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The Role of Different Toothpastes on Preventing Dentin Erosion: An SEM and AFM study®

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Summary: The aim of the present *in vitro* study was the evaluation of new formulation toothpastes on preventing dentin erosion produced by a soft drink (Coca Cola®), using atomic force microscopy (AFM) and scanning electron microscopy (SEM). Fifty dentin specimens were divided in treatment and control halves and were than assigned to 5 groups of 10 specimens each: group 1a: intact dentin, group 1b: dentin + soft drink, group 2a: intact dentin + Biorepair Plus-Sensitive Teeth®, group 2b: dentin + soft drink + Biorepair Plus-Sensitive Teeth®, group 3a: intact dentin + Biorepair Plus-Total Protection®, group 3b: dentin + soft drink + Biorepair Plus-Total Protection®, group 4a: intact dentin + Sensodyne Repair & Protect®, group 4b: dentin + soft drink + Sensodyne Repair & Protect®, group 5a: intact dentin + Colgate Sensitive Pro Relief®, group 5b: dentin + soft drink + Colgate Sensitive Pro Relief®. The surface of each specimen was imaged by AFM and SEM. Comparing specimens of group a and b (no demineralization and demineralization), a statistically significant difference ($p < 0.01$) in R_{rms} values was registered. Comparing b groups, all the analyzed toothpastes tended to remineralize the dentine surface in different extent. Biorepair Plus-Total Protection® and Sensodyne Repair & Protect® provided higher protective effect against dentin demineralization. SCANNING 36:301–310, 2014.

Key words: AFM, dentin, SEM, surface roughness, toothpastes

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Introduction

Erosive tooth wear in the oral environment has gained more importance from the dental practice since the decline in dental caries in many industrialized countries (Tantbirojin *et al*., 2008). Changes in dietary habits have led to excessive consumption of acidic food and beverages, which are the most widespread extrinsic factors that cause dental erosion (Ramalingam *et al*., 2005). Dietary acids are the most extensively studied etiological agents and can be said to be the most important extrinsic factor (Hemingway *et al*., 2006). Typical acid sources come from the diet, medications, occupational exposure, and lifestyle activities (Lussi *et al*., 2006).

In the oral environment, tooth structure undergoes continuous demineralization and remineralization: if this balance is interrupted, demineralization will lead to a progressive deterioration of tooth structure (Barbour *et al*., 2006; Yamaguchi *et al*., 2006).

Several studies investigated the characteristics of enamel erosion both *in vivo* and *in vitro*, and the remineralizing properties of different toothpaste formulations, but little is known about dentin demineralization and remineralization processes.

Dentin is the tissue underlying the enamel that forms the bulk of the tooth. The dentin matrix is formed by about 45–50 vol% mineral in the form of a carbonated hydroxyapatite, 30–35 vol% of organic matter, mostly as type I collagen with associated noncollagenous protein (Marshall *et al*., 2000). Apatite in dentin has a much smaller crystallite size, higher carbonates content and is more susceptible to acidic dissolution than enamel apatite. Hence, once the demineralization process involves dentin, its rate will be accelerated (Bertassoni *et al*., 2010).

A better understanding of the steps leading to remineralization of dentin is desirable. In dentin, apatite occurs in two specific regions, within the fibrils (interfibrillar mineral) and between the fibrils (extrafibrillar mineral). The interfibrillar mineral has been suggested to be crucial for the normal mechanical properties of the tissue; it might act as a site for apatite

nucleation and re-growth thus facilitating remineralization and recovery of mechanical properties (Kinney *et al*., 2003).

Biological and chemical factors in the oral environment influence the progress of dental erosion. Saliva provides protective effects by neutralizing and clearing the acids; it is also a source of inorganic ions necessary for the remineralization process (Hemingway *et al*., 2006); this is the reason why patients with diminished salivary flow are more exposed to dental erosion and decay (Hay and Thomson, 2002).

Enamel and dentine have no spontaneous capability to repair when affected by specific dental pathologies such as caries, abrasions, or fractures because enamel contains no cells and dentin apposition occurs only toward the pulp tissue (Reynolds, '98). Therefore, when both enamel and dentin are exposed to oral environment the only possibility to be reconstructed depends on the application of alloplastic materials, which provides to a sort of prosthetic restoration. Toothpastes have been considered effective and accessible vehicles to improve enamel and dentin resistance to further erosive attacks (Lussi *et al*., 2004).

Fluoride dentifrices have some protective effect on enamel/dentin eroded to brushing abrasion when immersed *in vitro* in a cola drink. Currently, conventional fluoride-containing toothpastes do not appear to be able to protect efficiently against erosion (Magalhães *et al*., 2007).

Recently, new toothpastes formulations have been introduced that claim to contrast enamel and dentin erosion. Biorepair Plus-Sensitive Teeth® (Coswell, Bologna, Italy) and Biorepair Plus-Total Protection® (Coswell) are based on Zinc Hydroxyapatite (MicroRepair®) (Hannig *et al*., 2012). Sensodyne Repair & Protect® (GlaxoSmithKline, Brentford, Middlesex, UK) is made of Calcium Sodium Phosphosilicate (Novamin®) (Rajesh *et al*., 2012). Colgate Sensitive Pro Relief® (Colgate-Palmolive, Rome, Italy) is based on Arginine 8% (Pro-Argin®) (Docimo *et al*., 2011).

Atomic force microscopy (AFM) gives images with atomic resolution with minimal sample preparation. This technique has been widely used to characterize the erosion of enamel and dentin (Finke *et al*., 2000; Marshall *et al*., 2000; De-Deus *et al*., 2006). In a previous study AFM have been used to investigate the protective effect of a mouthrinse on enamel softened by a demineralizing beverage (Poggio *et al*., 2009).

