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Effect of citric acid concentration and etching duration on the surface roughness of prepared human tooth enamel: an *in vitro* study

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ABSTRACT

BACKGROUND: Citric acid, commonly encountered through the consumption of fruits and juices, is a frequent factor affecting teeth in daily life. It contributes to enamel dissolution, exposing the underlying dentin in the crown region and potentially leading to significant tooth surface loss. On the other hand, acid etching is widely used in restorative dentistry to enhance the adhesion of restorative materials to tooth surfaces. This process partially dissolves and demineralizes the enamel's inorganic matrix, creating micropores and microgrooves that increase surface roughness. Pre-etching of enamel improves the bond strength of resin-based adhesives compared to non-etched enamel. Orthodontists also employ acid etching to create retention surfaces, which enhances bracket adhesion but simultaneously weakens adjacent enamel prisms, increases permeability, promotes biofilm adhesion, and reduces enamel's protective properties.

AIM: To evaluate the effect of citric acid exposure time and etching duration on the surface properties of dental hard tissues, including enamel and dentin.

MATERIALS AND METHODS: Three enamel samples were obtained from a maxillary molar extracted from a 21-year-old male patient. The tooth was embedded in epoxy resin for easier sectioning. Sectioning parameters on the IsoMet 4000 (Buehler, Switzerland) were set to a disk rotation speed of 2500 rpm and a feed rate of 10 mm/min. Three distinct areas were selected for analysis. Each sample was embedded in epoxy resin again for subsequent grinding and polishing, which was performed simultaneously for all three samples using the MetaServ 250 (Buehler, Switzerland). The prepared enamel surfaces were examined using an optical microscope (Stemi 305; Carl Zeiss, Germany) equipped with a color video camera (Axiocam 105; Carl Zeiss, Germany) under reflected light. The samples were etched as follows: Sample 1 with 0.5 wt.% citric acid (pH=2.3), Sample 2 with 3 wt.% citric acid (pH=1.86), and Sample 3 with 5 wt.% citric acid (pH=1.71). The pH was measured using Cobra4 Mobile-Link (PHYWE, Germany). Atomic force microscopy of enamel surface topography before and after etching was performed using the NANOEDUCATOR microscope (NT-MDT, Russia) with a tungsten probe operating in non-contact mode. Surface roughness (*Ra*) was measured at each etching stage for each sample using the Gwyddion software (Czech Metrology Institute, Czech Republic).

RESULTS: Optical microscopy provided images of enamel, dentin, and the dentinoenamel junction, confirming the healthy condition of the examined samples. The findings suggest that 5 wt.% citric acid is more suitable for controlled etching than 37% phosphoric acid, as prolonged exposure to phosphoric acid can cause severe enamel damage. AFM analysis demonstrated the surface characteristics of each sample before and after etching at different time intervals. A linear increase in surface roughness was observed in all samples after 45 s of etching.

CONCLUSION: For achieving significant surface roughness and visualizing enamel prisms within a reasonable timeframe, etching with 5 wt.% citric acid is recommended. However, further studies on the adhesive properties of enamel post-etching with citric acid are necessary for clinical implementation.

Keywords: citric acid; etching; tooth enamel.

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Влияние концентрации лимонной кислоты и времени травления на шероховатость поверхности эмали подготовленного человеческого зуба: исследование *in vitro*

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АННОТАЦИЯ

Обоснование. Распространённым источником воздействия на зубы в процессе ежедневного функционирования является лимонная кислота (при употреблении фруктов или соков). Она способствует растворению эмали, покрывающей нижележащий дентин в области коронки зуба. Это может привести к потере значительной части поверхности зуба. С другой стороны, для увеличения сцепления реставрационных материалов с поверхностью зуба широко используется кислотно-локализованное травление. Оно обеспечивает частичное растворение и деминерализацию неорганического матрикса поверхности эмали, в результате чего формируются микропористости и микропазы, что увеличивает шероховатость поверхности. Предварительное травление эмали улучшает прочность сцепления систем связывания на основе смолы с эмалью по сравнению с использованием тех же систем без травления. Ортодонты также используют кислоту для формирования удерживающих поверхностей, что, с одной стороны, улучшает фиксацию брекета, но с другой — ослабляет структуру смежных эмалевых призм, повышает проницаемость и адгезию биоплёнки к эмали и снижает её защитные свойства.

Цель исследования — оценка влияния времени воздействия лимонной кислоты и времени травления на свойства поверхности твёрдых тканей зуба, включая эмаль и дентин.

Материалы и методы. Из верхнечелюстного моляра, который удалили у пациента (мужчина, 21 год), получены три образца эмали. Зуб был залит эпоксидной смолой для удобства резки. Параметры резки на станке IsoMet 4000 (Buehler, Швейцария): скорость вращения диска 2500 об./мин, скорость подачи образца 10 мм/мин. Выбраны три области в качестве образцов для исследования. Каждый образец снова был залит эпоксидной смолой для обеспечения возможности последующего шлифования и полировки, что было проведено одновременно для всех трёх образцов на станке MetaServ 250 (Buehler, Швейцария). После подготовки образца поверхность зубов исследовали с помощью оптического микроскопа Stemi 305 (Carl Zeiss, Германия), оснащённого цветной видеокамерой Axiocam 105 (Carl Zeiss, Германия) в отражённом свете. Образец 1 травили кислотой в концентрации 0,5 мас.% (pH=2,3), образец 2 — в концентрации 3 мас.% (pH=1,86), образец 3 — в концентрации 5 мас.% (pH=1,71). Измеряли pH (Cobra4 Mobile-Link; PHYWE, Германия). Атомно-силовую микроскопию топографии поверхности эмали для каждого образца до и после травления проводили на микроскопе NANOEDUCATOR (NT-MDT, Россия), оборудованном вольфрамовым зондом. Устройство работало в бесконтактном режиме. Шероховатость поверхности *Ra* измеряли на каждом этапе травления для каждого образца с использованием программного обеспечения Gwyddion (Чешский метрологический институт, Чешская Республика).

