

Abrasion of 6 dentifrices measured by vertical scanning interference microscopy

Florence PASCARETTI-GRIZON¹, Guillaume MABILLEAU^{1,2}, Daniel CHAPPARD¹

1- LUNAM University, Groupe Études Remodelage Osseux et bioMatériaux (GEROM – LHEA), Institut de Biologie en Santé, Centre Hospitalier Universitaire d'Angers, Angers, France.

2- LUNAM University, Service Commun d'Imagerie et Analyses Microscopiques (SCIAM), Institut de Biologie en Santé, Centre Hospitalier Universitaire d'Angers, Angers, France.

Corresponding address: Daniel Chappard - GEROM - LHEA - Institut de Biologie en Santé - LUNAM Université - Centre Hospitalier Universitaire d'Angers - Angers - France - Phone: (33) 244 688349 - Fax: (33) 244 688351 - e-mail: daniel.chappard@univ-angers.fr

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ABSTRACT

Objectives: The abrasion of dentifrices is well recognized to eliminate the dental plaque. The aims of this study were to characterize the abrasive powders of 6 dentifrices (3 toothpastes and 3 toothpowders) and to measure the abrasion on a test surface by Vertical Scanning Interference microscopy (VSI). Material and Methods: Bright field and polarization microscopy were used to identify the abrasive particles on the crude dentifrices and after prolonged washes. Scanning electron microscopy and microanalysis characterized the shape and nature of the particles. Standardized and polished blocks of poly(methylmethacrylate) were brushed with a commercial electric toothbrush with the dentifrices. VSI quantified the mean roughness (Ra) and illustrated in 3D the abraded areas. Results: Toothpastes induced a limited abrasion. Toothpowders induced a significantly higher roughness linked to the size of the abrasive particles. One powder (Gencix[®]) produced a high abrasion when used with a standard testing weight. However, the powder is based on pumice particles covered by a plant homogenate that readily dissolves in water. When used in the same volume, or after dispersion in water, Ra was markedly reduced. Conclusion: Light and electron microscopy characterize the abrasive particles and VSI is a new tool allowing the analysis of large surface of abraded materials.

Keywords: Toothpaste. Toothpowder. Tooth abrasion. Interference microscopy. Surface properties.

INTRODUCTION

Since the ancient times, the incorporation of abrasive powder in toothpastes or powders has been done. Various abrasive materials have been proposed such as bone ash (which is mainly composed of β -tricalcium phosphate³), coral, shell, marble or pumice powders. These powders are held either in suspension in a pasty thickening hydrogel containing a wetting agent in the case of toothpastes or mixed with other powders such as metal oxides, phosphates or plant extracts¹¹. Several studies have been conducted to evaluate the abrasion of both dentifrices and toothbrush filaments⁸. It is classical in the dental literature to compare the abrasion of different types of toothpastes' formulations and in 1995, the ISO 11609 standard was proposed following the original

works of Hefferen on Radioactive Dentine Abrasion (RDA)¹². Briefly, permanent teeth from <40 y.o. subjects are used to prepare radioactive dentine specimens following ³²P irradiation. The specimen surfaces are then brushed with an electric toothbrush under standard procedures (time, tension...). The toothpastes are compared with values obtained with a reference abrasive (calcium pyrophosphate in an hydrogel composed of glycerol and carboxymethyl cellulose)¹⁰. At the end of brushing, the slurries are vortexed and put in a scintillation counter for radiation detection and abrasion is expressed in count *per* minute divided by the weight of dentine sample. Because the method is difficult and requires health hazardous procedures, other techniques have been proposed such as contact or non-contact profilometry^{1,22}.

The aims of the present study were 1) to characterize the abrasive powders entering in the composition of a variety of dentifrices by light and scanning electron microscopy¹³; 2) to measure the abrasion of each formula on polished poly(methylmethacrylate) (PMMA) surfaces using Vertical Scanning Interference microscopy (VSI).

