

## ORIGINAL PAPER

# Atomic force microscopy study regarding the influence of etching on affected and sclerotic dentine

A. GEORGESCU, GIANINA IOVAN, SIMONA STOLERIU,  
CL. TOPOLICEANU, S. ANDRIAN

Department of Odontology–Periodontology,  
Faculty of Dental Medicine,  
"Grigore T. Popa" University of Medicine and Pharmacy, Iassy

### Abstract

Aim of study is to compare the effect of etching with *ortho*-phosphoric acid on sound dentine, affected dentine and sclerotic dentine through AFM analysis. *Material and Methods.* The group study included 30 extracted third molars, 20 with acute and chronic carious lesions and 10 intact teeth. Teeth were sectioned in long axe to prepare sections with carious lesions surrounded by sound dentine. The sound teeth were sectioned to a depth of dentine comparable with carious lesions depth. The sections were etched with 37% *ortho*-phosphoric acid. The surface roughness was determined initially and after etching using AFM analysis. The results were expressed as relative variation of squared roughness. *Results.* The values of relative roughness indices were between 2.78 and 3 for sclerotic dentine, 3.18 and 3.26 for sound dentine, 3.32 and 3.38 for affected dentine. The highest values of roughness index were recorded for the affected dentine samples. Significant statistically values were recorded when comparing relative roughness indices for sclerotic dentine with relative roughness indices for affected dentine and sound dentine. *Conclusions.* Sclerotic dentine has significant higher resistance to the action of *ortho*-phosphoric acid than affected dentine and sound dentine. The lowest resistance to the action of etching agent was recorded for the affected dentine.

**Keywords:** affected dentine, infected dentine, etching, AFM analysis.

### □ Introduction

The new concepts of modern dental medicine focused on less invasive dental treatments, favoring the enamel and dentine preservation. The removal of high infected humid dentine [1–3] followed by the sterilization and remineralization of the affected dentine and perfect sealing of cavity can stop the evolution of carious process [4–6]. The removal of dentine partially demineralized and with low bacterial concentration, can be considered as a removal of relative healthy tissue and can induce pulp exposure [7].

The composite resins are favorite materials for dental practitioners when carious lesions or non-cariogenic lesions are treated. Numerous clinical situations are associated with the necessity to perform adhesion to modified dentinal substrate, affecting the long-term clinical performance of composite resins restorations. The morphology and nature of dentinal surface influence the adhesion process to dentinal hard tissues [8]. The dentine adhesion strategy associated with a separate etching step is based on dentine demineralization and hybrid layer formation [9]. Sometimes the adhesive penetrates only partially the demineralized dentine areas [10] favoring, on long-term, the adhesive failure and microleakage [11]. The atomic force microscopy (AFM) is a valuable method for demineralization studies and for the assessment of the influence exerted by different solutions or factors in oral environment. This method highlights the induced structural and dimensional changes of the dentine samples.

The aim of study is to compare, using AFM method, the effects of etching with 15% *ortho*-phosphoric acid on sound dentine, affected dentine and sclerotic dentine.

### □ Material and Methods

The group study included 30 extracted third molars, 20 with acute and chronic carious lesions and 10 intact teeth. The teeth with carious lesions were sectioned in long axe with diamond discs under water-cooling to result laser fluorescence values of 40–50 (DIAGNODent, KaVodental, Biberach, Germany). Sections included carious lesions surrounded by sound dentine. The sound teeth were sectioned to a depth of dentine comparable with carious lesions depth. The removal of infected dentine was performed with spheric burs at conventional speeds. The clinical assessment of differences between infected dentine, affected dentine and sclerotic dentine was performed accordingly to dentine aspect and hardness. The checking of the total removal of infected dentine was performed using laser fluorescence technique related to value 15 in lesion center [12]. The sections were etched with 37% *ortho*-phosphoric acid using Scotchbond Etching Gel (3M ESPE) for 15 seconds and washed with deionized water for total removal of etching agent. The surface roughness was determined before and after etching using AFM analysis. The results were expressed as relative variation of squared roughness, accordingly to formula  $\Delta R = \text{values of square roughness indices after demineralization} -$

values of square roughness indices before demineralization/values of square roughness indices before demineralization.

