

Mechanical characterization of sclerotic occlusal dentin by nanoindentation and nanoscratch

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ABSTRACT: Purpose: To evaluate the mechanical properties of occlusal wear lesions identified as sclerotic. **Methods:** Nanoindentation and nanoscratch techniques have been applied to determine elastic modulus (E), hardness (H) and wear resistance of different types of sclerotic dentin. Nanoscratch testing was applied to evaluate the tribological behavior. Mechanical properties of sclerotic dentin were evaluated together with scanning electron micrographs to show the different degree of tubular occlusion. **Results:** The higher the degree of sclerosis, the lower were the measured mechanical properties. The highest values (E = 20 GPa and H = 0.67 GPa) were obtained in normal dentin, and the lowest (E = 11.4 GPa and H = 0.51 GPa) in severe sclerotic lesions. These differences were statistically significant ($P < 0.05$). The groove width in the nanoscratch tests was also higher for moderate or severe sclerosis than for normal dentin. (*Am J Dent* 2010;23:108-112).

CLINICAL SIGNIFICANCE: Dentin containing severe sclerotic occlusal regions exhibited lower wear resistance than undamaged dentin.

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Introduction

Sclerotic dentin is defined as a hypermineralized dentin that affects both intertubular and peritubular zones, accompanied by partial or complete obliteration of the dentin tubules with acid-resistant mineral deposits, as a response to slowly progressive or mild irritations. The sclerotic dentin is bright, translucent or transparent, and may exhibit changes in color. The sclerosis can be generated at different locations: cervical and occlusal regions, or beneath carious zones or restorations. Different degrees of clinical sensitivity can be detected, according to the level of tubular occlusion. The sclerosis is usually associated with alterations in dentin physical properties such as hardness (H), and elastic modulus (E).¹⁻³ Furthermore, sclerotic lesions appear to respond in a different way than normal dentin to etching and bonding treatments, probably as a result of increased mineralization and tubular obstruction.⁴⁻⁹ Most studies of sclerotic dentin have been focused on non-carious cervical regions,^{10,11} and to a lesser extent on sclerotic dentin beneath carious lesions.¹²⁻¹⁴ Nevertheless, other areas of transparent dentin exist; particularly, the wear caused by aging and chewing, enhanced by occlusal disorders or bruxism, may produce considerable occlusal and incisal lesions, leading to enamel damage and exposure of underlying dentin, that becomes translucent and sclerotic due to hypermineralization.¹⁵

The determination of the physical properties, such as H, E and wear resistance of sclerotic dentin, is essential to predict the mechanical behavior of this tissue. Nanoindentation techniques have been used in the past for this purpose. H and E are the properties most frequently measured.¹⁶⁻¹⁸ Nanoindentation equipment allows the continuous and simultaneous measurement of the indentation force and the depth of penetration history over a complete loading cycle. The final result is a force-displacement curve from which H and E can be derived. Due to the extremely small force and displacement resolutions, often as low as 1 μ N and 0.2 nm, respectively, nanoindenters are able to characterize

Table 1. Dentin sclerosis scale²⁰ characteristics.

Degree	Sclerotic lesion characteristics
I	No sclerosis is evident. Dentin is light yellow or whitish color with little discoloration. Dentin is opaque, with little translucency or transparency.
II	More than category 1 but < 50% of way between categories 1 and 4.
III	Less than category 4 but > 50% of way between categories 1 and 4.
IV	Significant sclerosis present. Dentin is dark yellow or even discolored (brownish). Glassy appearance of dentin, with significant translucency or transparency evident.

small volumes of materials with a very high precision.

Nanoscratch testing is less common. This technique makes it possible to analyze the wear resistance of the dentin by means of a constant load applied on a spherical tip, while it is being moved through the sclerotic area. The width of the residual groove provides a qualitative measurement of the wear resistance.¹⁹ This parameter is very important especially on occlusal lesions exposed to masticatory wear.

This study determined the H, E and scratch resistance of occlusal sclerotic lesions, by means of nanoindentation and nanoscratch tests. Additionally, the specimens were analyzed by scanning electron microscopy (SEM) to evaluate the structural characteristics of each lesion and to confirm the degree of sclerosis.

Material and Methods

Selection and preparation of specimens - Five mandibular molars, extracted for periodontal reasons, all free of caries, with enamel attrition and exposed transparent dentin lesions on occlusal areas were selected. Informed consent was obtained from the patients in order to collect the teeth. The age of subjects ranged from 50-65 years. The specimens were classified according to their degree of sclerosis evaluated following the North Carolina dentin sclerosis scale, as indicated in Table 1.^{20,21} There was one tooth allocated to each group.