MATERIAL AND METHODS

PMMA blocks

PMMA blocks were prepared by using a purified monomer containing 1% of the polymerization accelerator benzoyl peroxide. 0.2% of the substituted amine N-N dimethylaniline were added to initiate polymerization⁵. The accelerated and initiated mixture was poured in Peel-a-way embedding molds (25 ml/mold - Polyscience Inc., Warrington, PA, USA) and polymerization took place in a cold environment (+4°C in a refrigerator with a water bath to limit the temperature peak). Because polymerization is not completed when the blocks have hardened, a one month period separated their preparation from further use¹⁷. Blocks were then ground on an automatic plane grinding machine Tegramin-30 (Struers, Copenhagen, Denmark) using ascending grads of polishing paper (1200, 2000 and 4000 during 2 min each). Final mirror polishing was made with a MD-Nap paper spray with DiaPro

1 µm solution. Polished PMMA blocks were affixed onto plastic slides with a hand-press (Struers) in order to have a perfectly horizontal surface parallel to the slide.

The tooth brushing system

A commercial electric toothbrush was used in this study (Oral-B®, Braun, France) with the following conditions: load of the toothbrush standardized at 250 g (verified with the internal pressure control of the brush), brushing time: 2 min (as recommended by the manufacturer); soft hardness toothbrush head, rotation sense changing every 30 sec. For each type of dentifrice, a new toothbrush was used after an overnight incubation in distilled water. The toothbrush head was in direct contact with the PMMA block and the dentifrice. For the toothpastes, 0.2 g were weighted on a high precision scale, diluted in 500 µl of distilled water and applied directly on the PMMA block with a syringe. For one of the powder (Gencix®) with a very low mass-weight, the volume used appeared considerable (more than a pea: trial "Gencix® full volume") and a smaller volume (similar to those used with the other powders) was used in an additional trial (trial: "Gencix® reduced volume"). A third trial was done according to the manufacturer instructions where the toothbrush is soaked in powder-containing water as follows: a volume of toothpaste equivalent to a pea (i.e.

Name	Mass density (g/cm ³)	Main components	Conservative	Abrasive	stain
Parodontax®	2	Plant extracts (rathania, myrrha, chamomilla, mentha, salvia) glycerol, cocamidopropyl betain, alcohol	Na benzoate	Na bicarbonate	Iron Oxide
Denivit®	1	Sorbitol, propylene glycol, pentasodium triphosphate, cellulose gum, PEG 60, castor oil, NaF, Na saccharin	Methylparaben	Hydrated silica, Aluminum oxide	TiO ₂ (CI 77891)
Colgate Total®	1.33	Glycerol, sorbitol, Na lauryl sulfate, PVM/MA copolymer, limonene, NaF, NaOH, Na saccharin, cellulose gum, carrageenan	Trichlosan	Hydrated silica	TiO ₂ (CI 77891)
Per Blan®	1.25	Plant extracts (chamomilla, salvia, melissa, hamamelis, potentilla, rosmarinus, glycyrrhiza, aloe, citrus) xylitol, Na methyl cocoyl taurate, NaCl	none	Ca Carbonate	none
Kontrol®	1	Sorbitol, Na lauryl sulfate mentha, PVP, Na undecylenate, Na saccharin, dimethicone	Methylparaben	Dicalcium phosphate	none
Gencix®	0.66	Plant homogenate (Carica papaya)	none	Pumice powder	none

Figure 1- Composition of the 6 dentifrices (3 toothpastes, 3 toothpowders) studied

~100 mg of the powder was dissolved in 1 ml of distilled water). The toothbrush was soaked into the mixture, then applied to the surface of the PMMA block (trial: "Gencix® diluted"). For each dentifrice, the experiment was conducted 3 times on 3 different blocks. A control was done in triplicate with a new toothbrush moistened with 500 µl of distilled water.