## Results

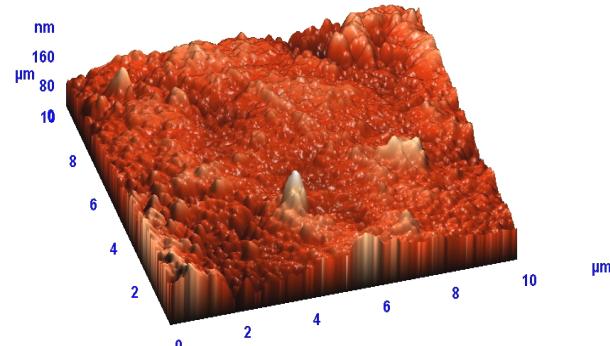
The values of relative roughness indices for dentine samples are presented in Table 1.

**Table 1 – Values of relative roughness indices for sound dentine, affected dentine and sclerotic dentine**

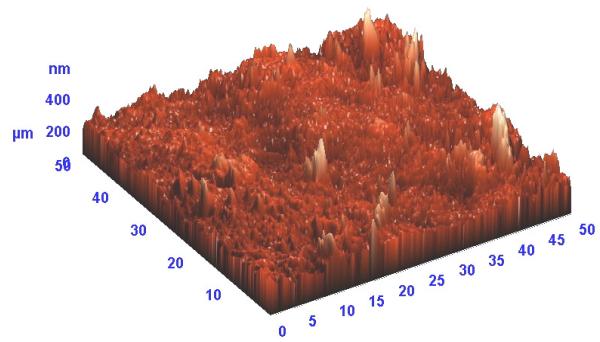
Sclerotic dentine	Sound dentine	Affected dentine
2.78	3.19	3.32
2.98	3.22	3.36
3	3.28	3.33
3.05	3.24	3.37
2.87	3.26	3.35
2.96	3.2	3.31
2.91	3.25	3.37
2.84	3.18	3.38
2.98	3.27	3.34
2.84	3.26	3.36
2.92	3.24	3.35

The values of relative roughness indices were between 2.78 and 3 for sclerotic dentine, 3.18 and 3.26 for sound dentine, 3.32 and 3.38 for affected dentine. The relative roughness index for sclerotic dentine presented lowest values comparing with sound dentine and affected dentine. The highest values of roughness index were recorded for the affected dentine samples. Significant statistically values were recorded when comparing relative roughness indices for sclerotic dentine with relative roughness indices for affected dentine and sound dentine.

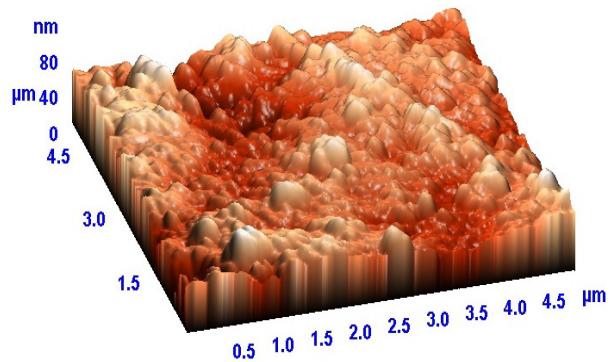
The AFM aspect of affected dentine sample before and after demineralization is presented in Figure 1 (10 µm section), Figure 2 (50 µm section) and Figure 3 (5 µm section). The profile of 5 µm section demonstrates the presence of variable roughness dimensions (Figure 4). The values of roughness were in range of 40–150 nm. The aspect of the same dentine sample after demineralization is presented in Figure 5 (10 µm section), Figure 6 (50 µm section) and Figure 7 (5 µm section). The profile of 5 µm section demonstrates the presence of more advanced roughness degrees comparing with sections before demineralization (Figure 8). The values of roughness presented in histogram were in range of 100–200 nm.