Результаты. Представлены результаты оптической микроскопии эмали, дентина и дентиноэмалевой границы. Чётко видны изображения, которые указывают на здоровое состояние исследуемых образцов. Сделан вывод, что лимонная кислота в концентрации 5 мас.% больше подходит для контролируемого травления, чем 37% фосфорная кислота. Известно также, что при длительном воздействии фосфорной кислоты эмаль может быть серьёзно повреждена. При атомно-силовой микроскопии показана поверхность каждого из трёх образцов до и после травления в различные временные промежутки. После травления в течение 45 с на всех образцах наблюдалось линейное увеличение шероховатости поверхности.

Заключение. Для получения значительной величины шероховатости поверхности и визуализации эмалевой призмы в разумные сроки можно рекомендовать травление 5 мас.% лимонной кислотой. Для клинической реализации полученных результатов необходимы дополнительные исследования адгезионных свойств эмали после травления лимонной кислотой.

Ключевые слова: лимонная кислота; травление; эмаль зуба.

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BACKGROUND

Teeth are routinely exposed to various acids during daily functions. A common source is citric acid, present in fruits and juices. Despite the exceptional strength of human dental enamel, which covers the underlying dentin in the crown region, acidic foods and beverages promote its dissolution [1–3], potentially causing substantial dental hard tissue loss. In contrast, acid etching is widely applied in clinical practice to enhance the adhesion of restorative materials to tooth surfaces. This technique partially dissolves and demineralizes the inorganic matrix of enamel, producing micropores and microgrooves [4–8]. As a result, enamel surface roughness increases. Pre-etching of enamel can improve the bond strength of resin-based adhesive systems compared with the same systems applied without prior etching [9, 10]. Several acids have been used for this purpose [11–13]. In orthodontics, acid etching is also employed to create retentive surfaces, which facilitate bracket bonding. However, this process simultaneously weakens adjacent enamel prisms, increases permeability, promotes biofilm adhesion, and reduces the protective properties of enamel [14].

In scientific research, the controlled application of acid etching enables visualization of the structural elements of dental enamel for further study. Atomic force microscopy (AFM) is widely used to investigate the effects of acid etching on enamel surface properties. Serbanoiu et al. [15] examined how different methods of enamel preparation may influence the appearance of artifacts during AFM measurements. Watari [16] studied the evolution of tooth surface microgeometry using three acidic agents. Simmer et al. [17] applied AFM to evaluate the ability of a toothpaste containing tricalcium silicate to remineralize acid-etched human enamel *in vitro*. Loyola-Rodriguez et al. [18] compared enamel surface roughness and absolute depth profiles before and after treatment with four different concentrations of phosphoric acid, assessed by AFM. In the present study, we used AFM to assess the average surface roughness (*Ra*) of three human enamel specimens obtained from a single tooth. The samples were subjected to etching with citric acid at varying concentrations and durations. For each combination of “acid concentration–etching time,” *Ra* values were quantified in three directions—horizontal, vertical, and diagonal—followed by surface profiling and AFM imaging.

This study aimed to evaluate the effect of citric acid exposure time and etching duration on the surface properties of dental hard tissues, including enamel and dentin.

METHODS

Study Design

We conducted an interventional, single-center, uncontrolled study of enamel, dentin, and the dentinoenamel junction using optical microscopy and AFM.

Eligibility Criteria

Inclusion criteria:

- Intact teeth
- Teeth extracted for orthodontic indications.

Non-inclusion criteria:

- Teeth with dental caries.

Study Setting

The study was conducted at the Shared Research Facility of the Scientific and Educational Center “Materials” and the Department of Chemistry, Don State Technical University. The tooth was extracted at the Dental Department of the Rostov State Medical University Clinic.

Study Duration

The experiments were carried out over a 6-month period.

Sample Preparation

Three human enamel specimens were obtained from a maxillary molar extracted for orthodontic reasons from a 21-year-old male patient.

After extraction, the tooth was immersed in 1% sodium hypochlorite (NaClO) solution for 10 minutes, then stored in Hanks’ balanced salt solution (HBSS) at 4 °C with thymol granules to prevent fungal growth (thymol-to-HBSS ratio 1:1000). The tooth was embedded in epoxy resin to facilitate sectioning. Sectioning was performed on an IsoMet 4000 machine (Buehler, Switzerland) with the following parameters: disk rotation speed, 2500 rpm; feed rate, 10 mm/min. Cooling was maintained by continuous application of coolant (Cool 2; Buehler, Switzerland). Two longitudinal cuts were made so that the second cut passed through the middle portion of the tooth, including a white spot lesion (the sample was positioned relative to the disk using the machine’s built-in micrometer). As a result, two longitudinal sections containing sound enamel were obtained. At the second stage, each of these sections was cut transversely, perpendicular to the first cut. Sectioning was carried out on the same machine under the same parameters, but with a cubic boron nitride disk and the same coolant. Three areas were selected as specimens for further study. Each specimen was embedded again in epoxy resin to allow subsequent grinding and polishing, which were performed simultaneously for all three samples using the MetaServ 250 grinder-polisher (Buehler, Switzerland). Grinding and polishing were carried out under a load of 10 N, with a disk rotation speed of 100 rpm. Tap water was used as a lubricant. Detailed grinding parameters are presented in Table 1.

The same load and disk rotation speed were applied during polishing. A lubricant composed of glycerin and propylene glycol mixed in a 1:1 ratio was used. Detailed polishing parameters are presented in Table 2.

Between each stage of grinding and polishing, specimens were ultrasonically cleaned in a bath with distilled water (Sonorex RK 31; Bandelin, Germany) for 5 minutes, and for 10 minutes after the final polishing step.