The aim of the present *in vitro* study was to evaluate the role played by different toothpastes (Biorepair Plus-Sensitive Teeth®, Biorepair Plus-Total Protection®, Sensodyne Repair & Protect®, Colgate Sensitive Pro Relief®) on preventing/repairing dentin erosion. AFM and SEM were used to quantitatively compare the impact of the four mentioned toothpastes on contrasting dentin erosion produced by a soft drink.

Materials and Methods

The overall experimental design is shown in Figure 1. Specimens were prepared from 50 extracted human permanent incisors free of caries and defects, extracted for parodontal reasons. After the extraction, the teeth were cleansed of soft tissue debris and inspected for cracks, hypoplasia, and white spot lesions; they were disinfected in 5.25% sodium hypochlorite solution for one hour (Hemingway *et al*., 2006) and stored in artificial saliva (pH 7.0, 14.4 mM NaCl; 16.1 mM KCl; 0.3 m mM $Cl_2{\cdot}6H_20$; 2.9 mM K_2HPO_4; 1.0 mM $CaCl_2{\cdot}2H_2O$; 0.10 g/100 ml sodium carboxymethylcellulose) during the whole experimentation (Lussi *et al*., 2004). The specimens were cut longitudinally, with a high-speed diamond rotary bur with a water-air spray; one half served as a control, and the other half as treatment. The labial surfaces near the enamel dentin junction were ground using silicon carbide papers under water irrigation to produce flat dentin surfaces (Ramalingam *et al*., 2005). Samples were placed into Teflon moulds measuring 10 mm × 8 mm × 2 mm, embedded in flowable composite resin and polymerized. The baseline root mean-square roughness, R_{rms}, was measured for all the specimens before starting experimentation. No statistical difference in R_{rms} values was recorded, suggesting that the specimens may be comparable. A soft drink (Coca Cola, Italy) was chosen for the demineralization process. The pH at 20°C, buffering capacity, concentration of calcium and phosphate of the beverage were measured (Oshiro *et al*., 2007). Measurements were performed in triplicate and average values calculated. Four toothpastes were used: Biorepair Plus-Sensitive Teeth, Biorepair Plus-Total Protection, Sensodyne Repair & Protect, Colgate Sensitive Pro Relief. The samples were then assigned to 5 groups, each made of 10 teeth:

- Group 1a: intact dentin.
- Group 1b: dentin + soft drink.
- Group 2a: intact dentin + Biorepair Plus-Sensitive Teeth.
- Group 2b: dentin + soft drink + Biorepair Plus-Sensitive Teeth.
- Group 3a: intact dentin + Biorepair Plus-Total Protection.
- Group 3b: dentin + soft drink + Biorepair Plus-Total Protection.
- Group 4a: intact dentin + Sensodyne Repair & Protect.
- Group 4b: dentin + soft drink + Sensodyne Repair & Protect.
- Group 5a: intact dentin + Colgate Sensitive Pro Relief.
- Group 5b: dentin + soft drink + Colgate Sensitive Pro Relief.

