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Clinical Research

In Situ Surface Biodegradation of Restorative Materials

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Clinical Relevance

Understanding the surface characteristics of restorative materials submitted to *in situ* biodegradation is an essential issue toward the assessment of the clinical longevity of restorative materials.

SUMMARY

This study aimed to evaluate the surface characteristics of restorative materials (roughness, hardness, chemical changes by energy-dispersive spectroscopy [EDX], and scanning electron microscopy [SEM]) submitted to *in situ* biodegradation. Fifteen discs of each material (IPS e.max [EM], Filtek Supreme [FS], Vitremer [VI], Ketac Molar Easymix [KM], and Amalgam GS-80 [AM]) were fabricated in a metallic mold (4.0 mm × 1.5 mm). Roughness, hardness, SEM, and EDX were then evaluated. Fifteen healthy volunteers used a palatal device containing one disc of each restorative material for seven days. After the biodegradation, the roughness, hardness, SEM, and EDX were once again evaluated. Data obtained from the roughness and hardness evaluations were submitted to Kolmogorov-Smirnov and Tukey-Kramer tests ($p<0.05$). All esthetic restorative materials showed a significant increase in the roughness after biodegradation. Before biodegradation, significant differences in the hardness among the materials were seen: EM>AM>FS>KM>VI. After biodegradation, the hardness was significantly altered among the materials studied: EM>AM>FS=KM>VI, along with a significant increase in the hardness for AM, KM, and VI. SEM images indicated degradation on the surface of all materials, showing porosities, cracks, and roughness. Furthermore, after biodegradation, FS showed the presence of Cl, K, and Ca on the surface, while F was not present on the VI and KM surfaces. EM and AM did not have alterations in their chemical composition after biodegradation. It was concluded that the dental biofilm accumulation *in situ* on different restorative materials is a material-dependent parameter. Overall, all materials changed after biodegradation: esthetic restorative materials showed increased roughness, confirmed by

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SEM, and the ionomer materials and silver amalgam showed a significantly higher hardness. Finally, the initial chemical composition of the composite resin and ionomer materials evaluated was significantly altered by the action of the biofilm *in situ*.

INTRODUCTION

Biofilms form not only on dental hard and soft tissues, but also on restorative biomaterial surfaces used in the oral cavity; in addition, biofilms are the major cause of caries and periodontal diseases.[1] However, the adhesion and aggregation of microorganisms are different among materials with different compositions and surface properties .[2,3]For most restorative materials, acid metabolites produced by cariogenic biofilm can cause surface damage such as corrosion, softening, and a roughness increase, which is known as biodegradation.[4,5] This is a complex process and includes disintegration and dissolution in saliva and other types of chemical/physical degradation, such as wear and erosion caused by food, chewing, and bacterial activity.[6] Thus, in order to present a satisfactory performance, the priority of restorative materials should be the resistance to that adverse condition.

There is limited knowledge about the influence of cariogenic biofilms on the surface of restorative materials. Long-term *in vitro* studies show an increase in the roughness and morphology damages for resin composites, polyacid-modified composites,[7] and ionomeric materials.[5] An *in situ* study found a lower hardness for Vitremer after 14 days of biodegradation.[8] Metallic materials, such as gold and amalgam, were observed to have thick biofilms covering their surfaces *in vivo*, though their cells were found to be barely viable.[9] This probably caused less deterioration of the materials' properties. Conversely, biofilms on ceramic biomaterial, which is considered the most inert of all dental materials used for restorations,[10] were found to be relatively thin but highly viable (from 34% to 86%).[9] All of these findings reflect the complex environment and biome observed in oral conditions.[8]

It is known that no *in vitro* test is capable of reproducing the complex biodegradation process. Consequently, many studies choose lactic acid as a representative of dental biofilm since this is the most important metabolic product from *Streptococcus mutans* in the biofilm exposed to sucrose.[11] Nevertheless, it is possible that the concentration, pH, and effective contact of this acid solution *in vitro* would differ from oral conditions, thus overestimating the degradation effects. In this context, the *in situ* model is a recognized experimental design that has been successfully used to evaluate the formation of cariogenic dental biofilm.[12] There are few studies on the influence of biofilm on the surface characteristics of restorative materials *in situ*.[8,13,14] Therefore, the aim of this study was to evaluate the effects of the *in situ* biodegradation on the surface characteristics of restorative materials. The hypothesis tested was that restorative materials subjected to seven days of biofilm interaction have significant modifications in regard to their roughness, hardness, and microstructure.

METHODS AND MATERIALS

Specimen Preparation and Storage Groups

Fifteen specimens of each restorative material (described in Table 1) were fabricated according to

Table 1: *Materials Used in This Study*

Materials	Classification	Contents (Manufacturer Information)
IPS e.max (Ivoclar Vivadent, Schaan, Liechtenstein)	Glass ceramic	Powder: 97% SiO_2, Al_2O_3, P_2O_5, K_2O, Na_2O, CaO, F, 3% TiO_2, and pigments
		Liquid: water, alcohol, chloride
Filtek Supreme (3M ESPE, St Paul, MN, USA)	Composite resin	Bis-GMA, Bis-EMA, UDMA, TEGDMA Zirconia/silica cluster filler and a nonagglomerated silica filler
Vitremer (3M ESPE, St Paul, MN, USA)	Resin-modified glass ionomer	Powder: fluoroaluminosilicate glass; redox system
		Liquid: aqueous solution of a modified polyalkenoic acid, HEMA
Ketac Molar Easymix (3M ESPE, St Paul, MN, USA)	Glass ionomer cement	Powder: fluorosilicate glass, strontium and lantanium
		Liquid: polycarbonic and tartaric acids and water
Amalgam GS-80 (SDI, Victoria, Australia)	Silver amalgam	Powder: 40% Ag, 31.3% Sn, 28.7% Cu
		Liquid: mercury

Abbreviations: Bis-EMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

the manufacturer's instructions, by using metal rings (4 mm diameter; 1.5 mm depth), at a temperature of 23°C ± 1°C, and a relative humidity of 50% ± 5%.