Optical Microscopy

After specimen preparation, tooth surfaces were examined using an optical microscope (Stemi 305; Carl Zeiss, Germany) equipped with a color video camera (Axiocam 105; Carl Zeiss, Germany) under reflected light (see Fig. 1). Enamel, dentin, and the dentinoenamel junction were clearly visible in the images, confirming the healthy condition of the studied specimens. Samples 1, 2, and 3 were etched with 0.5% (w/w) citric acid (pH 2.3), 3% (w/w) citric acid (pH 1.86), and 5% (w/w) citric acid (pH 1.71), respectively. The pH was measured with Cobra4 Mobile-Link (PHYWE, Germany), calibrated against standard buffer solutions of known pH. Etching was performed on each specimen at five sequential time points: 1, 15, 30, 45, and 60 seconds. For the 1-second

Table 1. Grinding parameters

Grinding stage	Grit size	Abrasive paper	Grinding time, s
1	P800	Siawat 1913, SiC-based (Sia Abrasives, Switzerland)	60
2	P1500	Smirdex, SiC-based (Smirdex, Greece)	30
3	P2500	Smirdex, SiC-based (Smirdex, Greece)	30

Table 2. Polishing parameters

Polishing stage	Particle size, μm	Abrasive suspension	Polishing time, s	Lubricant	Cloth
1	6	MetaDi (Buehler, Switzerland)	120	Glycerin + propylene glycol	Trident
2	3	MetaDi (Buehler, Switzerland)	30	Glycerin + propylene glycol	Trident
3	1	MetaDi (Buehler, Switzerland)	360	Glycerin + propylene glycol	Veltex
4	0.05	MasterPrep (MasterKit, Italy)	480	Distilled water	Veltex

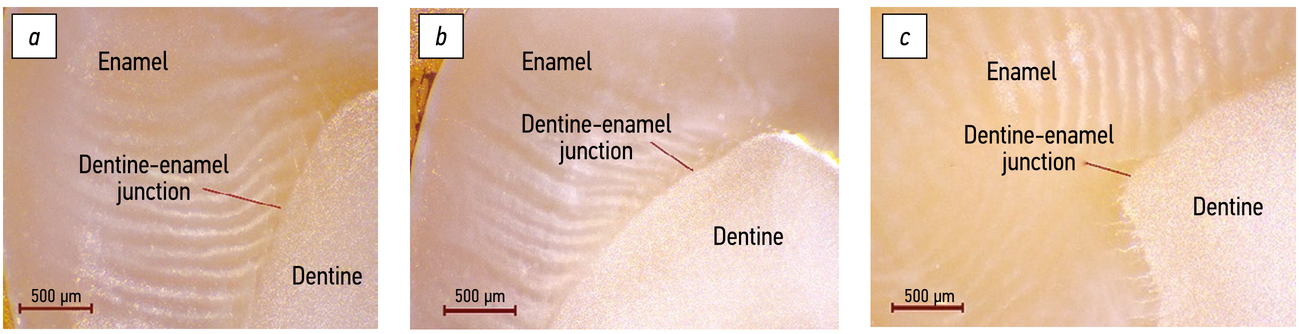

Fig. 1. Selected surface areas for atomic force microscopy scanning: *a*, Specimen 1; *b*, Specimen 2; *c*, Specimen 3.

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exposure, the enamel surface was contacted with a lint-free tissue saturated with citric acid. For the other four time intervals, etching was carried out by immersing the specimen in the corresponding acid solution. After each etching step, specimens were ultrasonically cleaned in distilled water.

Atomic Force Microscopy

Surface topography of enamel was examined for each specimen before and after etching using an atomic force microscope (NANOEDUCATOR; NT-MDT, Russia) equipped with a tungsten probe. The device operated in non-contact mode. All images were acquired at a resolution of 256 × 256 pixels with a scan rate of 20 mm/s. Positioning was performed with a USB optical microscope mounted on a stand.

Ethics Approval

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki (2013), GOST R 52379-2005, Good Clinical Practice, and Order No. 200n of the Ministry of Health of the Russian Federation (April 1, 2016), On the Approval of Good Clinical Practice Rules. The protocol was approved by the Ethics Committee of Rostov State Medical University (No. 13/22, September 8, 2022). Written informed consent was obtained from the patient.

RESULTS AND DISCUSSION

Surface Analysis Using Optical Microscopy

Enamel, dentin, and the dentinoenamel junction were clearly visualized in the images, confirming the healthy condition of the examined specimens (see Fig. 1). Hunter–Schreger bands were also visible in all samples.

Surface Analysis Using Atomic Force Microscopy

The surface of each of the three specimens before and after 1-second etching is shown in Fig. 2; after 15 and 30 seconds in Fig. 3; and after 45 and 60 seconds in Fig. 4. Fig. 5 presents the surface profiles of each specimen after every etching interval. Hydroxyapatite monocrystals were observed in the AFM images obtained with the Nano Compact atomic force microscope (Phywe, Germany), particularly in the horizontal surface profile of Specimen 1 after 60 seconds of etching (see Figs. 6 and 7).

The scan field was 1.01 × 1.01 µm. The microscope was equipped with a monocrystalline silicon probe with aluminum coating, a resonance frequency of 19,060 kHz, and a spring constant of 48 N/m. Surface scanning was performed in dynamic mode at a scan rate of 0.3 ms per line. Axial resolution was 1.1 nm, and the root mean square noise level in height mode was 0.5 nm.

Surface Roughness Measurements

Surface roughness (*Ra*) was measured at each etching stage for each specimen using Gwyddion software (Czech Metrology Institute, Czech Republic). The measurements were based on AFM surface profiles obtained from the collected AFM data (see Figs. 2–5). Because of potential irregularities in the visualized structure, roughness assessment based on a single profile in one direction was considered insufficient. Therefore, in this study, surface roughness was measured in three directions: horizontal, vertical, and diagonal. Five profiles were constructed in each direction. Five profiles were constructed in each direction. The mean value of these five profiles was taken as the absolute in this direction (Ra_{avg}). The mean roughness across all three directions was defined as the absolute roughness of the surface (Ra_{absol}). The measurement results for Specimens 1 through 3 are presented in Tables 3–5, respectively.