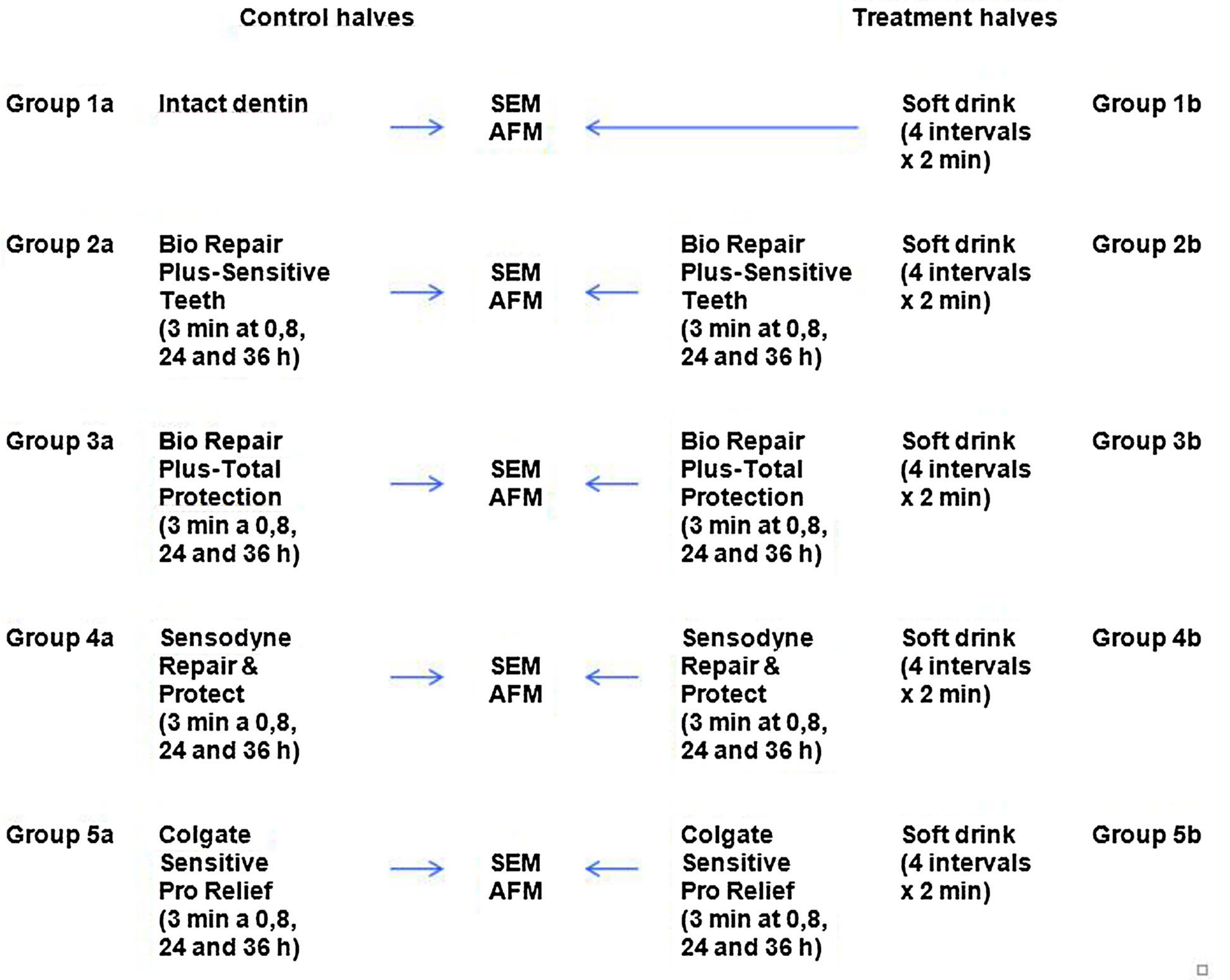

Fig 1. Flow chart.

The specimens of groups 1b, 2b, 3b, 4b, and 5b (each made of five teeth) were immersed in 6 ml of the soft drink for 2 min at room temperature before rinsing with deionized water. Four consecutive intervals of the immersion procedure were carried out (Barbour *et al.*, 2005).

The toothpastes were applied neat onto the surface of the specimens to cover the dentin surface without brushing in the groups 2a, 3a, 4a, and 5a wiped off with distilled water washing; the control specimens (group 1) did not receive any treatment. The toothpastes were applied to the dentin surfaces for 3 min at 0, 8, 24, and 36 h; during these intervals the specimens were kept in artificial saliva.

Atomic Force Microscopy (AFM) Observations

The specimens of each group were observed with an AFM AutoProbe CP 100 (Themormicroscopes, Veeco, Plainview, New York), equipped with a piezoelectric scanner, which can cover an area of 100 × 100 μm^2 with a range of 7 μm in the z-direction. The root mean-square roughness, R_{rms}, was obtained from the AFM images by testing, for each sample, at least 10 different film areas of 30 × 30 μm^2 with a resolution of 256 × 256 pixels. The data were obtained by averaging on at least 20 selected lines of the image (Hegedus *et al.*, '99). Measurements were performed on the treatment specimens and on the matching controls.

Statistical Analysis

Differences in the averaged values among the groups were analyzed by ANOVA test. Statistical difference was set at $p < 0.01$.

SEM Observations

The specimens were gently air dried, dehydrated with alcohol, sputter-coated with gold, and observed under SEM (440 SEM Whith Oxford EDS/ WDS, LEO). Serial

SEM microphotographs of the surfaces of each specimen at 1,000× original magnification were obtained.

Results

The pH of the soft drink at 20°C was 2.44; the buffering capacity was 0.0056. Concentration of calcium was 20.83 mg/ml, concentration of phosphate 175.7 mg/ml.

AFM Images

The mean R_{rms} values recorded before experimentation was 265, the mean R_{rms} value of group 1a (intact dentin) after immersion in artificial saliva was 268, suggesting that the groups were comparable (no statistical difference).

Table I reports mean R_{rms} values obtained through the demineralization and the remineralization process.

Comparing all the specimens of groups a and b (no demineralization and demineralization), a statistically significant difference ($p < 0.01$) in R_{rms} values was registered.

Comparing the R_{rms} values of groups 2b and 5b with group 1b (demineralized dentin), no statistical difference was recorded ($p > 0.01$), suggesting low protective effect of Biorepair Plus-Sensitive Teeth and Colgate Sensitive Pro Relief. Comparing the R_{rms} values of groups 3b and 4b with group 1b a statistical difference ($p < 0.01$) was registered, suggesting a protective effect against dentin demineralization of BioRepair Plus-Total Protection and Sensodyne Repair & Protect.

Figure 2 shows AFM images of intact dentin and a demineralized specimen surface (groups 1a and 1b). Figure 3 shows AFM images of groups 2b and 5b (little protective effect recorded). Figure 4 displays AFM images of groups 3b and 4b (protective effect recorded).

SEM Images

Figure 5 shows SEM images of intact dentin and a demineralized specimen surface (groups 1a and 1b). Figure 6 shows SEM images of groups 2b and 5b (little protective effect recorded). Figure 7 displays SEM images of groups 3b and 4b (protective effect recorded).

Discussion

In the present *in vitro* study, AFM and SEM were used to verify the protective effect of four toothpastes on dentin exposed to erosive action of a soft drink.

AFM was used to study tooth surfaces in order to compare the pattern of particle distribution in the outermost layer of the tooth surfaces (Farina *et al.*, '99). It was found that AFM gives high-contrast, high-resolution images and is an important tool as a source of new structural information: tapping mode AFM (TM-AFM) images treated with demineralizing solutions are able to show net differences between exposed and unexposed enamel areas (Watari, 2005).

In a previous study, the chemical properties of Coca Cola and Gatorade were evaluated (Quartarone *et al.*, 2008): their pH, buffering capacity, concentration of calcium and phosphate were measured at 20°C. Their pH level was below of 4, so it was to be expected that exposure to each of them resulted in a progressive loss of enamel. It was demonstrated that erosion is correlated to pH; moreover, there was a negative correlation between calcium concentration and erosion, but no clear relationship between phosphate concentration and erosion (Hemingway *et al.*, 2006). For these reasons, the beverage with the lowest pH and highest concentration of calcium was chosen for the present *in vitro* study: Coca Cola.