The specimens, with the exception of the ceramics, were covered with an acetate strip (Probem Ltda, Catanduva, SP, Brazil) and pressed onto a glass slide to compact the material. Filtek Supreme (FS) and Vitremer (VI) were photoactivated for 40 seconds each at the upper and lower surfaces of the matrix by a curing light (Elipar Trilight, 3M ESPE, St Paul, MN, USA), with an intensity of up to 750 mW/cm^2, and checked by a light-curing meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc, Turkey). Ketac Molar Easymix (KM) and Amalgam GS-80 (AM) were allowed to set at room temperature for 15 minutes. After the setting reactions were completed, the ionomeric specimens were superficially protected with petroleum jelly. For IPS e.max (EM), specimens were fabricated in a prosthetic laboratory by using the pressing process in an oven (Programat P500, Ivoclar Vivadent, Schaan, Liechtenstein), which simulates the clinical reality.

All of the restorative materials were stored at 37°C and 100% relative humidity for 24 hours. Afterwards, each specimen surface was polished according to the manufacturer's instructions. For FS, VI, and KM, Sof-Lex discs (3M ESPE, St Paul, MN, USA) were used. AM was polished with a polishing kit (KG Sorensen, Cotia, SP, Brazil). For the finishing of EM, discs were ground flat with an aluminum oxide jet (50 μm, Bio-Art, São Carlos, SP, Brazil) followed by a diamond drill (4138F, KG Sorensen, Barueri, SP, Brazil); the polishing of these specimens was performed with a rubber tip (KG Sorensen), and specimens were then washed in an ultrasonic bath (UNIQUE, São Paulo, SP, Brazil) and glazed.

Roughness Measurements

After the finishing and polishing procedures, all specimens were washed through sonication for 10 minutes, dried, and fitted to a roughness-measuring instrument (Surfcorder SE1700, Kosaka Corp, Tokyo, Japan). The roughness analysis was performed prior to the hardness assessment in order to avoid interference with their results. Moreover, each specimen was divided in the middle, with the left side being used for the roughness analysis and the right side for the hardness assessment. To record the roughness measurements, the needle moved at a constant speed of 0.5 mm/sec with a load of 0.7 mN. The cut-off value was set at 0.25 mm to maximize the filtration of the surface waviness. The measurement of roughness for each specimen was taken across the diameter over a standard length of 0.25 mm. The mean surface roughness values (μm) of the specimens were obtained from three successive in-line measurements from the center to the boundary of each disc at different angles (0°, 45°, and 90°). A calibration was done periodically to check the performance of the roughness-measuring instrument.

Hardness Measurements

Hardness tests were carried out with a hardness tester (Shimatzu, Tokyo, Japan) by using a Vickers indenter, with a load of 500 g for the ceramic and of 200 g for the composite resin, glass ionomer cements, and silver amalgam. All materials had a dwell time of 15 seconds. Five readings were taken for each specimen, which were then used to calculate the mean hardness. The mean hardness was calculated before and after the biodegradation.

Surface Morphology Assessment and Energy-Dispersive X-ray Analysis

Before and after the *in situ* biofilm experiment, three additional representative specimens of each group were rinsed, dried, and mounted on a holder using a double-sided adhesive carbon tape. Carbon was then sputtered on the specimens before the analysis. Energy-dispersive X-ray analysis (EDX) was performed before and after the biodegradation. The EDX measurements were calibrated by a certified engineer, using the standard samples of Cr_2O_3, titanium, silica, and $CaSiO_3$, as described by Statham.[15] Afterwards, these same specimens were examined with a JEOL scanning electron microscope (Model JSM 5600 LV, Tokyo, Japan), operating at a 1000× magnification.

Panelists and Ethical Aspects

Fifteen healthy adults participated in the study (ages 21-30 years). The volunteers were selected according to the following inclusion criteria: good general and oral health, normal salivary flow rate, absence of antibiotic use for two months before the experiment, absence of prosthesis or orthodontic devices, no signs of gingivitis or caries, and ability to comply with the study.[13] Visual oral examinations were carried out by an experienced dentist. All of the volunteers agreed to participate and signed an informed written consent form. The study design was approved by the local Ethics Committee (protocol 136/2009).

