimed to evaluate how repeated use of milling diamond burs with different coarseness affects surface roughness, and marginal and internal adaptation of CAD/CAM veneers. *Methods:* Forty leucite-reinforced glass-ceramic veneers were milled in 2 groups based on the milling mode (with fine or extra-fine bur sets). In each group, every 10 veneers were milled with a new bur set. All veneers were cemented to bovine teeth and then polished. Labial surface roughness was measured before cementation, and after polishing. Marginal and internal discrepancies were measured using a field emission scanning electron microscope. Three-way and two-way mixed repeated measures ANOVA were applied to assess changes in surface roughness values of veneers and discrepancy values, respectively. The Bonferroni correction was applied for multiple comparisons. *Results:* Repeated use of a milling diamond bur set had a significant effect on surface roughness of the veneers (P < .001). Mean surface roughness of the fine milling mode was significantly higher in comparison to that of extra-fine mode before (P = .002) and after (P = .01) polishing. After polishing a significant decrease in surface roughness

mode before (P = .002) and after (P = .01) polishing. After polishing a significant decrease in surface roughness occurred in fine (P = .02), but not in extra-fine milling mode (P = .99). Repeated use of milling burs significantly affected marginal and internal adaptation between some repeated uses.

Conclusions: Marginal and internal adaptation were significantly affected by repeated use of milling diamond burs up to 10 times between some repeated uses. However, no specific pattern could be established.

Clinical significance: Repeated use of milling burs could affect surface roughness, surface microcracks, critical defects, and adaptation of CAD/CAM restorations. Therefore, it plays a major role in clinical success of the restorations.

1. Introduction

The computer-aided design/computer-aided manufacturing (CAD/ CAM) systems have been improved dramatically during the last decades [1]. CAD/CAM technology allows a completely digital workflow, from impression to final framework, with clinical reliability [2] and good patients feedback [3]. The chairside use of new CAD/CAM materials has made many treatments feasible in one visit [4, 5]. Single visit dentistry offers fundamental advantages of time efficiency and predictability [6, 7], and eliminating interim restorations with its disadvantages [8]. Ceramic veneers are among the various restorations that are designed and milled by these chair side systems. Successful ceramic veneers rely on factors such as desirable bonding, mechanical strength, acceptable surface roughness, and marginal and internal adaptation [9, 10, 11]. Poor adaptation might result in excessive exposure of resin cement to oral environment, microleakage, recurrent caries, marginal discoloration, and fracture of cemented veneers [12, 13].

The CAD/CAM restoration adaptation could be affected by several parameters during scanning, designing, and milling [14, 15]. Diamond burs are usually used for milling of ceramic restorations in chair side milling machines. The quality of these burs affects surface roughness, surface microcracks, and critical defects of milled ceramics [16, 17].

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These defects on the intaglio surface of restorations act as stress concentrators and could induce crack propagation [18, 19]. In laboratory made restorations, this roughness on the outer surface is decreased by glazing. However, in chair side process, dentists could polish the restorations in order to decrease the number of appointments.

If it is possible to mill high-quality restoration with repeated use of the same diamond bur without setting a new one, the fabrication cost will be reduced efficiently. However, the life cycle of the diamond burs is likely to decline when they are used repeatedly [18, 20]. However, not much is known about defining the number of milling cycles in which the diamond burs could be securely used without needing to be replaced [21, 22, 23]. Moreover, there is no study indicating this number could be altered by the type of restoration and the material chosen. Roperto et al. [22] claimed that CEREC CAD/CAM milling burs could endure a larger number of milling times than determined by the manufacturer without reducing the restoration quality. However, they have only assessed the bur wear, not the restoration quality.

Thus, the purpose of this in vitro study was to evaluate how repeated reuse of milling diamond burs with different coarseness affected surface roughness, and marginal and internal adaptation of leucite-reinforced glass-ceramic CAD/CAM veneers. The null hypothesis was that repeated use of milling burs couldn't change surface roughness, and marginal and internal adaptation of the veneers made with two milling mode.