The dentifrices

Three toothpastes were investigated: Colgate Total® (Unilever, Rueil-Malmaison, France); Denivit® (Schwartzkopf & Henkel, Boulogne-Billancourt, France), Parodontax® (Glaxo-Smith-Kline GSK, Marly-le-Roi, France). Three toothpowders were analyzed in parallel: Kontrol® (Omega Pharma, Chatillon, France); Per Blan® (Per Blan, Paris, France) and Gencix® (Esprit d'Ethique, Nantes, France). The raw composition of each dentifrice (as provided by the manufacturers) appears on Figure 1 together with its mass density. For each dentifrice, an analysis was conducted by light and electron microscopy. First, a small amount of paste or powder was placed on a microscopic glass slide, covered with a thin glass lamella and imaged by bright field illumination. Then, 0.5 g of each dentifrice was dissolved in 10 ml of distilled water and stirred for 5 min with a magnetic stirrer to dissolve all the organic/inorganic soluble components. The mixture was centrifuged, the supernatant was discarded and the residue was washed several times with distilled water. Finally, a drop of the residue was placed on a glass slide as previously. Light microscopy was performed on an Olympus BX51 microscope (Olympus, Rungis, France) using bright field and polarized light illumination at a magnification of 100x. Residues were also placed on a brass stub, carbon-coated by sputtering (10 nm thick) and analyzed with a Zeiss EVO LS10 scanning electron microscope (Carl Zeiss Ltd, Nanterre, France) equipped with an energy X-ray microanalysis system (INCA XMAX, Oxford Instruments, Oxford, UK) to determine the atomic composition of the non-soluble abrasive component.

The Vertical Scanning Interference microscopy (VSI)

Measurements were done using a Wyko NT 9100 vertical scanning interference microscope (Bruker Nano Surface, Palaiseau, France), a system originally dedicated to the measurement of roughness in material samples. VSI is based on light interferometry and operates as a non-contact optical profiler in vertical scanning mode to produce 3D topography maps of the sample surface. Briefly, a white light source is emitted by conventional light source and is split into two beams which pass through a Mirau's interferometry objective. The incident beams are reflected from the reference mirror and the sample surface respectively. The light reflected from this