There is a clear relationship between erosion and temperature of the beverages (Barbour and Rees, 2004). In this study, the beverage was kept at a constant temperature of 20°C. Although erosion proceeds more

TABLE I Mean roughness values (R_{rms}) obtained in the (a) group a and (b) group b

Groups	R_{rms} (nm)
(a)	
Group 1a : intact dentin	159 ± 19
Group 2a: intact dentin + BioRepair Plus-Sensitive Teeth®	265 ± 18
Group 3a: intact dentin + BioRepair Plus-Total Protection®	311 ± 18
Group 4a: intact dentin + Sensodyne Repair & Protect®	359 ± 19
Group 5a: intact dentin + Colgate Sensitive Pro Relief®	257 ± 14
(b)	
Group 1b: dentin + soft drink	317 ± 15
Group 2b: dentin + soft drink + BioRepair Plus-Sensitive Teeth®	324 ± 15
Group 3b: dentin + soft drink + BioRepair Plus-Total Protection®	282 ± 20
Group 4b: dentin + soft drink + Sensodyne Repair & Protect®	285 ± 13
Group 5b: dentin + soft drink + Colgate Sensitive Pro Relief®	413 ± 10

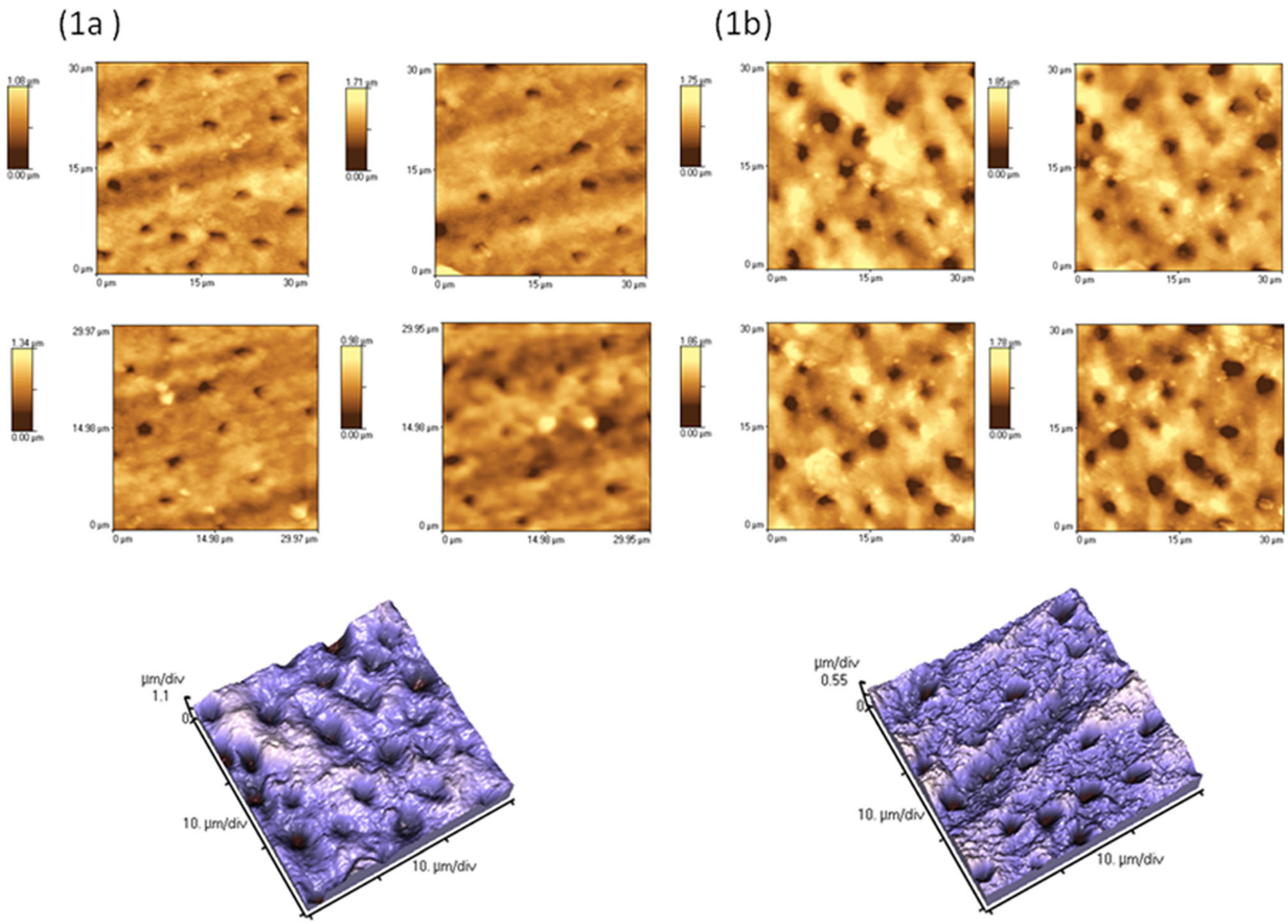

Fig 2. AFM images of intact dentin and a demineralized specimen surface (groups 1a and 1b).

slowly *in vivo* than *in vitro* owing to the protective effect of saliva and acquired pellicle, the effect of temperature can be expected to be significant (Barbour and Rees, 2004). In order to stress their deminaralizing potential, the cola drink was replenished every 2 min to ensure that it was carbonated and to reduce the buffering effect from ions dissolved from the enamel surface (Tantbirojin *et al*., 2008).