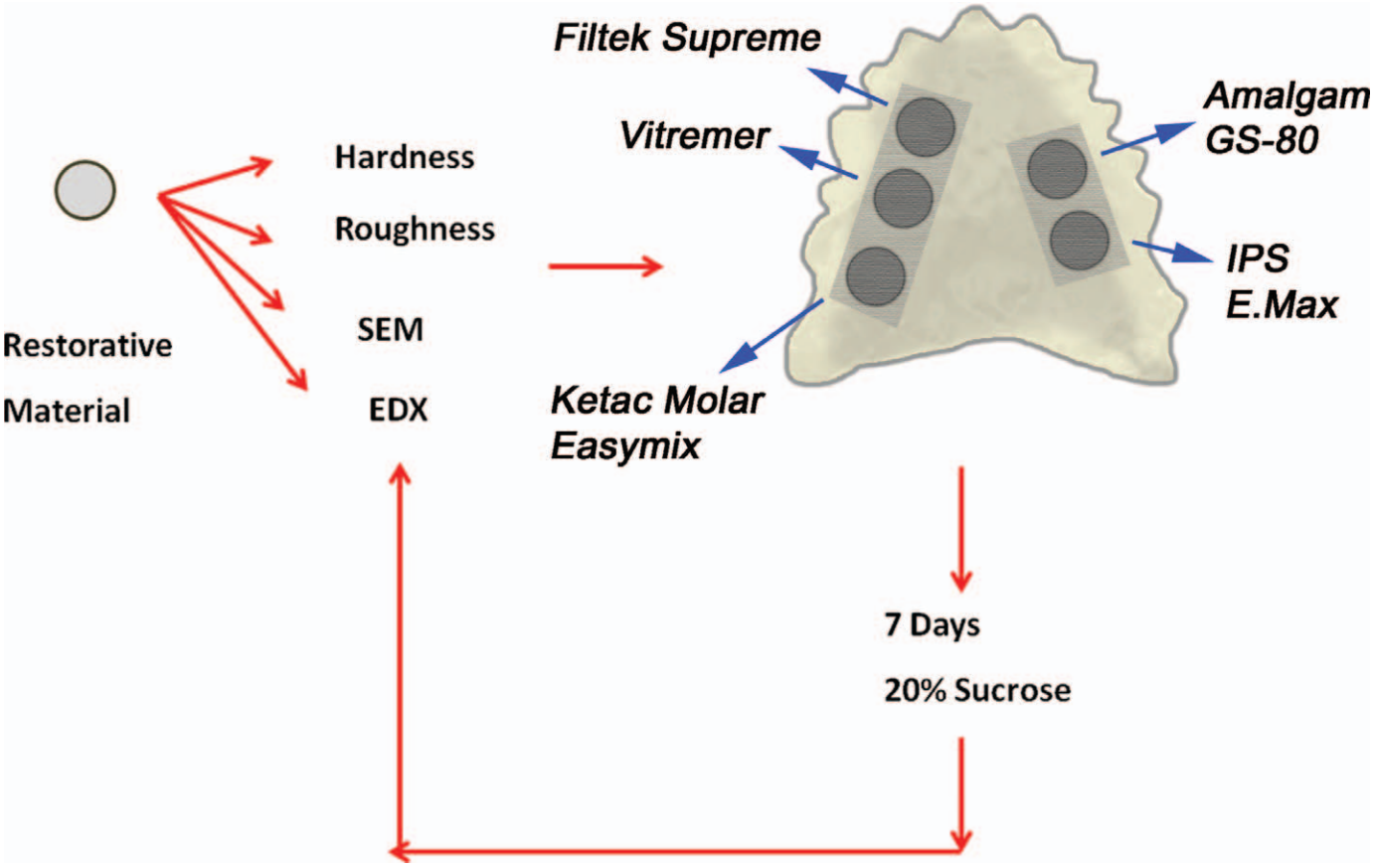

Figure 1. *Experimental design.*

In situ *Phase*

The volunteers' teeth were impressed with alginate (Jeltrate, Dentsply, Petrópolis, RJ, Brazil), and type III gypsum models were obtained. An individual intraoral acrylic resin palatal device, containing five 2.5-mm deep wells (one restorative material per well) was prepared for each volunteer,[9] as shown in Figure 1. A plastic mesh was fixed on two sides of the intraoral device, leaving a 1-mm space for the accumulation of the dental biofilm on the specimens. To assure their acceptability for the study, oral and written instructions of the *in situ* protocol were given to the volunteers before receiving the intraoral devices. There were no restrictions on the volunteers' diet. The only recommendation was to remove the device during meals and before ingesting any beverages or food, and to keep the oral devices moist in plastic boxes provided by the researchers. Volunteers were instructed to perform oral hygiene three times per day with a standardized fluoride dentifrice (1100 mg F/g as NaF). Only the palatal region of the device was extraorally brushed to avoid disturbing the biofilm. The cariogenic challenge was provided by the application of a 20% sucrose solution extraorally on the specimens (10×/day). The volunteers removed the devices from the mouth, excess saliva was cleaned with gauze, and one drop of the solution was dripped onto each specimen at 8:00, 9:30, 11:00, 12:30, 14:00, 15:30, 17:00, 18:30, 20:00, and 21:30 hours.[16] The sucrose was gently dried after 5 minutes and the device was reinserted into the mouth. After seven days, the specimens were carefully removed from the device and washed in an ultrasonic bath for 10 minutes; the final measurements for roughness, hardness, and surface morphology were then performed.

Statistical Analysis

The measurements were analyzed by using the Kolmogorov-Smirnov test at a 5% level of significance to assess the normality of the distribution. A methodology of mixed models for repeated measurements and Tukey-Kramer statistical tests at a 5% level of significance were used with a PROC MIXED SAS statistical software (Cary, North Carolina, USA).