2. Methods and materials

In this study, approved by the Research Ethics Committee, School of Dentistry, Tehran University of Medical Sciences, under the approval ID: IR.TUMS.VCR.REC.1398.444, forty recently extracted bovine mandibular incisors were used. They were stored in a 10% formalin solution for 3 days. Calculus deposits and soft tissues were removed from the teeth with a scaler and cleaned with a bristle brush and non-fluoridated flour of pumice. The teeth were then preserved in 1.0% Chloramine T tri-hydrate [24] (Merck Schuchardt OHG, Hohenbrunn, Germany) for 7 days and in normal saline (9% NaCl) throughout the study. Teeth were mounted in additional silicon (Swiss TEC Hydro Xtreme, Coltène/Whaledent AG, Altstätten, Switzerland) up to 2 mm below the cementoenamel junctions (CEJ), and were scanned with intraoral scanner (CEREC Omnicam, Sirona Dental Systems, Bensheim, Germany) before preparation. Then,



Figure 1. Flowchart of study design. A, fine group. B, extra-fine group.

they were prepared to receive ceramic veneers. A .3 mm deep long chamfer finishing line was prepared 1 mm above the CEJ and extended to half of the proximal surfaces.

Prepared teeth were scanned with CEREC Omnicam intraoral scanner. The margin was defined manually whenever the software was unable to detect it automatically. The veneers were designed in CAD software (CEREC premium 4.0 software, Dentsply Sirona, Bensheim, Germany) using initial scans before preparation and a biogeneric copy design option. Veneer thickness was set to be minimum .5 mm [25], and reduction was done wherever it was thicker. Cement space was set to 40 μ m [26]. Then, they were randomly divided into 2 group based on the milling mode (Fine and extra-fine diamond burs) in milling machine (CEREC MC XL, Sirona Dental Systems, Bensheim, Germany). Leucite-reinforced glass-ceramic blocks (IPS Empress CAD, Ivoclar Vivadent, Schaan, Liechtenstein) were used to mill veneers in the milling machine (one block per specimen). In each group (fine and extra-fine), every 10 specimens were milled with a new bur set. Thus, a total number of 4 new bur sets were used (Figure 1).

External surface roughness (Ra) was measured using a contact type surface profilometer (Nano Pajuhan RAGA co, Iran) after milling. Three consecutive measurements of the veneers were taken from different regions (one central, one right, and one left) with a cutoff of .25, and the arithmetic mean roughness (Ra) was obtained [27].

After try-in and approval of best fit, the intaglio surface of each veneer was first conditioned with 9.5% hydrofluoric acid (Porcelain Etchant, Bisco Inc., Schaumburg, IL, USA) for 60 s, rinsed with water, dried with oil-free air, and then treated with one coat of silane coupling agent (Bis-Silane; Bisco) according to the recommendation of manufacture. The teeth were treated with 37% phosphoric acid (Ultradent Products Inc., South Jordan, UT, USA) for 15 s, washed with water, and dried for 10 s. Two coats of a universal adhesive (All-Bond Universal, Bisco Inc., Schaumburg, IL, USA) was painted on the teeth, air dried for 10 s, and then light cured for 10 s. A very thin coat of a HEMA-free unfilled resin (Porcelain Bonding Resin, Bisco Inc., Schaumburg, IL, USA) was applied on the intaglio surface of veneers and subsequently thinned with air. Each veneer was lined with adequate amount of resin cement (Choice 2, Bisco Inc., Schaumburg, IL, USA) and gently seated on its corresponding tooth. A clean glass slide was placed on top of a stainless-steel jig and pressed with a 9.8N load for 20 s to attain a uniform cement thickness. Excess cement was removed with a disposable microbrush moistened with bonding agent. The cement was light cured for 40 s per side.

The margins were finished with fine finishing and polishing rubber rotary instruments. Veneers were polished with a polishing kit (Optrafine kit, Ivoclar Vivadent AG, Schaan, Liechtenstein). "Light blue" instrument was used as the pre-polishing step; afterwards polishing was done with "dark blue" instrument. Then polishing paste was used to have a highgloss polished surface. All polishing procedures were performed by single operator using moderate pressure for 15 s for each rubber wheel or disc. Labial surface roughness of the veneers were measured after polishing again.