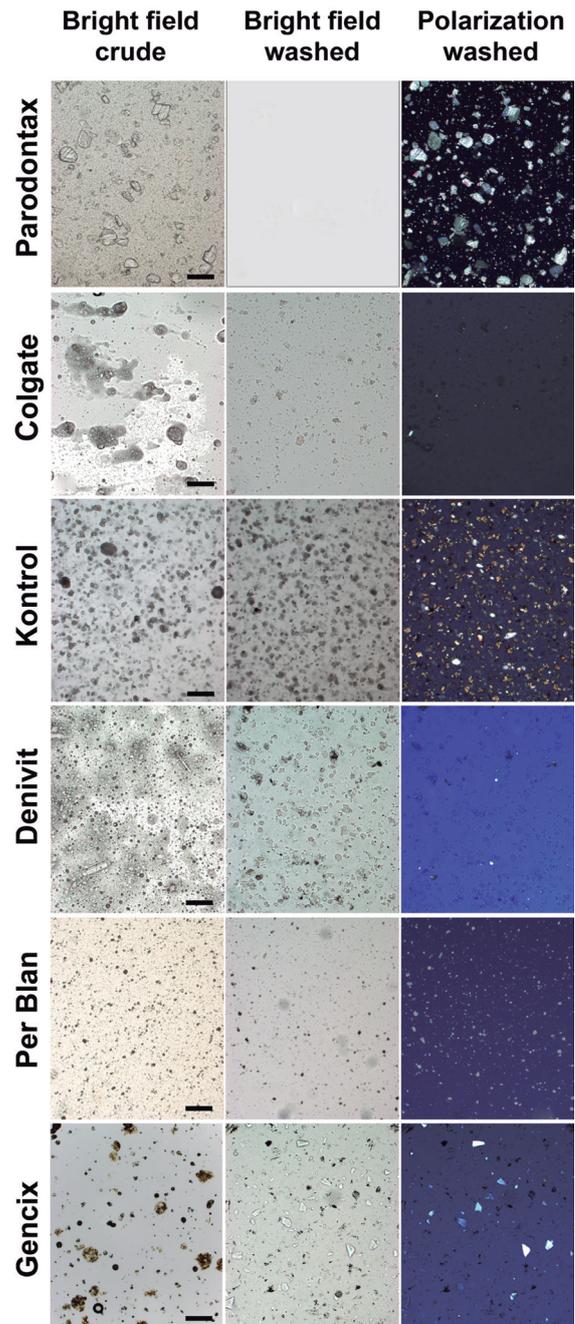


Figure 2- Light microscopic aspect of the six dentifrices studied at the same magnification (100x; the bar stands for 200 µm). For each of them, the image of the 1st column corresponds to the appearance of the crude dentifrice under glass slide and coverslip in bright field microscopy. The 2nd column corresponds to the abrasive powder obtained after extensive washes and examined under glass slide and coverslip in bright field microscopy. Note the considerable differences in size and shape of the particles. In the 3rd column, the same field is examined in polarized light to see the aspect of the abrasive powder (excepted for Parodontax®, whose abrasive particles were dissolved during the washing steps, the image correspond to the polarization aspect of the 1st column). Note that abrasive particles can be birefringent or not

mirror combines with the light reflected from the sample to produce interference fringes (known as interferogram) where the best-contrast fringe occurs at best focus. The light and dark fringes are used in combination with the wavelength of the light to determine height difference between each fringe. A piezo-electric stage moves the sample vertically with a nanometer precision, which produces phase shifts in the interferogram. Interferograms were digitized using a CCD camera and data were analyzed to produce topographic surface maps at a magnification of 5x. For each block, 3 images (1.0x1.3 mm) were obtained in the 4 quadrants of the brushed surface (internal, medium and outer position). The software

Vision™ (release 4.10, Wyco) was used to acquire the data and compute the mean roughness (R_a , expressed in μm) on each image. For each block, the values were averaged on the 12 images and the mean value obtained on the 3 blocks was considered; so for each trial, the analyzed surface was 46.8 mm^3 .

Statistical analysis

Statistical analysis was performed using the Systat statistical software release 13.0 (Systat Software Inc., San José, CA, USA). All data were expressed as mean \pm standard deviation. Differences between groups were analyzed by a non-parametric ANOVA test (Kruskal-Wallis) with the Conover-