Table 2: *Surface Roughness Means (SD), in μm**

Groups	Biodegradation	
	Baseline	7 Days
Filtek Supreme	0.34 (0.07)Bc	1.74 (1.51)Aab
Vitremer	0.62 (0.17)Bb	1.87 (0.99)Aab
Ketac Molar Easymix	0.57 (0.17)Bb	1.37 (0.83)Ab
Amalgam GS-80	1.70 (0.66)Aa	2.81 (1.13)Aa
IPS e.max	0.86 (0.45)Bb	2.40 (2.19)Aa

** Means followed by different letters (upper-case letters in each row and lower-case letters in each column) differ significantly (p≤0.05). Kolmogorov Smirnov and Tukey-Kramer statistical tests.*

Table 3: *Surface Hardness Means (SD), in VHN**

Groups	Biodegradation	
	Baseline	7 Days
Filtek Supreme	105.47 (2.09)Ac	101.27 (4.93)Ac
Vitremer	62.57 (6.00)Be	73.73 (7.25)Ad
Ketac Molar Easymix	81.59 (3.53)Bd	105.03 (5.95)Ac
Amalgam GS-80	129.45 (5.92)Bb	161.39 (27.13)Ab
IPS e.max	581.05 (37.24)Aa	577.69 (21.41)Aa

** Means followed by different letters (upper-case letters in each row and lower-case letters in each column) differ significantly (p≤0.05). Kolmogorov-Smirnov and Tukey-Kramer statistical tests.*

RESULTS

Tables 2 and 3 show the mean and standard deviations of the roughness and hardness, respectively, for each material before and after biodegradation, *in situ*.

All esthetic restorative materials studied showed a significant increase in the roughness after the biofilm/material interaction. Only AM showed no statistical difference between the periods analyzed. Before the biodegradation, AM presented the highest roughness, followed by VI, KM, and EM, with FS showing the lowest. However, after biodegradation, AM and EM had a higher roughness than KM, while FS and VI presented intermediate roughness and had no statistical difference with the other materials.

It was observed that before the biodegradation, the hardness was statistically different between the materials studied, with the following sequence: EM>AM>FS>KM>VI. VI, KM, and AM presented significant differences between the experimental periods, with higher values after the biodegradation period. However, FS and EM did not show significant differences between the periods. After biodegradation, the hardness was as follows: EM>AM>FS=KM>VI.

By EDX analysis, presented in Figure 2, the initial chemical composition of AM and EM was not altered by the action of the biofilm *in situ*. AM showed the presence of Hg, Sn, Ag, Si, and Cu, while EM showed Si, Al, K, and Na, among others. However, FS, VI, and KM did present alterations in their spectra. EDX results for FS revealed that before and after biodegradation, Si is present in the highest amount, followed by P and C. However, there was an adsorption of ions on the material surface, possibly originating from saliva. In VI and KM, Al, Si, and Ca were present in the highest amount before and after biodegradation; the quantity of F decreased after biodegradation. Furthermore, there was also the incorporation of ions such as Ca^{2+} and Cl^{-} on the surface of VI and KM.

The scanning electron micrographs in Figure 3 show details of the surface morphology of the studied materials. Materials are presented in rows and periods in columns. Regarding the resin-based materials (FS and VI), the polishing of the resin produced an irregular surface with the loss of the organic matrix and the exposure of the filler particles. Furthermore, the biodegradation produced an irregular surface coating, with the displacement of some filler in the organic matrix. KM also presented exposed fillers and cracks on the surface before the biofilm interaction and after the *in situ* experiment; the ionomeric material surface showed cracks and biodegraded areas with filler displacements, as shown by the arrows. For AM, a subtle corroded aspect after the surface degradation was observed, while EM showed an increased amount of surface cracks associated with an increase in the size of the nodules when compared with the specimen before biodegradation.

DISCUSSION

The success of restorative procedures depends on many factors, from treatment planning and a patient's adequacy, to clinical steps and subsequent preservation and maintenance of the restoration performed. Thus, it is important to carefully select a restorative material able to withstand the functional force and chemical environment of the oral cavity. Fundamentally, the factors known to cause surface damage to restorative materials include low pH due to cariogenic biofilm, consumption of acid drinks or foodstuffs, and the action of enzymes, all of which can soften the outermost layers and damage restorative materials.[5,8,17-21]

Most conditions of the oral cavity can be simulated by an *in situ* study, such as saliva properties (salivary flow, buffer capacity, clearance, mineral and protein