The teeth were put in prefabricated metal molds and filled with clear polyester resin (Polynt Composites USA, Inc., Bergamo, Italy). After polyester polymerization, specimens were sectioned with a diamondcoated disk (Mecatome T201A, Technimeta, Persi, Grenoble, France) under copious water irrigation in 3 lines in incisocervical direction parallel to tooth long axis. The sectioned specimens were examined with a field emission scanning electron microscope (Nova NanoSEM 450, FEI, Thermo Fisher Scientific, Waltham, MA, USA) at ×200 magnification (Figure 2). The thickness of the resin cement was measured at 9 points (6 marginal and 3 internal areas). To investigate marginal adaptation, two variables of marginal discrepancy (MD) and absolute marginal discrepancy (AMD) were measured. AMD is the distance from the internal edge of the coping margin to the cavosurface angle of preparation finish line and was measured at the cervical margin. MD is the vertical distance from the internal surface of the coping to the margin of the preparation [28] and was measured at cervical and incisal margins. For each veneer,



Figure 2. Field emission scanning electron microscope image analysis of a sectioned specimen at $\times 200$ magnification.

6 marginal measurements were at mesioincisal, mesiogingival, midincisal, midgingival, distoincisal, and distogingival areas. The internal discrepancy (ID) was also defined as the perpendicular distance from the internal surface of the restoration to the axial wall of the preparation [28]. For each veneer, 3 internal measurements at middle of incisocervical distance in the 3 sections (MID, M, and D) were also made (Figure 3).

2.1. Statistical methods

Data analysis were performed using the statistical softwares SPSS 18.0.0. (SPSS Inc., IL, USA) and MedCalc 19.2.1. (MedCalc Software, Mariakerke, Belgium) P values less than 0.05 were considered statistically significant. The normality assumption was examined using the Shapiro-Wilk test. A three-way mixed repeated measures ANOVA with 2 within-subjects factors (polishing and repeated use) and one between-subjects factor (milling mode) was applied to assess changes in surface roughness values of veneers based on the main effects, two-way interactions, and a three-way interaction. Polishing status had 2 levels (before polishing, after polishing). The two different milling modes were "fine" and "extra-fine". These 2 milling modes reflect the 2 levels of the "between-subjects" factor. The Bonferroni correction was applied as posthoc test in terms of type I error control for multiple comparisons.

In addition, repeated measures ANOVA with one within-subjects factor (repeated use) and one between-subjects factor (milling mode) was applied to examine changes in secondary outcomes (discrepancies) based on the main effects, and a two-way interaction effect. Repeated measures ANOVA with one within-subjects factor (repeated use) was conducted for each milling mode, separately, when needed.

3. Results

3.1. Surface roughness

Mean surface roughness values before and after polishing for both milling modes during repeated use of the burs is shown in Figure 4.



Figure 3. A schematic picture showing AMD, MD and ID.

The results of three-way mixed repeated measures ANOVA are summarized in Table 1. There were significant main effects of "repeated use" for both fine (P = .001) and extra-fine milling modes (P < .001). The Bonferroni adjusted pairwise comparisons for the significant main effect of "repeated use" showed that first use of fine burs results in significantly less roughness compared to subsequently using of fine and extra-fine burs (P < .05). The mean surface roughness was significantly lower in extra-fine milling mode compared to that of fine milling mode before (P = .002) and after polishing (P = .01). For fine milling mode, the mean surface roughness decreased significantly after polishing compared to that of before polishing (P = .02). For extra-fine milling mode, this parameter did not differ significantly after polishing (Figure 5). The Bonferroni adjusted pairwise comparisons for the significant main effect of "Polishing" showed significant difference between 1^{st} and 5^{th} (P = .029), and 1^{st} and 9^{th} (P = .048) use of fine burs, and also 1st and 7th use of extra-fine burs (P = .042).

3.2. Discrepancies

Discrepancy values in both milling modes during repeated use of the burs are summarized in Table 2. Repeated measures ANOVA with one within-subjects factor (repeated use) and one between-subjects factor (milling mode) was conducted (Table 3). No specific pattern for the differences between the repeated uses was observed (Figure 6). Irrespective of the milling mode and number of repeated use, the mean values reported were within a range of $60.99-219.37 \mu m$ for cervical AMD, $53.85-184.32 \mu m$ for cervical MD, $109.02-305.63 \mu m$ for incisal MD, and $68.04-181.41 \mu m$ for ID. The minimum and maximum values were observed in cervical MD of 6^{th} , and incisal MD of 10^{th} use in extra fine mode, respectively.