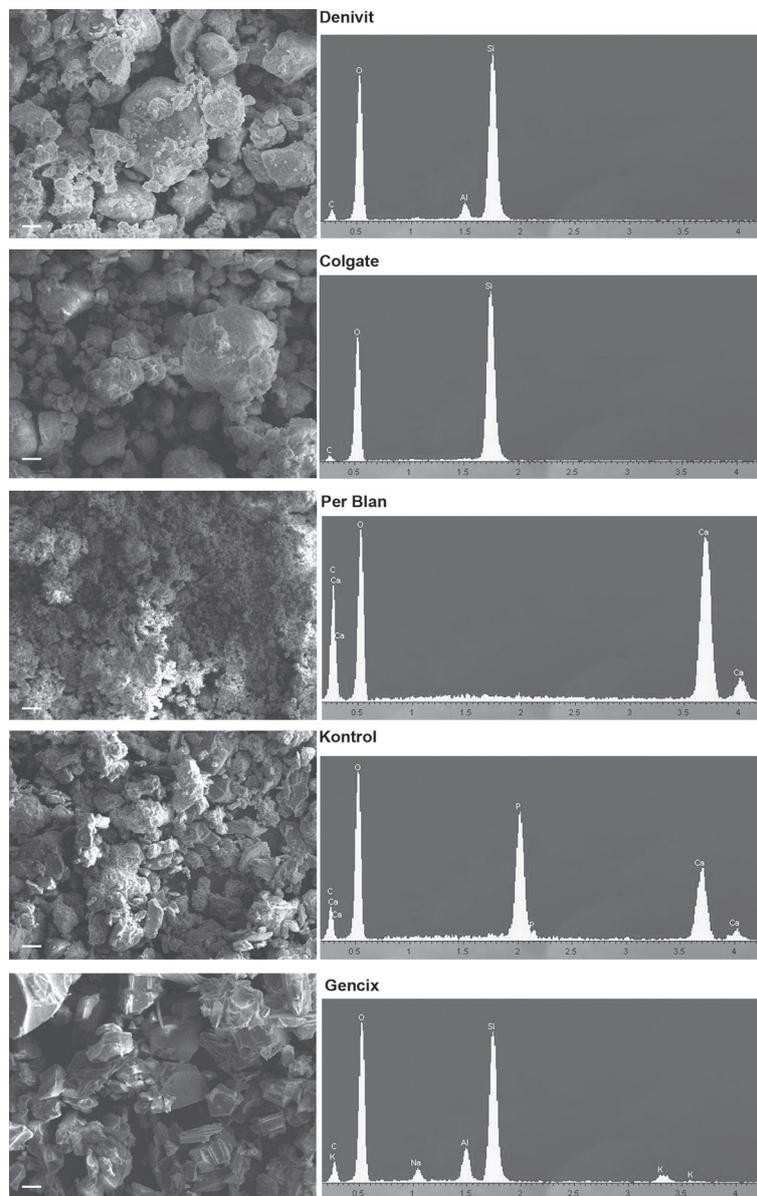


Figure 3- Scanning electron microscopy of the abrasive grains contained in the different types of dentifrices and obtained after extensive washing and centrifugation. The EDX spectrum of each type of particle appears on the right. All images were obtained at the same magnification (500x) and the white bar stands for $10 \mu\text{m}$. Note the considerable differences in shape and composition of the abrasive particles contained in the six dentifrices. The NaHCO_3 particles of Parodontax®, having being solubilized during the preparation steps, are not figured

Inman *post-hoc* test. Differences were considered significant when $p < 0.05$.

RESULTS

Light microscopy

The light microscopy aspect of the different dentifrices appears on Figure 2. All dentifrices contained inorganic particles used as abrasive but the size of the grains considerably differed between them. The larger grains were observed for Parodontax® and the smaller for Colgate Total® and Per Blan®. Dilution and washing of the different dentifrices was used to separate and enrich the slurry in these abrasive particles from the other components. However, the highly soluble grains of sodium bicarbonate contained in Parodontax® were completely dissolved. Polarization microscopy identified birefringent and non birefringent characteristics of the abrasive powders. Some dentifrices (Colgate Total®, Gencix®, Denivit®) appeared to be composed of both types of particles. Others (Per Blan® and Kontrol®) contained only birefringent ones. The NaHCO_3 particles of Parodontax® were birefringent in the undiluted phase.

Scanning electron microscopy

The image of the different types of abrasive grains appears on Figure 3 for all dentifrices

(except Parodontax®, in which the particles were dissolved during the preparation steps). The EDX analysis confirmed the presence of silicate particles only in Colgate Total®. Calcium oxide was evidenced in Per Blan® as a fine deposit. Calcium phosphate constituted all the particles of Kontrol®; Denivit® contained both silicates and aluminum oxides and the Gencix® particles coming from pumice were composed of sodium and potassium silicates together with traces of aluminum oxides.

VSI analysis

The surface of each PMMA block exhibited signs of abrasion even when the brush was used with distilled water. Typical images of eroded surfaces are illustrated in Figure 4. The statistical results for the surface roughness parameter R_a of the different dentifrices are graphically presented in Figure 5. R_a did not differ significantly between toothbrush without dentifrice and with Parodontax® or Gencix® used in reduced volume. Denivit® and Colgate Total® produced significantly higher erosion (resp. $p < 0.006$ and $p < 0.002$). The toothpastes were associated with a significant higher R_a than the different toothpastes or the brush alone ($p < 0.0001$) except Gencix®, used in reduced volume. Considerable differences exist when using the different modes for using Gencix® and the full volume trial induced the marked abrasion.