Tracking changes on the specimen surfaces during etching revealed several important details. The surfaces before etching (see Fig. 2, *a–c*) and after 1 second of etching (Fig. 2, *d–f*) did not exhibit distinct relief and were covered with a smooth, smeared layer formed during polishing. This layer consisted of residual enamel structural components and occasional abrasive inclusions from the grinding/polishing process [19]. Examination of the specimens after 15 seconds of etching still demonstrated the presence of the smeared layer in Specimens 1 and 2 (see Fig. 3, *a*, *b*), which had been treated with lower concentrations of citric acid. In contrast, irregularly rounded structural elements—enamel prisms—were clearly visualized on the surface of Specimen 3 (see Fig. 3, *c*). This finding suggests that 15 seconds of etching with 5% (w/w) citric acid was sufficient to completely remove the smeared layer. Another feature evident in this image was the differential dissolution rate of enamel prisms. Monitoring the surfaces after 30 seconds of etching (see Fig. 3, *d–f*) showed the presence of enamel prisms with round or drop-like shapes in all specimens. The number of visible structural elements increased, with more detailed features clearly observed in Specimens 2 and 3 (see Fig. 4, *e*, *f*). An additional feature noted in these and subsequent images was the variation in prism orientation, which may correspond to different regions of the same tooth. In Specimens 2 and 3, prisms appeared tilted, whereas in Specimen 1 they were more perpendicular to the surface. According to the trends in surface roughness (*Ra*) as a function of etching time in different directions (see Fig. 8) and the absolute surface roughness (Ra_{absol}) with increasing etching time (see Fig. 9), roughness increased steadily after 30 seconds of etching, with a linear rise in Ra_{absol} values observed after 45 seconds of etching.

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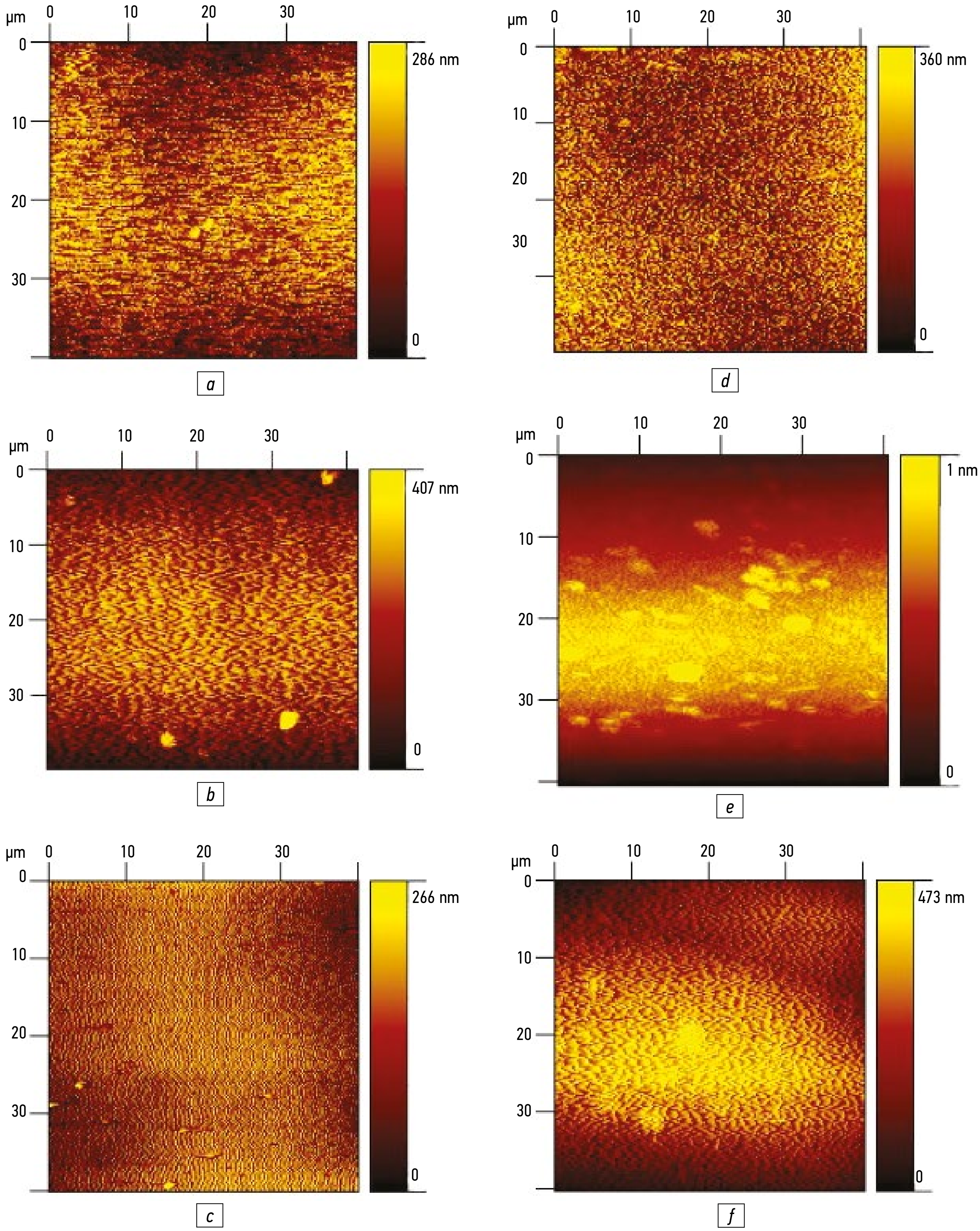

Fig. 2. Atomic force microscopy images of specimen surfaces before etching (*a*, Specimen 1; *b*, Specimen 2; *c*, Specimen 3) and after 1 second of etching (*d*, Specimen 1; *e*, Specimen 2; *f*, Specimen 3).
Images do not display detailed microstructural features. AFM, atomic force microscopy.