4. Discussion

According to the results of the present in vitro study, the null hypothesis was partially rejected. It was recognized that repeated use of a



Figure 4. Mean surface roughness and standard error values (µm) before and after polishing in both milling modes during repeated use of the burs. A, fine group. B, extra-fine group.

Table 1. Summarized results of repeated measure ANOVA for surface roughness.

Surface roughness outcome	Summarized results
"Repeated use \times polishing status \times milling mode" interaction effect	F(9,90) = .405, p = .93
"Repeated use \times polishing status" interaction effect	F(9,90) = 1.325, p = .24
"Repeated use \times milling mode" interaction effect	F(3.281,32.809) = .433, p = .75
"Polishing status \times milling mode" interaction effect	F(1,10) = 6.141, p = .03 sig
Results for each milling mode [#]	
Fine	
"Polishing status \times repeated use" interaction effect	(F(9,45) = .864, p = .56)
Main effect of "polishing status"	(F(1,5) = 12.036, p = .02) sig
Main effect of "repeated use"	(F(9,45) = 4.005, p = .001) sig
Extra fine	
"Polishing status \times repeated use" interaction effect	(F(9,45) = .866, p = .56)
Main effect of "polishing status"	(F(1,5) = .000, p = .99)
Main effect of "repeated use"	(F(9,45) = 4.640, p < .001) sig
Results for each polishing status	
Before polishing	
Main effect of "milling mode"	(F(1,10) = 16.47, p = .002) sig
After polishing	
Main effect of "milling mode"	(F(1,10) = 11.347, p = .01) sig
Main effect of "repeated use"	
	(F(3.281, 32.809) = 8.277, p < .001)

Abbreviations: F, F-value; p, p-value; RM-ANOVA, Repeated Measure ANOVA.

[#] Since there was a significant "polishing status \times milling mode" interaction effect, a repeated measures ANOVA with two within-subjects factor (polishing and repeated use) was conducted for each milling mode, separately.

milling diamond bur set had a significant effect on surface roughness of leucite-reinforced glass-ceramic CAD/CAM veneers. Even though these findings were heterogeneous, and pairwise comparisons did not follow a definite trend, roughness was specifically increased after the first use of the bur. Nevertheless, Surface roughness values even before polishing (Ra < 1.2 μ m) were in the range of the mean Ra values of glazed or polished ceramics [29, 30]. So, it is suggested to evaluate the effect of a more number of cycles on surface roughness. In addition, repeated use of burs up to 10 times displayed a significant effect on marginal and internal

accuracies between some repeated uses. However, no specific pattern was seen.

Contrary to surface roughness, adaptation was not affected by the milling mode. Surface roughness before polishing was significantly higher in fine milling mode in comparison with extra-fine. A previous study by Lebon et al. [21] also showed a quasilinear correlation between surface roughness of a milled restoration and diamond grit size. Fortunately, the polishing procedure could easily compensate it, resulted in significant decrease of surface roughness with fine milling mode.



Figure 5. Mean surface roughness and standard error values (µm) before and after polishing in both milling modes.