**Figure 1 – 3D aspect of affected dentine before and after etching (10 µm section).**



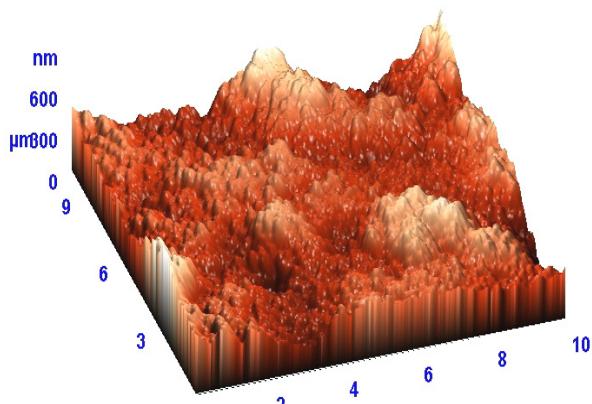
**Figure 2 – 3D aspect of affected dentine before and after etching (50 µm section).**



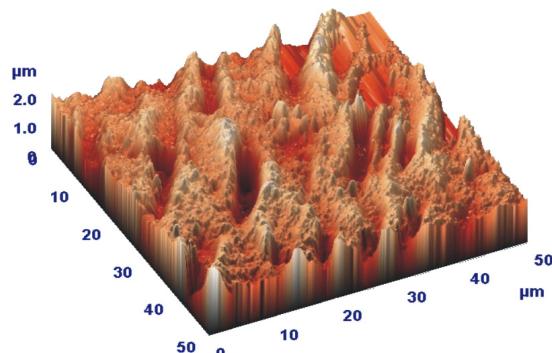
**Figure 3 – 3D aspect of affected dentine before and after etching (5 µm).**



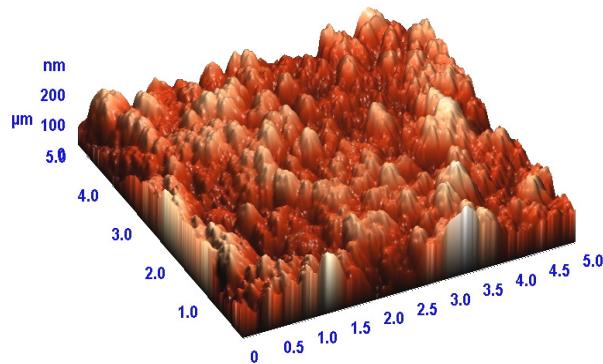
**Figure 4 – The profile of 5 µm section for affected dentine before etching.**



**Figure 5 – 3D aspect of affected dentine after etching (10 µm section).**



**Figure 6 – 3D aspect of affected dentine after etching (50  $\mu\text{m}$ ).**



**Figure 7 – 3D aspect of affected dentine after etching (5  $\mu\text{m}$  section).**



**Figure 8 – The profile of 5  $\mu\text{m}$  section for affected dentine after etching.**

Data were statistically analyzed using Mann–Whitney test. Significant statistically values were recorded when comparing relative roughness indices for sclerotic dentine with relative roughness indices for sound dentine (Tables 2a and 2b) and with relative roughness indices for affected dentine (Tables 3a and 3b).

**Table 2a – Results of statistical test of comparing relative roughness for sclerotic dentine and sound dentine**

<b>Ranks</b>				
	<b>GrdFI</b>	<b>N</b>	<b>Mean rank</b>	<b>Sum of ranks</b>
<i>Roughness</i>	Sclerotic dentine	10	5.50	55.00
	Sound dentine	10	15.50	155.00
		Total	20	

**Table 2b – Results of statistical test of comparing relative roughness for sclerotic dentine and sound dentine**

<b>Test statistics<sup>b</sup></b>	
	<b>Roughness indices</b>
Mann–Whitney U	.000
Wilcoxon W	55.000
Z	-3.784
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000 <sup>a</sup>

<sup>a</sup>Not corrected for ties.

<sup>b</sup>Grouping variable: GrdFI.