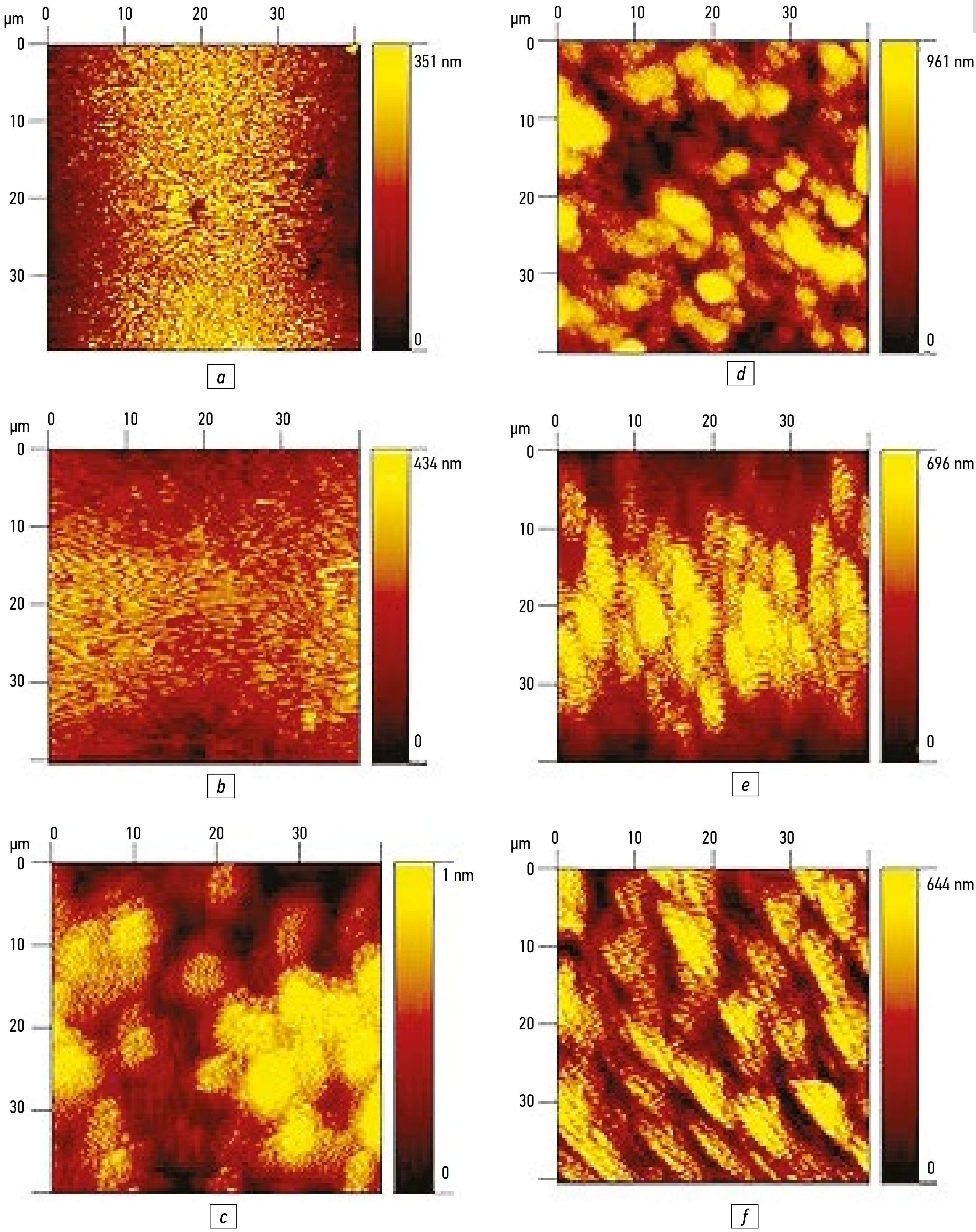

Fig. 3. Atomic force microscopy images of specimen surfaces after 15 seconds of etching (*a*, Specimen 1; *b*, Specimen 2; *c*, Specimen 3) and after 30 seconds of etching (*d*, Specimen 1; *e*, Specimen 2; *f*, Specimen 3). Qualitative changes in surface microgeometry are observed AFM, atomic force microscopy.