Reuse	Cervical marginal discrepancy		Cervical absolute marginal discrepancy		Incisal marginal discrepancy		Internal discrepancy	
	Fine(n = 6)	Extra fine $(n = 6)$	Fine (n = 6)	Extra fine $(n = 6)$	Fine (n = 6)	Extra fine $(n = 6)$	Fine(n = 6)	Extra fine $(n = 6)$
First use	174.90 ± 54.70	121.70 ± 48.62	167.73 ± 91.18	136.62 ± 46.41	262.75 ± 59.47	170.21 ± 111.31	132.69 ± 62.41	147.62 ± 48.93
Second use	84.00 ± 30.50	158.24 ± 37.76	$\textbf{92.47} \pm \textbf{33.70}$	179.05 ± 66.89	159.84 ± 45.68	$\textbf{203.64} \pm \textbf{99.64}$	$\textbf{87.76} \pm \textbf{24.02}$	118.00 ± 40.13
Third use	127.73 ± 40.71	$\textbf{70.22} \pm \textbf{9.07}$	136.17 ± 45.88	84.25 ± 29.87	187.18 ± 25.70	112.52 ± 39.92	117.24 ± 32.74	68.04 ± 38.84
Forth use	102.78 ± 33.68	115.46 ± 20.46	101.15 ± 23.56	128.46 ± 32.73	175.27 ± 95.47	198.46 ± 61.56	102.43 ± 27.58	$\textbf{99.37} \pm \textbf{37.68}$
Fifth use	167.31 ± 60.82	$\textbf{86.06} \pm \textbf{34.49}$	193.91 ± 79.88	$\textbf{96.87} \pm \textbf{48.02}$	185.34 ± 85.71	122.29 ± 31.54	158.19 ± 11.14	$\textbf{97.40} \pm \textbf{28.10}$
Sixth use	113.54 ± 55.44	53.85 ± 18.50	128.10 ± 68.06	$\textbf{60.99} \pm \textbf{17.15}$	109.02 ± 54.22	126.72 ± 36.86	89.24 ± 12.87	128.76 ± 55.52
Seventh use	91.04 ± 24.82	108.66 ± 31.44	$\textbf{86.07} \pm \textbf{23.86}$	131.48 ± 46.13	115.37 ± 57.11	226.55 ± 18.78	$\textbf{95.92} \pm \textbf{22.43}$	114.75 ± 54.20
Eight use	125.97 ± 69.08	136.70 ± 64.36	126.81 ± 54.70	148.38 ± 38.39	179.51 ± 47.81	162.10 ± 40.87	$\textbf{90.75} \pm \textbf{51.78}$	136.38 ± 57.91
Ninth use	102.33 ± 45.52	61.98 ± 27.76	152.84 ± 52.89	$\textbf{70.63} \pm \textbf{50.50}$	155.65 ± 24.52	184.03 ± 146.64	181.41 ± 40.35	143.28 ± 15.75
Tenth use	$\textbf{75.18} \pm \textbf{42.55}$	184.32 ± 56.54	81.99 ± 26.91	219.37 ± 15.76	222.15 ± 54.01	305.63 ± 25.64	157.18 ± 67.11	101.92 ± 38.28
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Table 2. Discrepancy values (µm) in both milling modes during repeated use of the burs.

Data are expressed as mean \pm SD.

Although surface roughness did not significantly change after polishing in extra-fine milling mode, it was still significantly lower than that of fine milling mode (Figure 4). Concerns about surface roughness are due to its high impact on clinical factors like dental plaque accumulation and esthetics [31]. Nevertheless, Surface roughness values found in this study before polishing (Ra < 1.2 μ m) were mostly comparable to the mean Ra values of glazed or polished ceramics [29, 30]. That is why these roughness values could not affect discrepancy values. In other words, although surface roughness was higher in fine group, marginal and internal adaptation did not significantly differ between these two groups. It should also note that in spite of standard roughness studies, in this study Ra values were measured in a curved surface, which made us perform it in very small areas of each specimen, resulting in relatively large standard error values (Figure 3).

Tomita et al. [32] declared that machining accuracy of ceramic veneers was not altered by the number of bur use even up to 51 times. However, photo observation showed chip marks at cervical contour from 21st to 51st crowns. This may be due to increasing diamond grain loss caused increased surface roughness [33]. Madruga et al. [23] evidenced

Several investigators [35] have evaluated marginal adaptation values but still there is no general consensus on a clinically acceptable marginal fit. Some considered marginal opening less than 75 μ m [14, 15], 120 μ m [36], or 150 μ m [37] as clinically acceptable. While others have declared a range between 7.5 μ m and 206.3 μ m [38, 39]. This extended range could be attributed to many factors like different adaptation definitions, measurement methods, measurement locations, restoration and

Table 3. Summarized results of repeated measure ANOVA for discrepancy outco

Tuble 5. Summarized results of repeated measure involvition discrepancy outcomes.	
Discrepancy outcome	Summarized results
Cervical marginal discrepancy	
"Repeated use \times milling mode" interaction effect	F(3.515,35.148) = 7.580, p < .0001
Results for each milling mode [#]	
Fine: "Repeated use" main effect	F(9,45) = 3.650, p = 0.002
Extra fine: "Repeated use" main effect	F(9,45) = 8.439, p < .0001
Cervical absolute marginal discrepancy	
"Repeated use \times milling mode" interaction effect	F(3.523,35.232) = 7.810, p < .001
Results for each milling mode [#]	
Fine	F(1.877,9.385) = 3.005, p = 0.10
Extra fine	F(2.618,13.089) = 7.971, p = 0.004
Incisal marginal discrepancy	
"Repeated use \times milling mode" interaction effect	F(9,90) = 3.209, p = 0.002
Results for each milling mode#	
Fine	F(9,45) = 3.646, p = 0.002
Extra fine	F(9,45) = 3.930, p = 0.00
Internal discrepancy	
"Repeated use \times milling mode" interaction effect	F(9,90) = 3.488, p = 0.001
Results for each milling mode#	
Fine	F(9,45) = 4.590, p < .001
Extra fine	F(3.150,15.749) = 2.544, p = 0.09