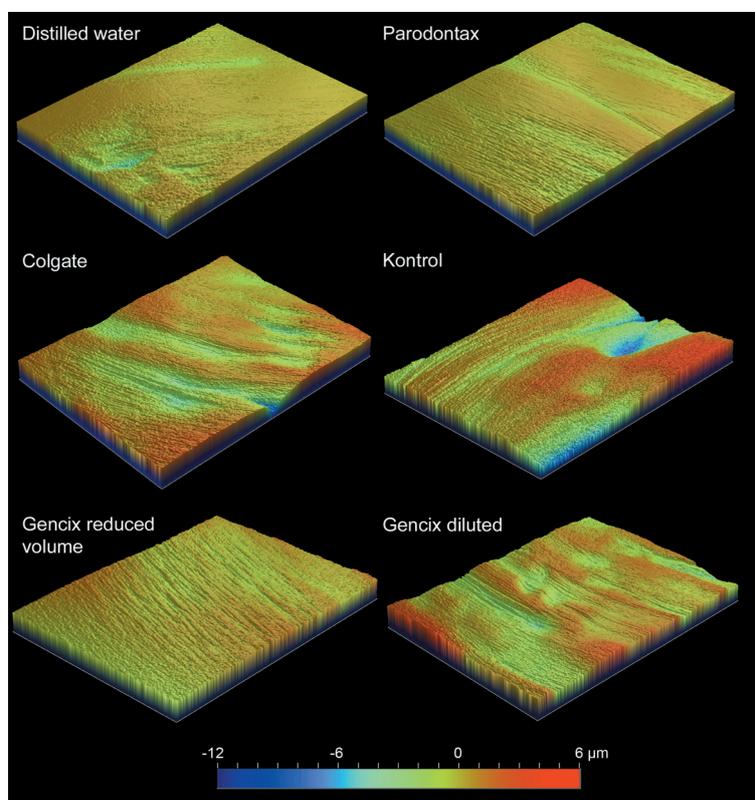


Figure 4- Vertical scanning interference microscopy of the surface of PMMA blocks after brushing with different types of dentifrices. Each image corresponds to a 1x1.3 mm surface area. The colored scale bar (ranging from -12 to +6 μm) represents the abrasion depth

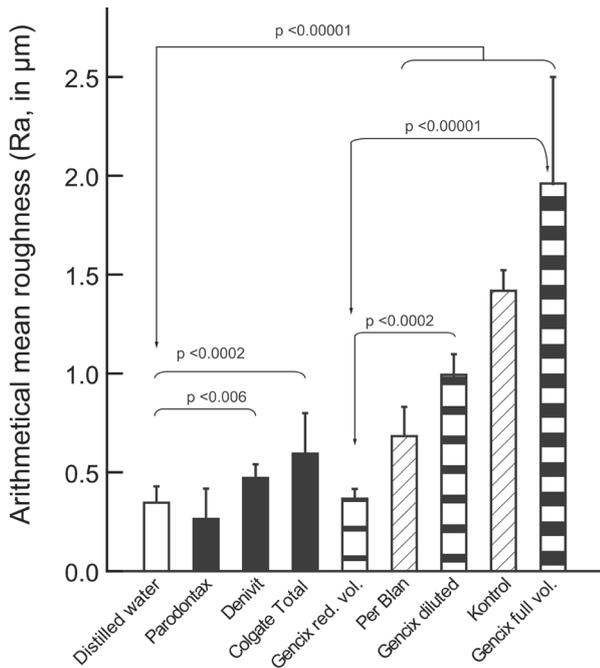


Figure 5- Surface roughness analysis after brushing with the different dentifrices. Bars correspond to the average value obtained on the 3 blocks analyzed per dentifrice (12 images *per* block). Data are expressed as mean±standard deviation. Toothbrushes used with distilled water: white bar; with toothpastes: black bars; with toothpowders: patterned bars. The different ways of using Gencix® appear with different types of horizontal patterned bars

DISCUSSION

The aims of the present study were to analyze the abrasive powders contained in 6 different dentifrices (3 pastes and 3 powders) and to evaluate their abrasive capacity on a synthetic polymer – PMMA – widely used in dentistry.