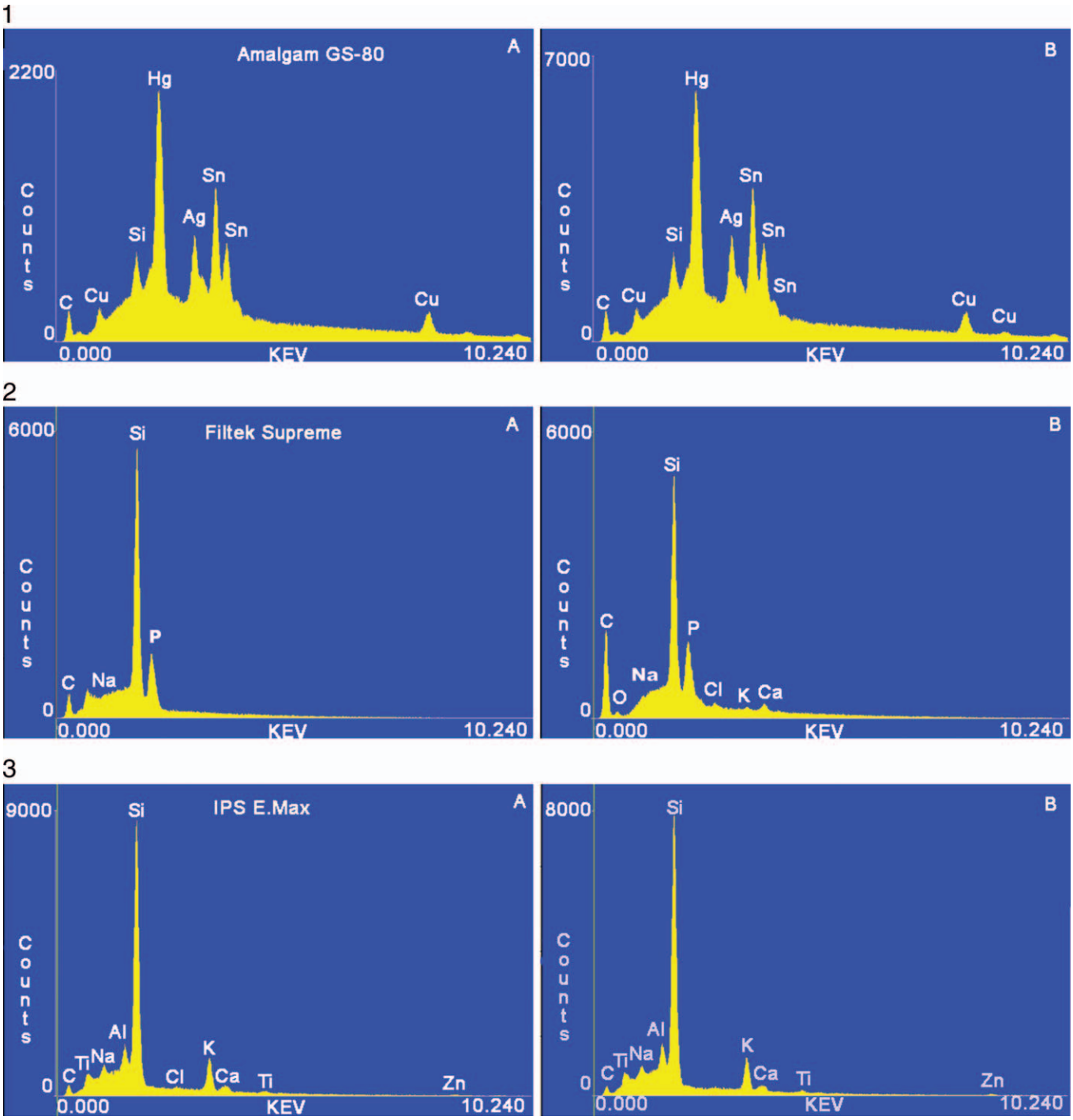

Figure 2. *EDX spectra of different restorative materials compared before (A) and after (B) biodegradation.*

content, and enzymes), biofilm accumulation (diversity of species, microorganism selection, succession, nutrient availability, and competition), temperature fluctuations, and aqueous environment, among others. However, little information is available regarding the surface degradation of restorative materials after the interaction with biofilms *in situ*. Among the available *in situ* studies, a focus was given on the evaluation of either the biofilm characteristics or the restorative material.[8,13,14] In this context, the present study allowed for the development of biofilm on different restorative materials using the *in situ* model