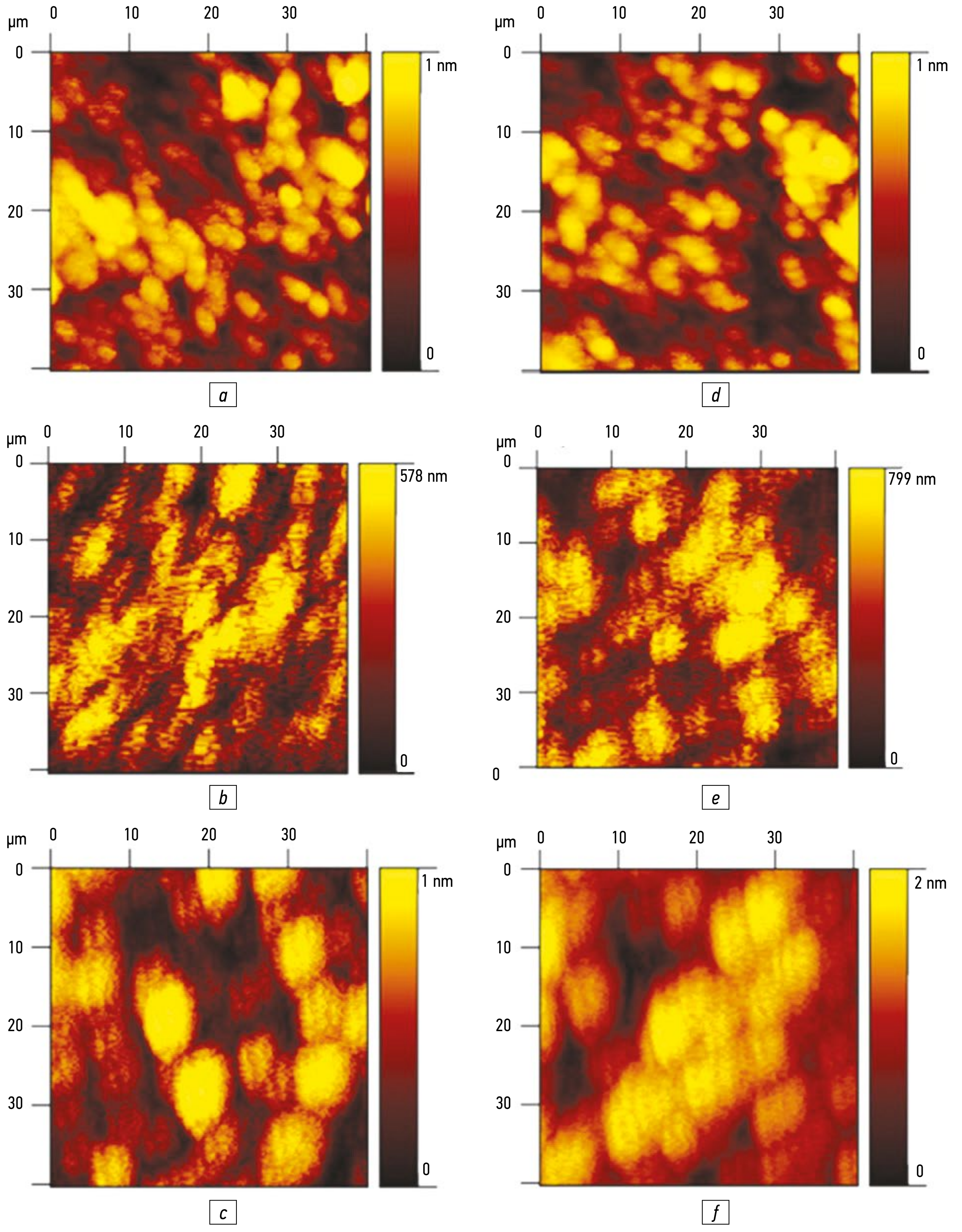

Fig. 4. Atomic force microscopy images of specimen surfaces after 45 seconds of etching (*a*, Specimen 1; *b*, Specimen 2; *c*, Specimen 3) and after 60 seconds of etching (*d*, Specimen 1; *e*, Specimen 2; *f*, Specimen 3).

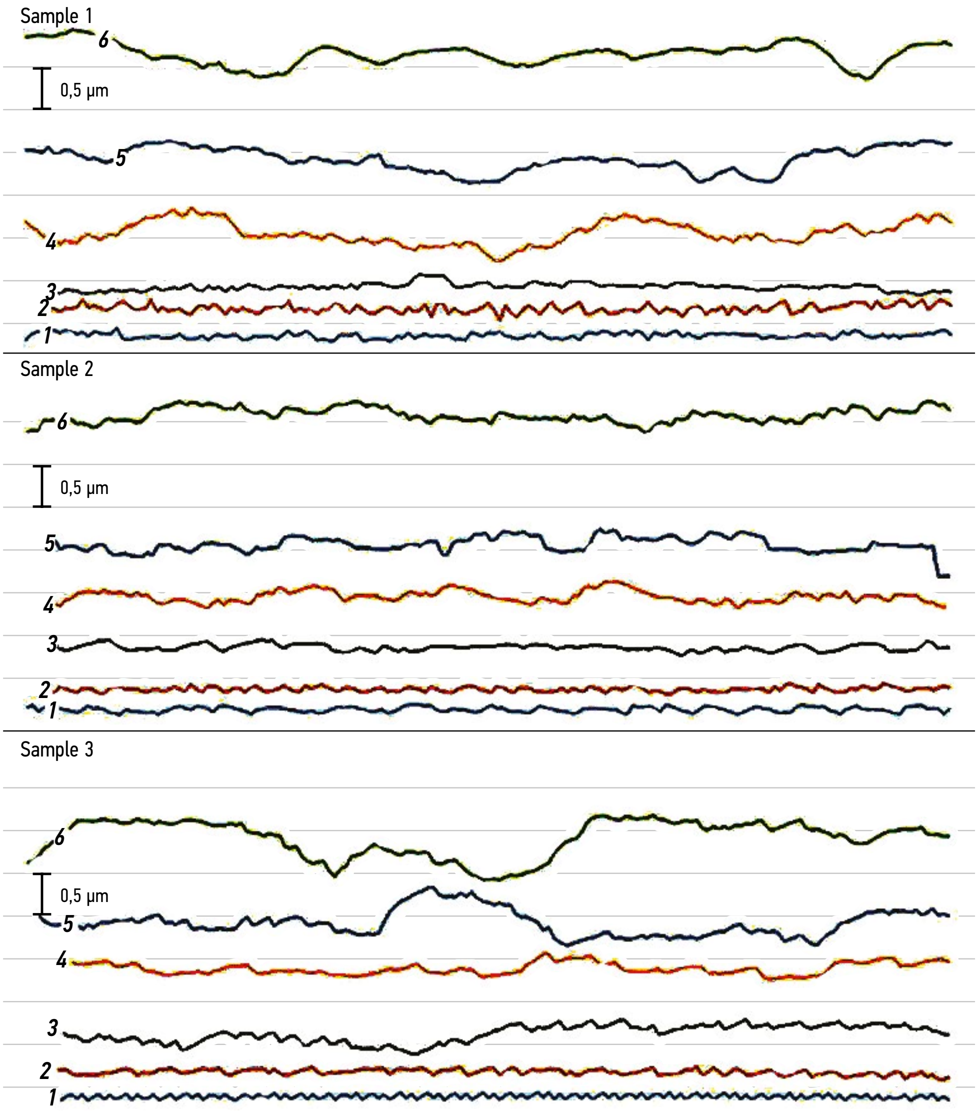

Fig. 5. Surface profiles of specimens after each etching stage: *1*, before etching; *2*, after 1 second; *3*, after 15 seconds; *4*, after 30 seconds; *5*, after 45 seconds; *6*, after 60 seconds.