Flat and polished specimens were used in the present study in an attempt to standardize specimens and remove natural variations in surface enamel between teeth and between different tooth sites and types, which may result in different responses to acid dissolution (Adebayo *et al*., 2009). However, it should be noted that natural tooth surfaces erode more slowly than polished surfaces (Ranjitkar *et al*., 2009). The same process was used for the preparation of dentin specimens (Bertassoni *et al*., 2010). The specimens were cleaned with 5% NaOCl for 1 h, which could not alter dentin/enamel surface (Farina *et al*., '99).

The AFM could analyze the topographical aspects of dentine samples and the possible effects of the demineralization/remineralization processes on the dentine morphology, in the presence of the protective agents listed above. The most common topographical parameter was therefore determined, such as the surface roughness (R_{rms}), which is an index of the surface quality; the opening of the tubules was also morphologically evaluated. As reported in Figure 2a, the intact dentin shows a fairly smooth surface with tubules of about 3 µm diameter. The R_{rms} of the intact dentine surface was 159 nm. After demineralization with an acidic substance such as Coca Cola, the surface appeared much rougher. The process of erosion caused, in fact, an increase of R_{rms} from 159 to 317 nm. The peritubular material is consumed and the diameter of the tubules increases up to about 5 µm (Fig. 2b). For what concerns the samples of intact dentin treated with the toothpastes (groups 2b, 3b, 4b and 5b), the AFM images showed a random distribution along the surface of the protective agents to form aggregates and/or precipitates of mineral substances, more or less abundant as it can be seen in the lighter areas of images (Figs. 3 and 4). This justifies roughness values, significantly higher than those expected when considering the intact dentin (see Table I). In contrast, the previously demineralized samples exhibit different morphological aspects, depending on the used mineralizing agent. These

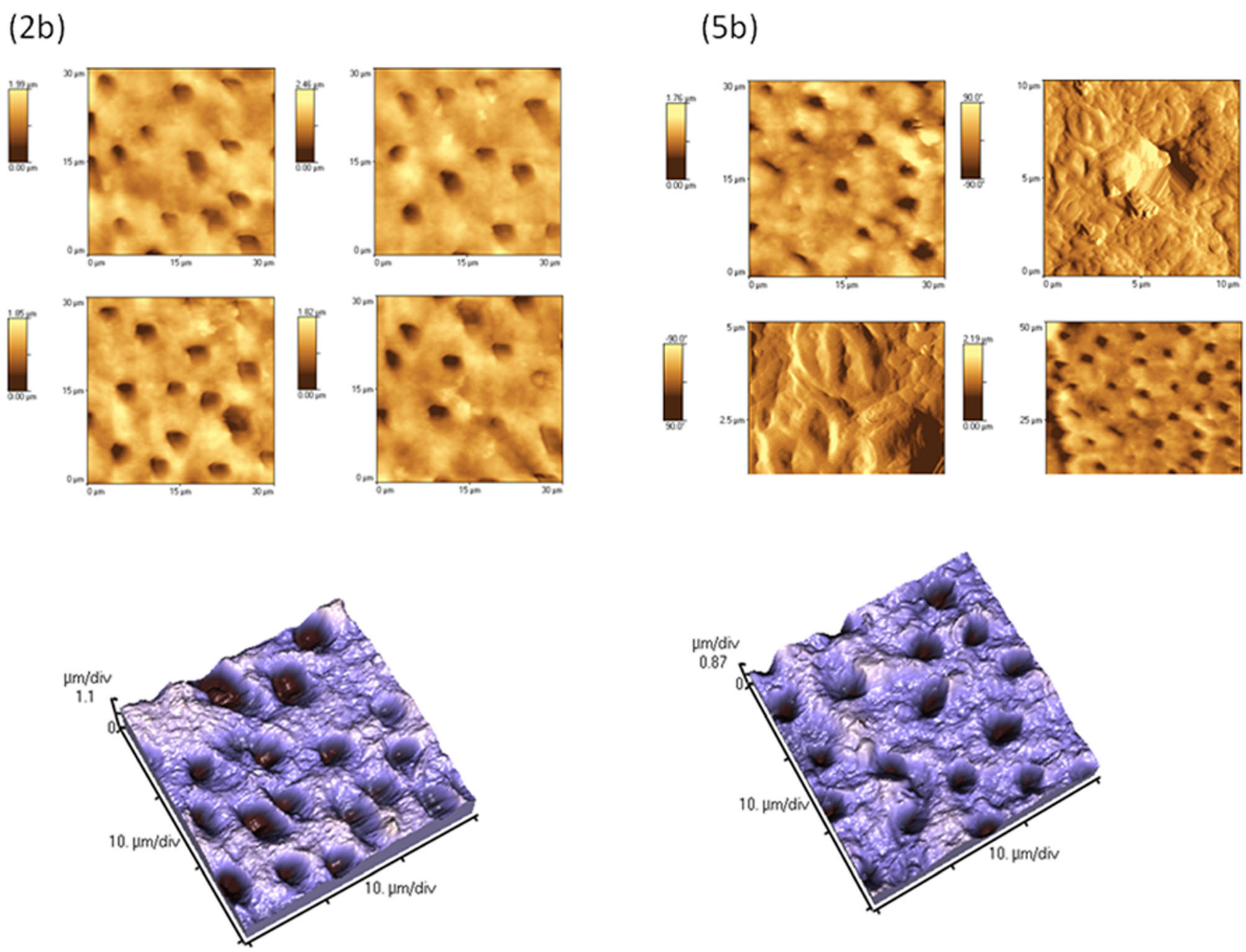

Fig 3. AFM images of groups 2b and 5b (little protective effect recorded).