It is well recognized that the capacity of a dentifrice to abrade can be attributed to the nature and size of its particulate abrasive components^{20,25}. Here, we found that Parodontax®, which contains large grains of sodium bicarbonate, did not produce a significantly rougher surface than the brush used with distilled water alone. It is likely that the high and rapid solubility in water of this compound (mainly used as a whitening agent that is fully soluble in water) had no significant effect on abrasion¹⁵. Bright field microscopy revealed that all grains were rapidly dissolved in water and could not be observed by polarization microscopy or SEM. Denivit® and Colgate Total® which contained some insoluble abrasive grains had a low but significantly higher abrasion on PMMA blocks. All toothpowders which contained insoluble abrasive grains were associated with a significantly higher effect than the control brushes with either distilled water or toothpastes. A

limitation of the present study is that the hardness of individual grains could not be determined. We were unsuccessful when trying to measure this parameter by nanoindentation due to the small size of individual grains and the impossibility to firmly anchor them for this type of analysis. It is likely that pumice powder grains are harder than calcium phosphate or calcium carbonate particles and the nature of the grain influences the abrasive powder^{20,25}.

The tooth brushing test was performed as recommended by the toothbrush manufacturer and it used the control system incorporated in the electric device to control the pressure and time of analysis. These parameters are those recommended in daily use^{4,24}. However, only a single 2 min brushing was used here contrary to a number of studies where prolonged brushing times or cycles are used^{2,18,25-27,29}. This short brushing time was chosen because 1) PMMA is by far less hard than enamel or dentine (i.e. the Young's modulus is 3.1 GPa and hardness 196 MPa for PMMA vs. 88 GPa and 4,800 MPa for enamel; 22 GPa and 800 MPa for dentine)^{9,19}; 2) VSI is a very accurate method used in industry to measure surface roughness of materials at the nanoscale level on larger surfaces than atomic force microscopy¹⁴; 3) PMMA is the major component of denture which are also cleaned by brushing^{2,6,7,20,23,25}. In addition, PMMA-based polymers are also used in veneers or soft liners which can also be abraded by dentifrices¹⁶.

We found that VSI provided reliable quantitative results and was able to image the surface of the material together with providing arithmetic mean roughness. The method has been used in only one paper to calculate Ra but no images were presented²². The toothbrush moistened with distilled water alone appeared to cause a limited abrasion of the polymer. It has previously been reported that the rigidity of the brush's bristles can influence the abrasion of synthetic acrylic polymers^{26,28}. Because all brushes used in this study were of the same model and only new brushes were used for each dentifrice, the abrasion due to the dentifrice itself is the real reason to produce additional scars at the surface of PMMA blocks. When the same weight of toothpaste was used, the abrasion noted on the PMMA blocks appeared roughly related to the size of the abrasive particles evaluated by bright field and polarization microscopy. However, as specified by the manufacturer, the Gencix® powder should not be used as a classical toothpowder. The pumice particles are mainly used to nucleate and facilitate the deposit of the plant homogenate and produce spherical capsules. As the plant homogenate is readily dissolved in water, only the pumice particles (composed of silicates and aluminum oxide) with sharp edges are liberated. So, when the test is done with a large amount of powder, this leads to the accumulation of a considerable volume of pumice

powder. When using the Gencix® toothpowder as recommended by the manufacturer (after dilution in water), the abrasion was in the same range than Per Blan® or Kontrol®. Because the mass density of the dentifrice is lower than the other powders, using the same volume of Gencix® powder did not produce significant erosion when compared to the brush used with distilled water or Parodontax®.

CONCLUSION

Bright field and polarization microscopy, together with SEM analysis, are simple methods to evaluate the morphology of the abrasive particles contained in a dentifrice, although these methods are only qualitative. VSI microscopy is a new powerful technique for the evaluation of material and biomaterial surface and to study their degradation at the nanoscale level^{19,21}. The technique allows both quantitative and qualitative evaluation of roughness. Abrasion due to a dentifrice appears linked to the amount and nature of the abrasive powder used.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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