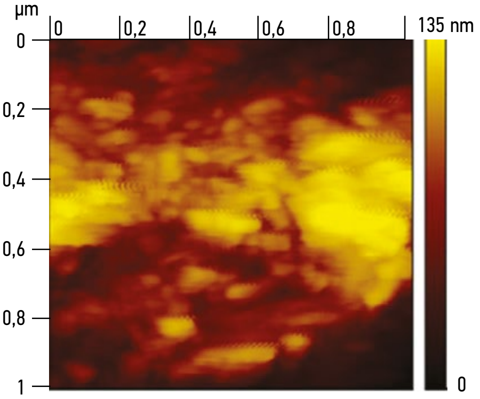

Fig. 6. High-resolution image of Specimen 1 after 60 seconds of etching.

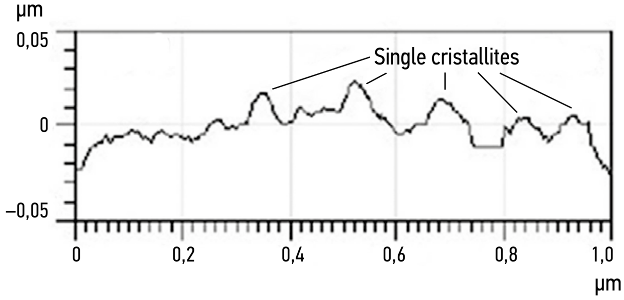

Fig. 7. High-resolution horizontal surface profile of Specimen 1 after 60 seconds of etching.

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Table 3. Surface roughness (*Ra*) of Specimen 1

Time, s	No. of selected profile points per base length (*n*)	Horizontal direction *Ra* (*n*)	Ra_{avg}	Vertical direction *Ra* (*n*)	Ra_{avg}	Diagonal direction *Ra* (*n*)	Ra_{avg}	Absolute surface roughness (Ra_{absol})
0	1	26.9	22.5	26.6	27.1	18.4	22.8	24.1
	2	26.3		27.1		21.3		
	3	20.8		26.6		21.3		
	4	21.0		28.7		28.4		
	5	17.3		26.5		24.3		
1	1	30.4	31.0	33.1	29.6	20.0	24.4	28.3
	2	31.5		28.7		27.0		
	3	30.0		25.8		20.7		
	4	27.8		28.9		30.4		
	5	35.3		31.3		23.8		
15	1	27.6	30.5	19.4	20.2	24.8	26.4	25.7
	2	30.1		10.0		32.8		
	3	31.6		20.4		21.8		
	4	35.6		18.4		32.1		
	5	27.6		26.0		20.5		
30	1	91.7	99.5	82.1	101.8	82.5	103.0	101.4
	2	153.0		125.3		103.6		
	3	107.4		110.9		147.8		
	4	67.0		110.1		110.2		
	5	78.5		80.4		70.9		
45	1	125.6	120.0	153.1	137.7	128.4	115.0	124.2
	2	137.9		123.8		115.9		
	3	133.2		86.4		126.4		
	4	104.3		153.3		126.6		
	5	99.2		161.8		77.6		
60	1	194.2	130.8	111.4	126.6	160.6	161.8	139.7
	2	142.0		145.6		150.6		
	3	94.5		123.8		130.3		
	4	133.1		134.4		187.0		
	5	90.1		117.9		180.6		

Table 4. Surface roughness (*Ra*) of Specimen 2 (conclusions)

Time, s	Horizontal direction Ra_{avg}	Vertical direction Ra_{avg}	Diagonal direction Ra_{avg}	Ra_{absol}
0	17.1	32.6	28.0	25.9
1	20.4	212	17.4	9.7
15	28.7	40.6	27.6	32.3
30	52.4	70.9	84.3	69.2
45	64.0	69.6	64.2	65.9
60	79.0	83.8	87.7	83.5