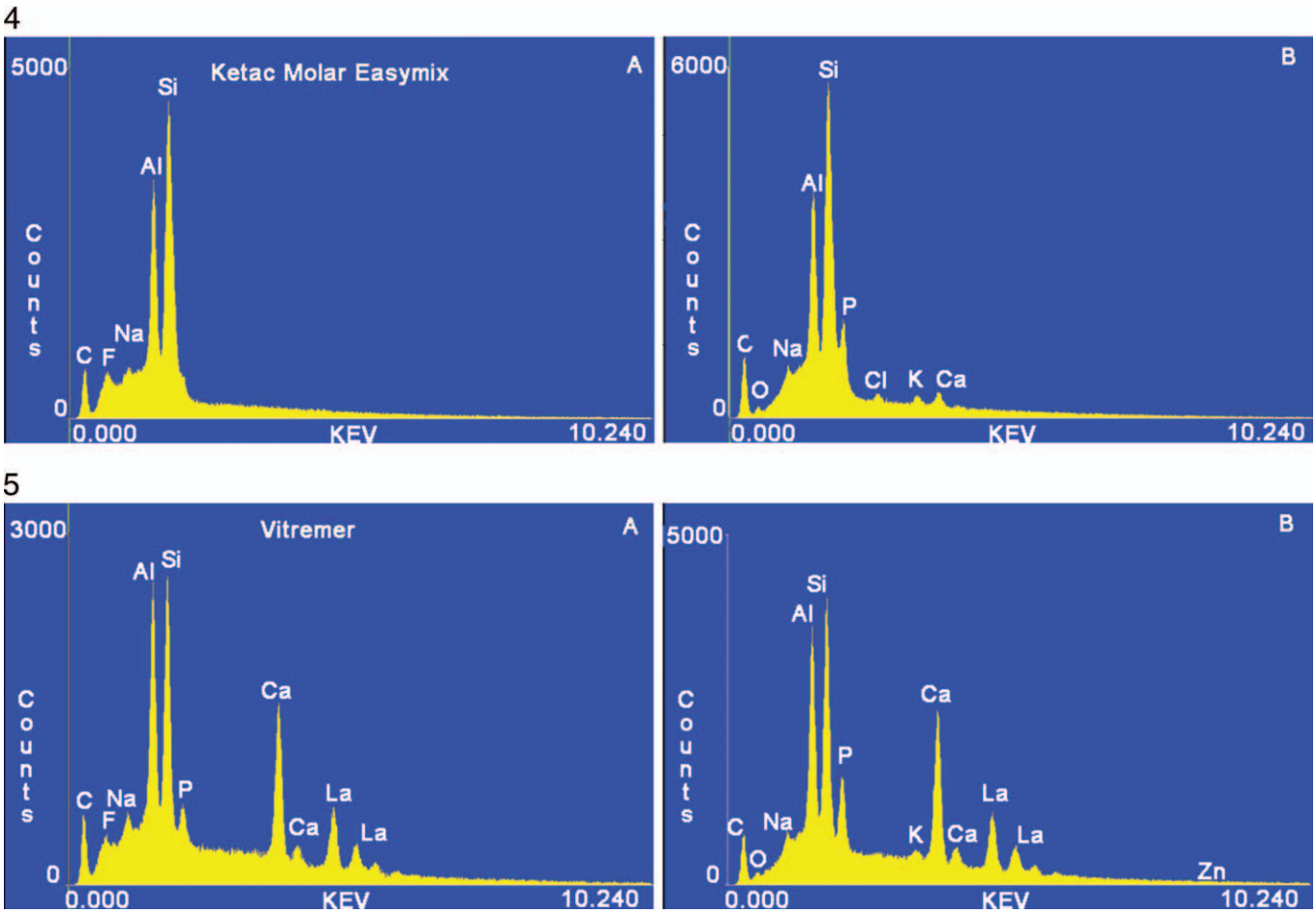

Figure 2. *Continued*

in order to analyze the consequences of this bio-interaction on the materials' surface, under frequent biodegradation.

Different groups of restorative materials (amalgam, composite resin, glass ionomers, and ceramic) were selected due to studies that suggested that the biofilm accumulation and biodegradation intensity are influenced by the surface upon which it is developing, which is directly related to the physical and chemical properties of the restorative materials.[8,14,22,23] Furthermore, the selected materials are representative of the different classes of restorative materials used in odontology, as well as of the classes established in materials science (ie, metals, polymers, ceramics, and composites).[24]

All materials were handled according to their manufacturer's recommendations, including photoactivation, setting time, and polishing procedures. The polishing procedure was performed since it improves the esthetic characteristics and the durability of the restoration, decreases the porosity of the surface, decreases the surface staining, and also improves its mechanical properties.[25] Furthermore, the polishing removes the organic matrix of different restorative materials and exposes the fillers particles, a fact that is confirmed by the scanning electron microscopy (SEM) images taken before biodegradation. These micrographs show that the polishing produced some scratches on the composites and amalgam surfaces, removing the matrix and exposing particle fillers on the direct restorative materials studied (Figure 3).

All esthetic materials in this study showed an increase in the roughness after the biofilm activity. The acid attack by bacterial metabolism can cause biodegradation through different ways for restorative materials. For Filtek Supreme, there is a release of TEGDMA and UDMA monomers from the resin matrix when it is in contact with salivary enzymes and bacterial acids.[26] During biodegradation, Vitremer releases HEMA, a highly hydrophilic cosolvent and the main component released from the