differences, however, are more qualitative than quantitative. In general, it was observed that all remineralizing agents tend to fill the dentin tubules. In particular, for Biorepair Plus-Total Protection and Sensodyne Repair & Protect (Fig. 4), the paste covers the entire surface of the dentine, leaving exposed only a few tubules. This observation is also confirmed by the corresponding SEM images (Fig. 7). As regards the other fractions of demineralized dentin then remineralized, the roughness data are entirely comparable, as well as the diameters of the tubules, which do not seem to undergo modifications if compared to the demineralized controls. Very smooth and homogeneous surfaces, without any crystalline precipitates can be observed in the case of Biorepair Plus-Total Protection and Sensodyne Repair & Protect. This aspect may be a probable index of a better association of the mineral agent with the organic network of collagen, as also observed in the SEM study. In the case of groups 3b (dentin + soft drink + Biorepair Plus-Total Protection) and 4b (dentin + soft drink + Sensodyne Repair & Protect) the tubules are smaller than the corresponding not demineralized fraction (groups 3a and 4a), suggesting a better protection against demineralization.

The topographical and numerical AFM analysis was also confirmed by SEM images. The present SEM study allowed to qualitatively understand the processes of demineralization of the dentine surface through the observation of specific morphological and structural features that characterize the dentin itself, such as opening and size of the tubules, the presence or absence of peritubular mineral, the evolution of the organic network of collagen. The remineralizing process by means of the analyzed different toothpastes has been interpreted through the analysis of mineral precipitation inside or outside the tubules, as well as the observation of the possible associations of these mineralizing substances with the collagen structure. The effect of demineralizing acids such as Coca Cola was originally studied by comparing the SEM images of dentin treated with the drink with those of the intact sample, shown in Figure 5. As expected, the surface layer of dentin treated with Coca-Cola shows larger tubule sizes than those observed in the untreated specimen, due to erosion of the

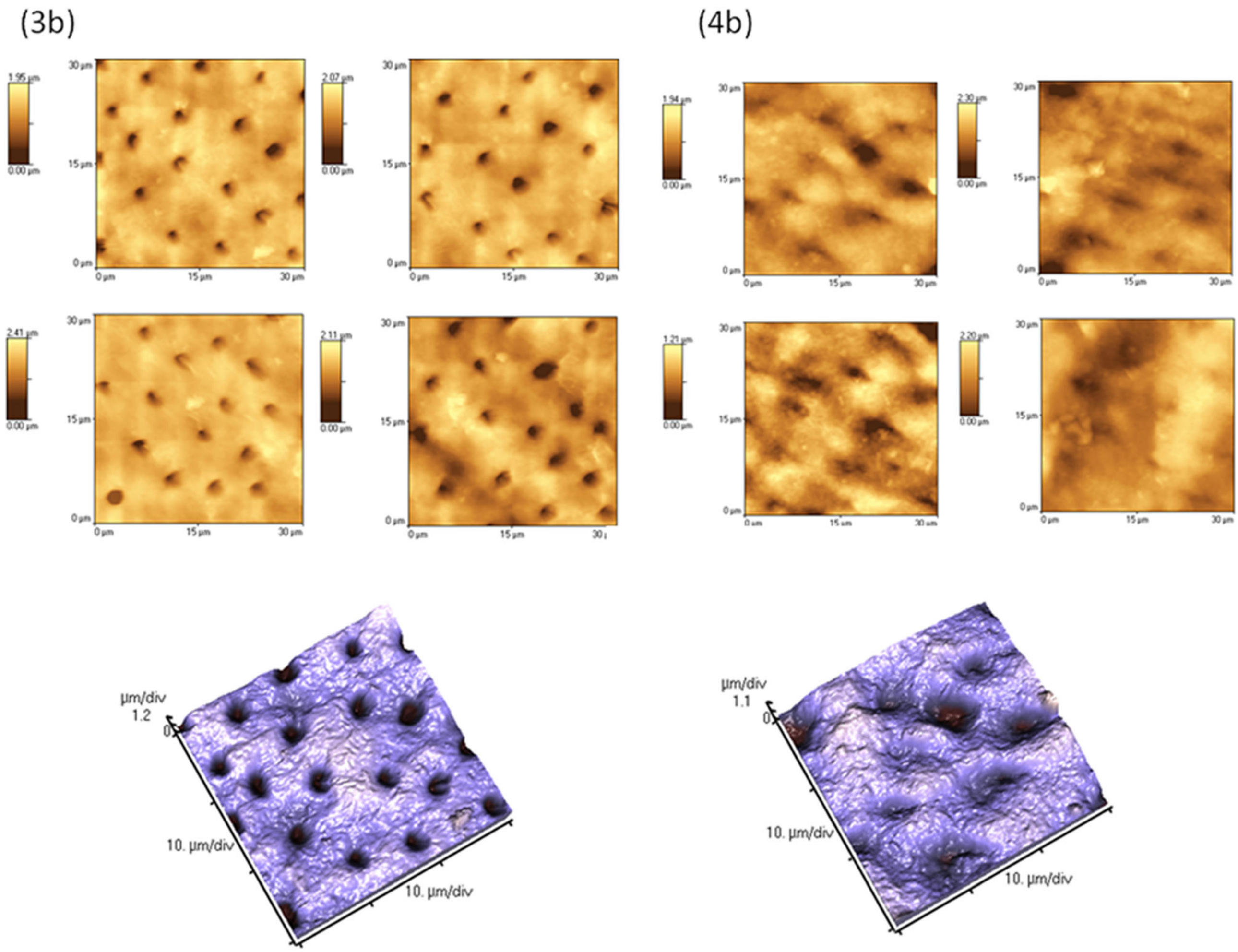

Fig 4. AFM images of groups 3b and 4b (protective effect recorded).