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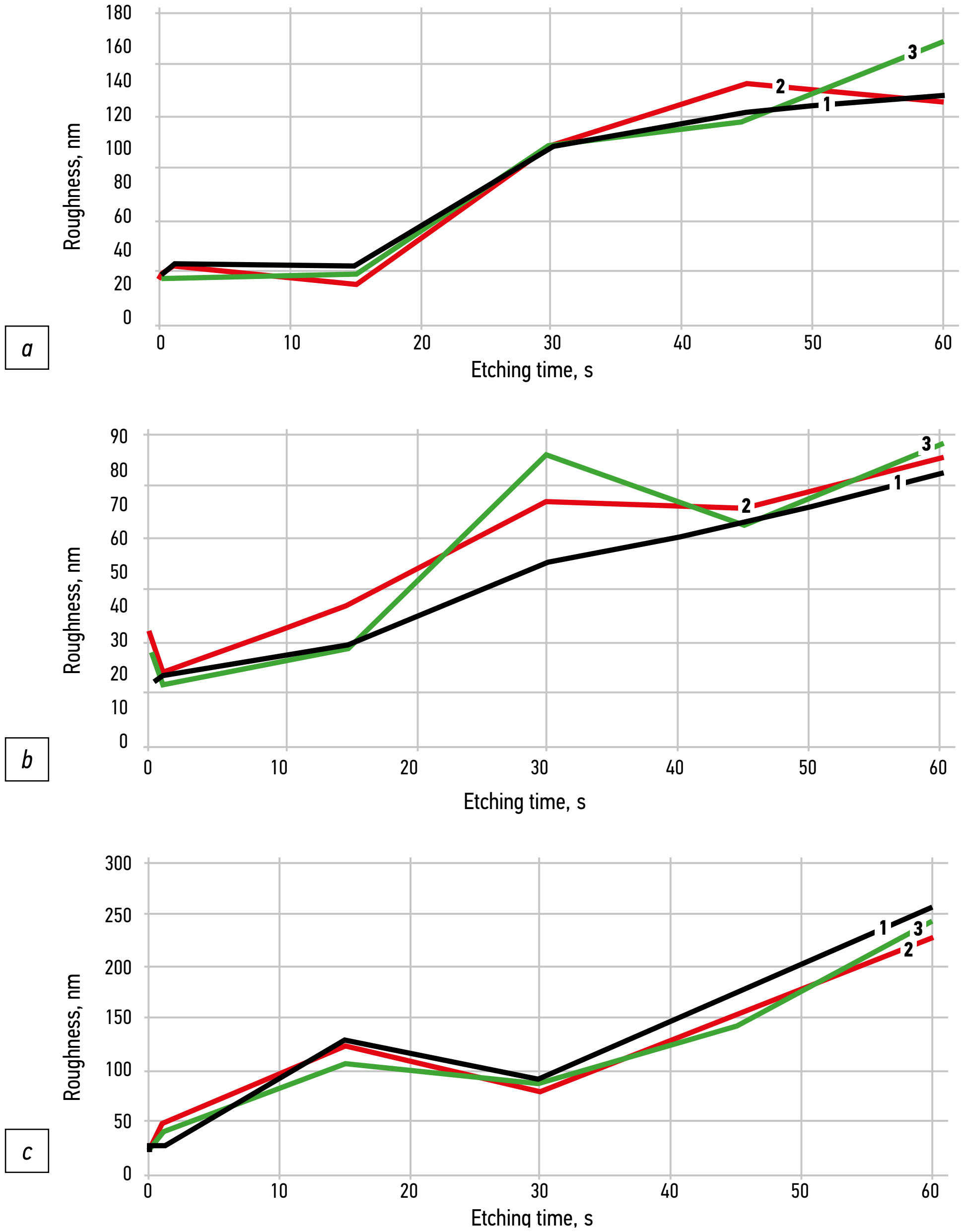

Fig. 8. Dependence of surface roughness (*Ra*) on etching time in different directions (*1*, horizontal; *2*, vertical; *3*, diagonal) for: *a*, Specimen 1; *b*, Specimen 2; *c*, Specimen 3.

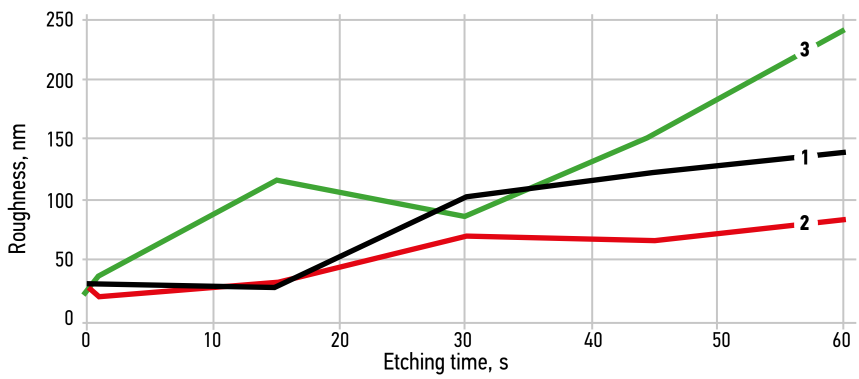

Fig. 9. Dependence of absolute surface roughness (Ra_{absol}) on etching time for all specimens. Numbers in the graph correspond to specimen numbers.

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Table 5. Surface roughness (*Ra*) of Specimen 3 (conclusions)

Time, s	Horizontal direction Ra_{avg}	Vertical direction Ra_{avg}	Diagonal direction Ra_{avg}	Ra_{absol}
0	25.7	19.0	15.8	20.2
1	26.6	43.6	38.2	36.1
15	126.3	121.7	106.7	118.2
30	89.0	77.5	87.2	84.6
45	171.1	151.9	142.0	155.0
60	255.8	225.0	242.0	241.0

Etching for 45 seconds resulted in more defined contours of the structural elements in Specimens 2 and 3 (see Fig. 4, *b*, *c*). The structure of Specimen 1 remained poorly organized. Unetched interprismatic substance was still present on the surfaces of Specimens 1 and 2 (Fig. 5, *a*, *b*). After 60 seconds of etching, no further substantial structural changes were observed in Specimens 1 and 3 (Fig. 4, *d*; see also Fig. 4, *e*). In Specimen 1, the acid concentration was still insufficient to completely remove enamel from the interprismatic regions. In Specimen 2, the structural elements increased slightly in size and acquired a more streamlined shape as interprismatic enamel was dissolved. With longer etching times, clearer prism boundaries were visible in the surface profiles of all specimens (see Fig. 5), although this effect was most pronounced in Specimen 3. When compared with published results of etching human enamel using 37% phosphoric acid [5]—which produced a mean surface roughness of 260.9 nm after 30 seconds—the etching process in the present study proceeded more slowly. Comparable surface roughness values were achieved after 60 seconds of etching with 5% (w/w) citric acid. Based on these findings, citric acid at this concentration appears more suitable for controlled etching than 37% phosphoric acid. It is also well established that prolonged exposure to phosphoric acid may cause severe enamel damage [20].

CONCLUSION

The dependence of absolute surface roughness values on etching time was calculated for all specimens. To achieve significant surface roughness and clear visualization of enamel prisms within clinically relevant etching times, etching with 5% (w/w) citric acid can be recommended. A linear increase in surface roughness was observed in all specimens after 45 seconds of etching. Further studies on the adhesive properties of enamel following citric acid etching are required for clinical implementation of these findings.

ADDITIONAL INFORMATION

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