Abbreviations: F, F-value; p, p-value.

[#] Since there was a significant "repeated use × milling mode" interaction effect, a repeated measures ANOVA with one within-subjects factor (repeated use) was conducted for each milling mode, separately and the results of "Repeated use" main effect is reported.



Figure 6. Discrepancy values (μ) in both milling modes during repeated use of the burs. A, cervical vertical discrepancy. B, cervical absolute discrepancy. C, internal discrepancy. D, incisal vertical discrepancy.

abutment type, and restorative materials. In addition, whether discrepancy is measured before or after cementation, and whether aging procedure has been done after cementation, are other influencing factors [40, 41, 42, 43].

Regardless of the milling mode, number of repeated use, and measurement locations, the mean marginal/absolute marginal discrepancy values measured in this study were within a range of 53.85–305.63 µm, which seemed quite large in comparison with those mentioned above. There are several possible reasons for this finding. In this study, as discrepancy measurements were made after cementation, so higher discrepancies were expected [41, 42] In addition, seating pressure during cementation might lead to chipping of the thin margin of veneers. This could also significantly affect the marginal discrepancy values [44]. But most importantly, it should be noted that the acceptable values cited in mentioned studies are about crowns. Crowns unlike veneers have a definite seat, so the clinician is able to use more pressure in order to better seat the restoration. Therefore, it is not far from the mind that veneers could have a more extended clinically acceptable range. Moreover, similar to values measured in this study, the results obtained in Al-Dwairi et al study [45] which have evaluated veneers adaptation, were out of that range.

Raposo et al. [46] as well assessed the marginal misfit with the 18 successive use of the diamond bur set. They stated after 11 times, the IPS e.max CAD crowns would be unacceptable due to the increased marginal misfit. However, their acceptance criterion was marginal discrepancy of less than 120 μ m.

One of the main study limitations was the low number of repeated use (10 cycles) of milling burs and specimens. In addition, the present report tested the burs with a single material. In fact, similar CAD/CAM materials produced from different manufacturer could present different hardness [47] or flexural resistance [48]. Therefore, the results of the present report should be considered carefully. The other limitation is that only ceramic veneers were evaluated in this study, while other types of restorations have different machined surface area. Some manufacturers state an average range of bur life (4–6 to 15 or more mills for diamond burs and 20–30 to 60 or more mills for carbide burs in CEREC/inLab MC

XL). However, individual bur life could usually vary based on different factors like the tip size of the bur, the size of block milled, material used, and complexity of the restoration. That is why, milling machines show a tool error according to an estimate of how much life of the bur is remained. In some milling machines like E4D a simple traffic light colour-coding shows minutes a bur used in milling, which could be a better criterion than simply counting the number of mills. In higher versions of CEREC software's, special formulas are used to calculate remaining bur life as a percentage. It should also be noted that there are many different roughness parameters in use (Ra, Rz, Rq, Rsk). Although, Ra is by far the most common, it is better to measure the other parameters too, and observe the correlation between them. Further in vitro and in vivo studies are recommended with a larger number of milling cycles, different materials, other types of restorations, and different roughness parameters.

5. Conclusion

Within the limitations of this study, the following conclusions can be drawn:

- 1. Repeated use of milling diamond burs up to 10 times displayed a significant effect on marginal and internal adaptation between some repeated uses. However, there was no specific pattern.
- 2. Milling mode could not affect restorations adaptation.
- 3. Surface roughness values of restorations milled by extra-fine milling mode were significantly lower compared to fine mode, both before and after polishing.

Declarations

Author contribution statement

Leila Payaminia, Marzieh Alikhasi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Shima Younespour: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Soudabeh Koulivand: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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