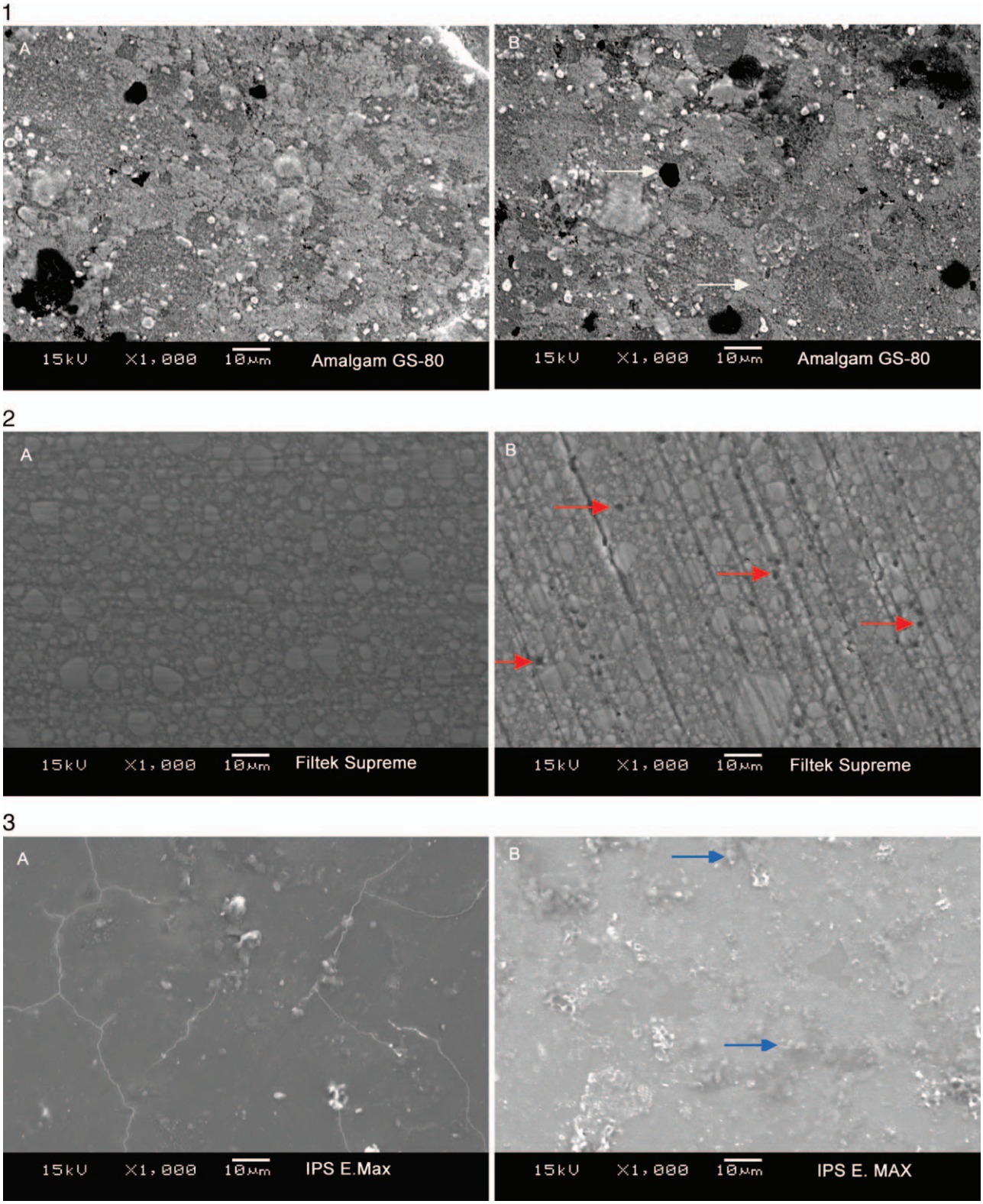

Figure 3. *SEM images of different restorative materials. Left, before biodegradation; right,after biodegradation (1000×). Red arrows show filler particles removed from the organic matrix; black arrows show cracks; white arrows show various phases; and blue arrows show surface nodules.*

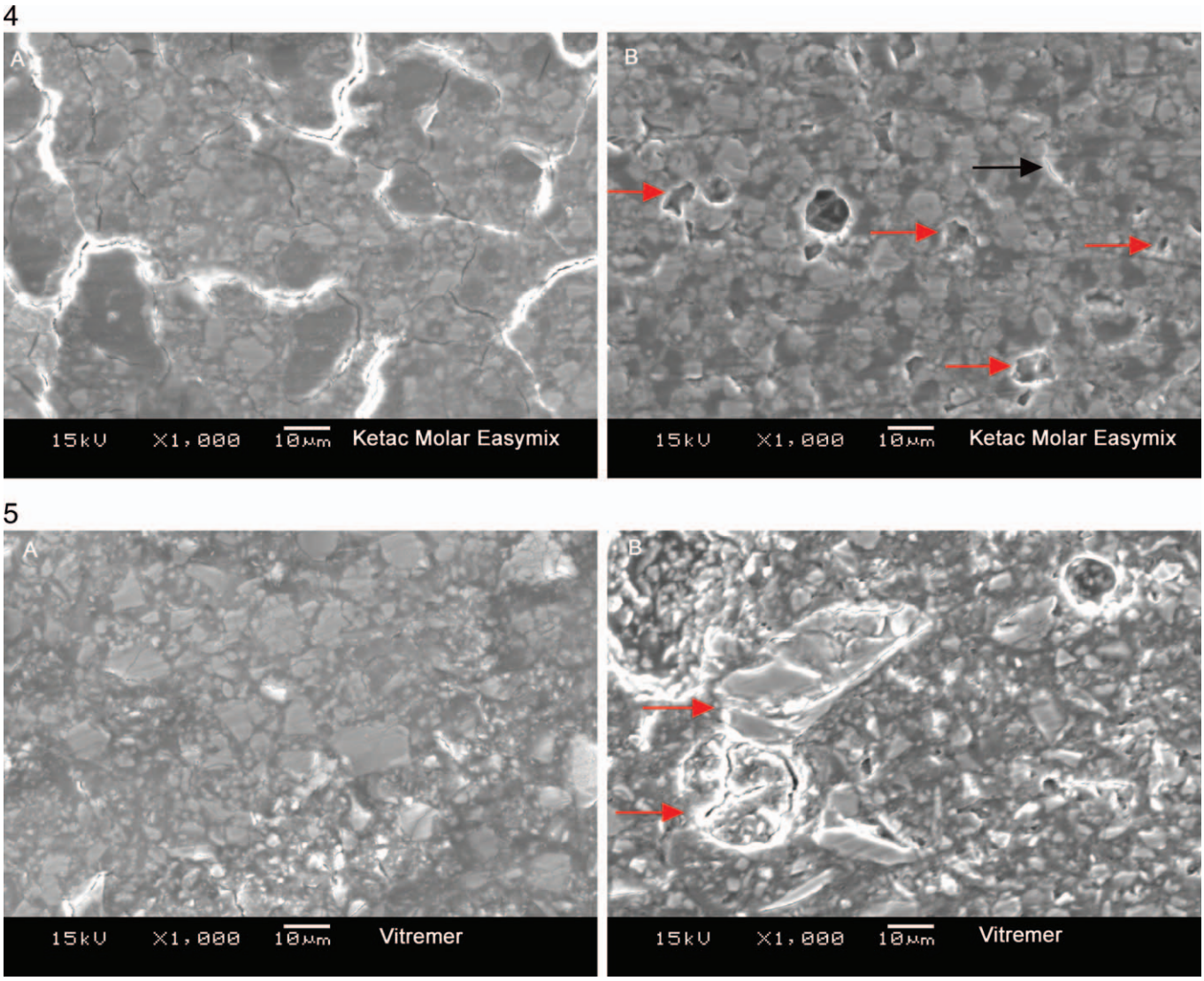

Figure 3. *Continued*

organic phase.[27] Glass ionomer cements withstand a complex process of absorption, disintegration, and outward transportation of ions, with an erosive loss of matrix components and leaching of glass particles. This process (absorption, disintegration) is more intense in acid medium.[6,28,29] As the biodegradation softens the organic matrix and releases ions from ionomeric materials, it is possible that the loss of components from two Vitremer matrixes (polyacrylate-inorganic and polymer-organic) and from the organic matrix of Filtek Supreme leads to changes in the roughness.