**Table 3a – Results of statistical test of comparing relative roughness for sclerotic dentine and affected dentine**

<b>Ranks</b>				
	<b>GrdFI</b>	<b>N</b>	<b>Mean rank</b>	<b>Sum of ranks</b>
<i>Roughness</i>	Sclerotic dentine	10	5.50	55.00
	Affected dentine	10	15.50	155.00
		Total	20	

**Table 3b – Results of statistical test of comparing relative roughness for sclerotic dentine and affected dentine**

<b>Test statistics<sup>b</sup></b>	
	<b>Roughness indices</b>
Mann–Whitney U	.000
Wilcoxon W	55.000
Z	-3.785
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000 <sup>a</sup>

<sup>a</sup>Not corrected for ties.

<sup>b</sup>Grouping variable: GrdFI.

## Discussion

In this study, the assessment of the affected dentine was performed accordingly to the scale of values recorded by Haak R *et al.* (2000) [12]. This method allowed us to establish, on the study group samples, clear limits between infected dentine areas and affected dentine or sclerotic dentine areas. The affected dentine areas present lower hardness than sound or sclerotic dentine areas representing tissue partially demineralized [13]. The demineralized areas are associated with porous dentine surfaces, explaining the increased values of square roughness indices before demineralization for affected dentine samples. The differences of structure and composition for the affected dentine layer influence the action of etching agent [14], conclusion sustained by the results of our study.

Sclerotic dentine represents an altered substrate formed partially as a natural response mechanism to aggressions and partially because of microbial colonization. The principal feature of sclerotic dentine is represented

by partial or total obliteration of the dentinal tubules with mineral crystals [15, 16]. Whitlockite crystals present lower diameters in the surface dentine layer and form mineral complexes that block the dentinal tubules. This hypermineralized layer makes difficult the action of etching agent, explaining the lowest values of relative roughness index recorded by our study. A second explanation of the less efficient action on sclerotic dentine could be related to the higher concentrations of fluorine ions integrated in more stable forms resistant to acid attacks [17–19]. The same decrease of etching efficiency to the sclerotic dentine was established by Kwong SM *et al.* (2000), though the study was performed on sclerotic dentine present in non-cariogenic cervical lesions [16].

The microstructural features and different behavior of the two types of dentinal substrate, modified by acid attack, can influence the mechanisms of composite resins adhesion, using different adhesion strategies. The total-etch adhesive systems seem less efficient in the case of the hypermineralized sclerotic dentine substrate, result similar with data offered by studies that assessed the interface dentine-adhesive system [20]. The affected dentine, submitted to the action of etching agent, presented a similar behavior with sound dentine, suggesting that total-etch adhesive systems could represent viable options for this particular dentine type, conclusion confirmed by Xuan W *et al.* (2010) [21].

## Conclusions

The sclerotic dentine presented a significantly higher resistance to the 37% *ortho*-phosphoric acid action comparing with affected dentine and sound dentine. The lowest resistance to the action of etching agent was recorded for the affected dentine, accordingly to the highest values of the relative roughness indices recorded by our study.