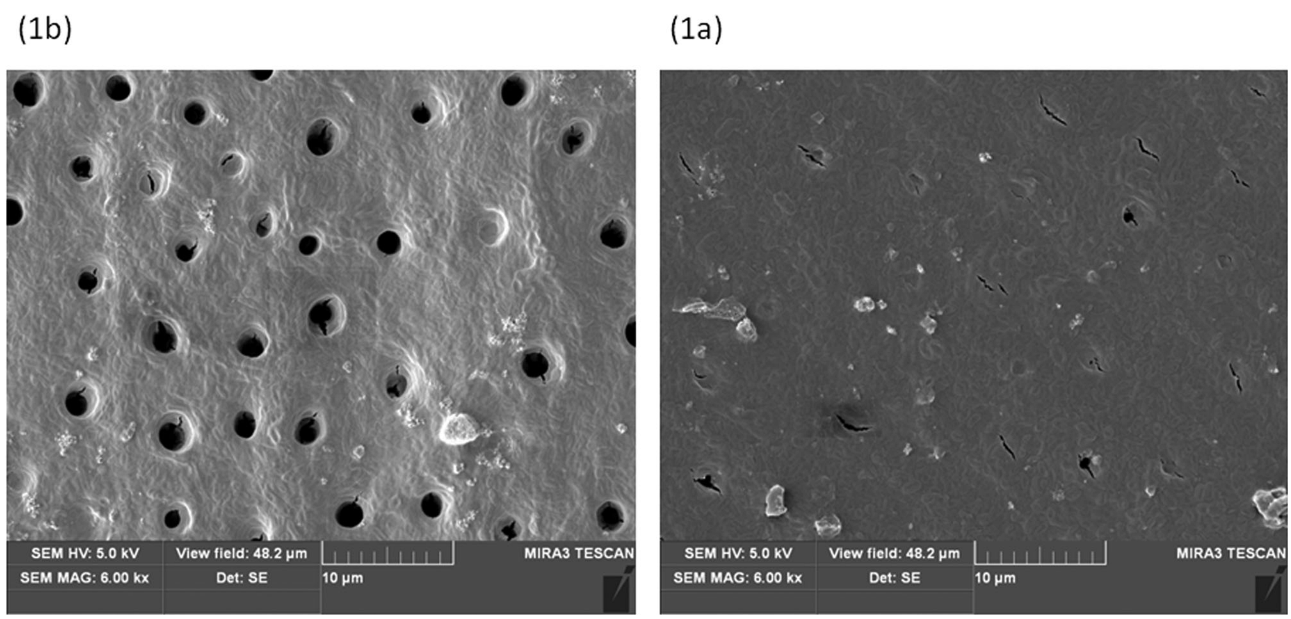

Fig 5. SEM images of intact dentin and a demineralized specimen surface (groups 1a and 1b).

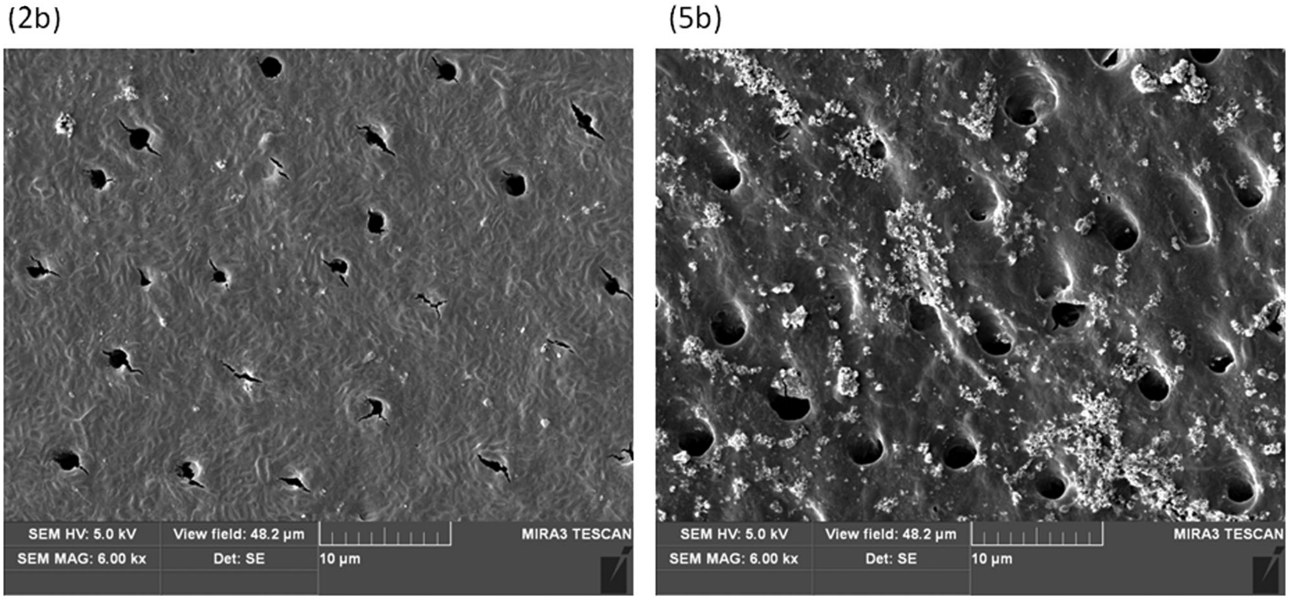

Fig 6. SEM images of groups 2b and 5b (little protective effect recorded).