For the dental ceramic, the initial increased roughness may be related to the characteristics of the polishing phase and not necessarily to the biodegradation. This result was not expected because the ceramics are considered the most inert of all dental materials used for restoration.[30] There are fundamentally two types of glaze application: the autoglaze and the overglaze. Fahmy and others[31] observed a crack length significantly smaller for the autoglaze group than for the overglaze group, while Atay and others[32] showed greater color stability for the autoglaze specimens than for the overglaze specimens; Zaki and Fahmy[33] showed that bleaching agents did not affect significantly the roughness of the autoglaze group. Thus, the autoglaze appears to have a more resistant surface to biodegradation than the overglaze, which is the one used in this study. In this way, the vitreous ceramic of the glaze possibly suffered biodegradation due to the increase of roughness. Besides, Chang and others[34] observed

an increase in particle grit sizes for ceramics, which form nodules, thus corroborating with the formation of nodules observed in our SEM images. This event possibly occurs due to the poor thermal conductivity of porcelain associated with the formation of large temperature spikes at the point of contact between the diamond bur and the porcelain.

However, the roughness of the amalgam did not alter significantly after the biodegradation, possibly due to some factors related to this material's characteristics: a high copper content, spherical copper particles, polishing, and a passive layer on the surface. The high copper content (>6%) was achieved through the optimization of amalgam alloys by Innes and Youdelis[35]; the introduction of spherical copper particles was further performed by Asgar[36]; the polishing leads to a substantial increase in the corrosion resistance once it removes the tinmercury alloy (gamma-2 phase) and decreases the concentration of electrolytic cells[37]; and, finally, the passive layer formed on the surface also contributes to the improvement of the corrosion resistance.[38] Considering the last aspect, studies on the mercury liberation from dental amalgams suggest the formation of a passive layer that is composed of an oxide film on the material surface, which interferes with the dissolution process of the metal components and substantially diminishes their lixiviation.[38-41] Furthermore, it is important to note that seven days is a relatively short time to promote considerable corrosion on silver amalgams.

Regarding the hardness, it was observed that the amalgam and ionomeric materials presented an increase in their hardness values after the biodegradation experiment, probably related to a posthardening process after the setting time. For ionomeric materials, this process could be explained by the slow rate of the acid-base reaction forming the polyacrylate salts (KM and VI) and the free-radical polymerization reaction (VI), which continued after light-irradiation.[42,43] A maturation over time could also occur with the amalgam. During the trituration process, the mercury dissolves the surface of the alloy particles and a plastic mass is formed by the setting and hardening of the amalgam. The amalgam crystallization can continue for several days,[30] according to our results.

In a different way, the rapid setting reaction of the resin composite is initiated by light exposure, and most parts of the conversion process end immediately after the photoactivation, leading to a reduced postirradiation polymerization.[44,45] Moreover, the presence of Bis-EMA and TEGDMA in the matrix composition possibly contributed to the hardness stability of Filtek Supreme. TEGDMA can decrease the surface softening caused by acids and increase the degree of polymerization of resin-based material,[46] while Bis-EMA showed a lower amount of released products and a higher stability.[47] In the sintering process of ceramics, the compacted particles suffer a coalescence phenomenon that leads to the increase of the solid density. Consequently, the hardness of these materials undergoes a significant increase as the mechanical integrity of the body is favored. However, after the sintering process, the hardness of these materials tends to suffer little alteration after the setting reaction,[31,48,49] corroborating our results.

According to the EDX results, the initial chemical composition of AM and EM was not significantly altered after biodegradation, while FS, VI, and KM did present alterations in their spectra. Thus, it was observed that the fluoride released from KM and VI after seven days possibly occurred due to their intrinsic characteristics, resulting in dissolution and diffusion processes, which occur mainly in an acid medium.[20,50] Furthermore, there was the adsorption of ions such as Ca^{2+} and Cl^{-} on the surface of some of the materials (FS, VI, and KM), probably from saliva, after the *in situ* experiment.

In the oral environment, an established or mature biofilm can accumulate at stagnant sites, such as interproximal surfaces, pits and fissures, and gingival crevices, beyond compatible levels of oral health.[51] This can develop into disease conditions, such as secondary caries, as well as into the demineralization process of marginal enamel and dentin.[52] Therefore, it would be important to assess patients individually regarding their salivary flow, caries risk, buffer capacity of saliva, diet and oral hygiene, among others, and then carefully select the restorative material for intraoral sites where the biofilm would be protected against dynamic shear forces from saliva and tongue and toothbrushing, which stimulates its accumulation and maturation.

The hypothesis that restorative materials subjected to a biofilm interaction have a significant difference on roughness, hardness, and microstructure, after seven days has to be partially accepted since there was a material dependence among the characteristics analyzed.

CONCLUSIONS

In conclusion, within their limits, the present findings show that the influence of dental biofilm

accumulation *in situ* on different restorative materials is a material-dependent event. All of the materials changed after biodegradation *in situ*. Thus, all esthetic restorative materials showed increased roughness, confirmed by SEM, while ionomer materials and the silver amalgam showed significantly higher hardness. The initial chemical composition of the composite resin and ionomer materials evaluated was significantly altered by the action of the biofilm *in situ*.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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