## References

- [1] KIDD EAM, JOYSTON-BECHAL S, BEIGHTON D, *Microbiological validation of assessments of caries activity during cavity preparation*, *Caries Res*, 1993, 27(5):402–408.
- [2] RICKETTS DNJ, KIDD EAM, BEIGHTON D, *Operative and microbiological validation of visual, radiographic and electronic diagnosis of occlusal caries in non-cavitated teeth judged to be in need of operative care*, *Br Dent J*, 1995, 179(6):214–220.
- [3] RICKETTS DNJ, *Management of the deep carious lesion and the vital pulp dentine complex*, *Br Dent J*, 2001, 191(11):606–610.
- [4] RANLY DM, GARCIA-GODOY F, *Current and potential pulp therapies for primary and young permanent teeth*, *J Dent*, 2000, 28(3):153–161.
- [5] MERTZ-FAIRHURST EJ, CURTIS JW JR, ERGLE JW, RUEGGEBERG FA, ADAIR SM, *Ultraconservative and cariostatic sealed restorations: results at year 10*, *J Am Dent Assoc*, 1998, 129(1):55–66.
- [6] ATAC AS, CEHRELI ZC, SENER B, *Antibacterial activity of fifth-generation dentin bonding systems*, *J Endod*, 2001, 27(12):730–733.
- [7] YIP HK, STEVENSON AG, BEELEY JA, *The specificity of caries detector dyes in cavity preparation*, *Br Dent J*, 1994, 176(4):417–421.
- [8] WENNERBERG A, SAWASE T, KULTJE C, *The influence of Carisol on enamel and dentine surface topography*, *Eur J Oral Sci*, 1999, 107(4):297–306.
- [9] EICK JD, GWINNETT AJ, PASHLEY DH, ROBINSON SJ, *Current concepts on adhesion to dentin*, *Crit Rev Oral Biol Med*, 1997, 8(3):306–335.
- [10] SANO H, YOSHIKAWA T, PEREIRA PN, KANEMURA N, MORIGAMI M, TAGAMI J, PASHLEY DH, *Long-term durability of dentin bonds made with a self-etching primer, in vivo*, *J Dent Res*, 1999, 78(4):906–911.
- [11] DE MUNCK J, VAN LANDUYT K, PEUMANS M, POITEVIN A, LAMBRECHTS P, BRAEM M, VAN MEERBEEK B, *A critical review of the durability of adhesion to tooth tissue: methods and results*, *J Dent Res*, 2005, 84(2):118–132.
- [12] HAAK R, WICHT MJ, NOACK MJ, *Does chemomechanical caries removal affect dentine adhesion?*, *Eur J Oral Sci*, 2000, 108(5):449–455.
- [13] OGAWA K, YAMASHITA Y, ICHIJO T, FUSAYAMA T, *The ultrastructure and hardness of the transparent layer of human carious dentin*, *J Dent Res*, 1983, 62(1):7–10.
- [14] WANG Y, SPENCER P, WALKER MP, *Chemical profile of adhesive/caries-affected dentin interfaces using Raman microscopy*, *J Biomed Mater Res A*, 2007, 81(2):279–286.
- [15] VAN MEERBEEK B, BRAEM M, LAMBRECHTS P, VANHERLE G, *Morphological characterization of the interface between resin and sclerotic dentine*, *J Dent*, 1996, 22(3):141–146.
- [16] KWONG SM, TAY FR, YIP HK, KEI LH, PASHLEY DH, *An ultrastructural study of the application of dentine adhesives to acid-conditioned sclerotic dentine*, *J Dent*, 2000, 28(7):515–528.
- [17] ARENDS J, CHRISTOFFERSEN J, RUBEN J, JONGEBLOED WL, *Remineralization of bovin dentine in vitro. The influence of the F content in solution on mineral distribution*, *Caries Res*, 1989, 23(5):309–314.
- [18] DAMEN JJ, BUIJS MJ, TEN CATE JM, *Fluoride-dependent formation on mineralized layers in bovine dentin during demineralization in vitro*, *Caries Res*, 1998, 32(6):435–440.
- [19] TEN CATE JM, DAMEN JJ, BUIJS MJ, *Inhibition of dentin demineralization by fluoride in vitro*, *Caries Res*, 1998, 32(2):141–147.
- [20] TAN JG, ZHOU LJ, FENG M, FENG HL, *Ultrastructural study of a self-etching adhesive to sclerotic dentin in non-carious cervical lesions*, *Zhonghua kou qiang yi xue za zhi (Chinese J Stomatol)*, 2005, 40(3):230–232.
- [21] XUAN W, HOU BX, LÜ YL, *Bond strength of different adhesives to normal and caries-affected dentins*, *Chinese Med J*, 2010, 123(3):332–336.

## Corresponding author

Andrei Georgescu, Junior Assistant, Department of Odontology–Periodontology, Faculty of Dental Medicine, “Grigore T. Popa” University of Medicine and Pharmacy, 16 University Street, 700115 Iassy, Romania; Phone +40232–301 618, e-mail: andgeorgescu@yahoo.com

Received: February 25<sup>th</sup>, 2010

Accepted: April 30<sup>th</sup>, 2010