peritubular region. For what concerns the remineralization, different aspects emerged from the comparison of SEM images on the samples of dentin treated with the various toothpastes. An often-observed phenomenon concerns the presence of crystals derived from the mineralizing agent, which are randomly distributed on the dentin surface, subjected to demineralization and subsequent remineralization. In the case of Biorepair Plus-Sensitive Teeth and Colgate Sensitive Pro Relief (Fig. 6), the dentin tubules, previously treated with Coca Cola, are larger than the intact sample but they tend to close during mineralization, because partially filled with the mineral agent. In the case of Biorepair Plus-Total Protection and Sensodyne Repair & Protect (Fig. 7), the tubules are almost completely filled, and in a greater extent than the previous agents; in addition, the mineral crystals precipitating during the remineralizing treatment appear better associated with the collagen network. In the sample immersed in Coca Cola and then remineralized, a complete filling of the dentine tubules by the mineralizing agent may be observed. In addition, the mineral substance appears to be well bound

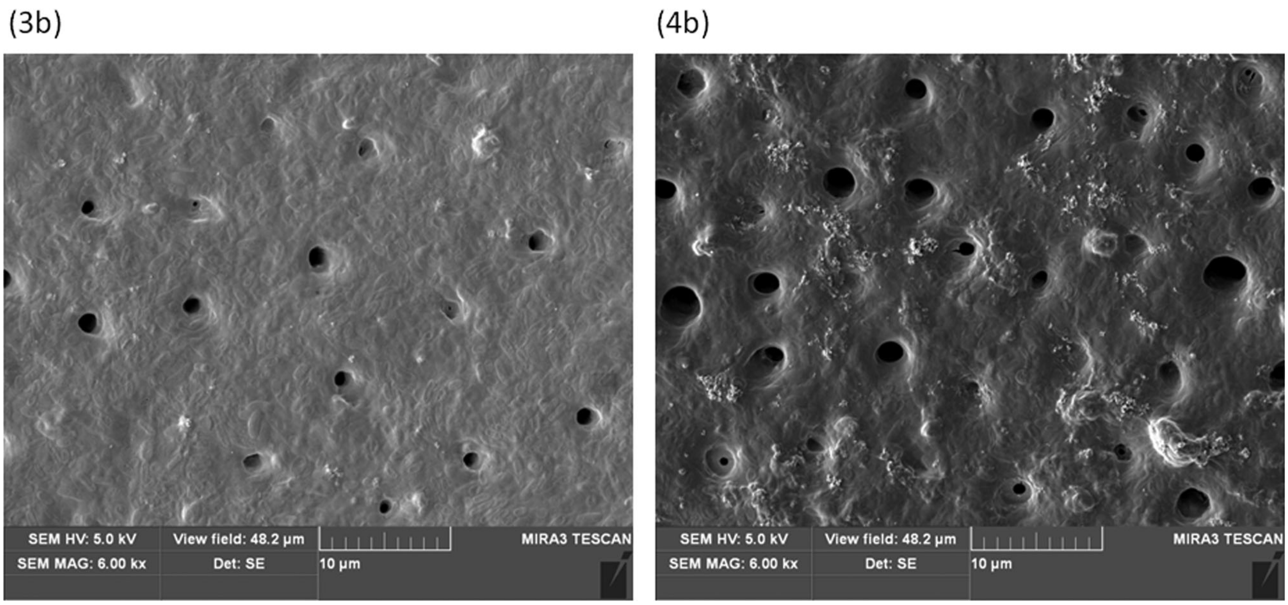

Fig 7. SEM images of groups 3b and 4b (protective effect recorded).

to the surface of the collagen and in a significantly greater extent than that observed for the other pastes.

However, dentine remineralization is more difficult than enamel remineralization due to the abundant presence of organic matrix in dentine (Zhang *et al.*, 2012). Scaramucci *et al.* (2011) evaluated the erosive potential of orange juice modified by food additives in enamel and dentin; they concluded that for dentin only the combination of calcium lactate pentahydrate and sodium linear polyphosphate reduced erosion. Tschoppe *et al.* (2011) evaluated the effects of nano hydroxyapatite toothpastes on remineralization of bovine enamel and dentine subsurface lesions *in vitro*. They concluded that with the *in vitro* conditions chosen, toothpastes containing nano hydroxyapatite revealed higher remineralizing effects compared to amine fluoride toothpastes with bovine dentine. In an *in vitro* study Kim et al. (2011) stated that the application of 0.2% and 2% Clorexidine seemed to be effective in promoting the remineralization of demineralized dentine. Diamanti *et al.* (2010) stated that the high fluoride toothpastes promoted remineralization and inhibited demineralization more effectively, than the calcium sodium phosphosilicate toothpastes. Calcium Sodium Phosphosilicate (Novamin) contained in toothpaste formulations adhered to exposed dentin surfaces. The layer formed was resistant to acid and mechanical challenges. Characterization of this layer indicated it was hydroxyapatite-like in nature (Earl *et al.*, 2011). Que *et al.* (2010) demonstrated that the new Pro-Argin formula toothpaste provided a significant reduction in dentin hypersensitivity when used over a period of 8 weeks in a clinical study. Wang *et al.* (2011) evaluated the effectiveness of novel bioactive glass-containing toothpaste on dentine permeability and remineralization they concluded that it may be useful for the treatment of dentine hypersensitivity and dentine remineralization.

Conclusions

In the light of these results, it can be concluded that all the analyzed agents tend in different extent to remineralize the dentin surface. Among these, however, Biorepair Plus-Total Protection and Sensodyne Repair & Protect appears to be more effective in both the filling of the tubules and in associating to the collagen structure of the demineralized dentin, which is important with regard to the reconstruction of the tissue and the recovery of its mechanical properties.

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