The selected teeth were stored in distilled water at room temperature until used. The storage time did not exceed 2 weeks. The occlusal surface, which contained the sclerotic lesions, was prepared using SiC papers up to 2400 grit finish, followed by polishing in an alumina slurry (up to 0.25 μm) to remove the smear layer. Finally, the specimens were treated with ultrasonic cleaner to eliminate dental debris deposited on the occlusal surface.

The roots of the teeth were cut off perpendicular to the principal axis of the tooth and parallel to the occlusal surface by means of a microtome.^a The specimens were glued to copper cylindrical supports of height no more than 5 mm, in order to facilitate the nanoindentation and nanoscratch tests.

Nanoindentation. - The experimental device used was a Nanoindenter XP.^b Before the tests, a standard fused silica specimen was chosen to calibrate the Berkovich indenter. Fifty tests were done with the Berkovich tip within the load range of the system to calibrate the area function of the indenter. In each lesion, 30 indentations (a matrix of 6 x 5) separated by 100 μm were performed on each sample using a Berkovich tip. The location of the indentation points was randomly assigned. In each indentation test, the load increased during 30 seconds up to 100 mN. This load is maintained constant during 5 seconds to avoid the viscoelastic effects on the unloading curve. After that, the unloading process took place during another 30 seconds. The load and the displacement were measured continuously, obtaining the corresponding force-displacement curve. Hardness and elastic modulus can be calculated following Doerner & Nix,²² and Oliver & Pharr.²³ The unloading slope at maximum load and the elastic modulus can be related through equation 1:

$$\frac{dP}{dh} = \frac{2}{\sqrt{\pi}} \cdot E^* \cdot \sqrt{A} \quad (1)$$

where, dP/dh is the slope at maximum load, A is the contact area at maximum load and E* is the combined elastic modulus.

The contact area can be determined by indenter geometry and parameters directly measured from the force-displacement curve, such as the maximum load, P_i, and the maximum displacement h_i, as shown in equation 2:

$$A = 24.5 \cdot \left[h_i - \frac{P_i}{(dP/dh)} \right]^2 \quad (2)$$

The hardness, H, follows from equation 3:

$$H = \frac{P_i}{A} \quad (3)$$

Finally, the elastic modulus, E, can be calculated from the combined elastic modulus, E*, through equation 4:

$$\frac{1}{E^*} = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i} \quad (4)$$

where E_i is the elastic modulus of the indenter (1140 GPa), and ν and ν_i the Poisson's ratios of the dentin (0.3) and the indenter (0.07), respectively.

In addition to the five teeth batch, a row of indentations was performed in another three specimens of grade 4 sclerosis, from an undamaged region to a severe sclerotic zone in order to capture the differences in mechanical properties of areas with different degrees of sclerosis in the same tooth.

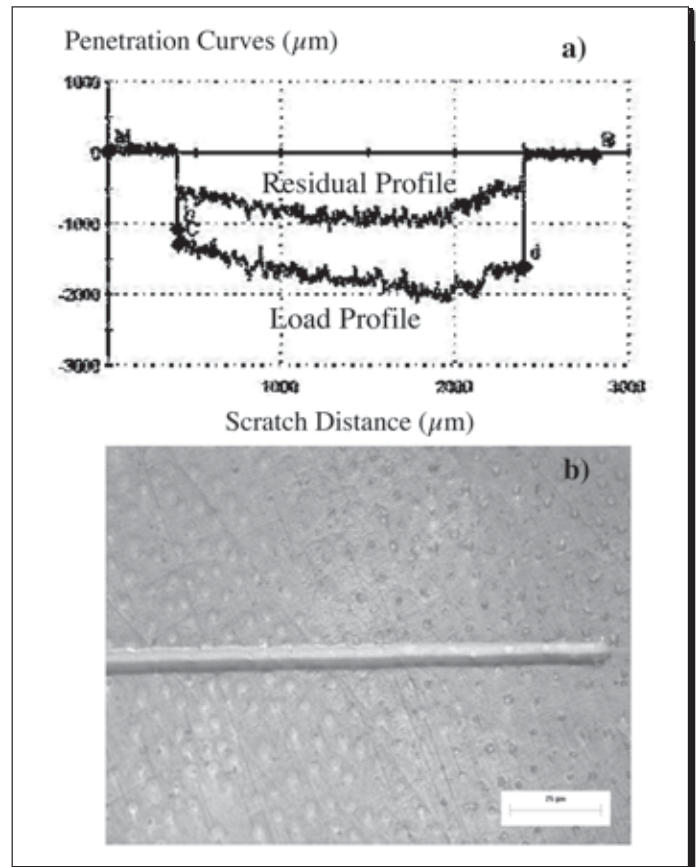


Fig. 1. Nanoscratch measurements made applying a constant load (100 mN) by a spherical indenter: (a) initial and residual profile, (b) groove image (2000 μm).

Nanoscratch - The wear properties of the different specimens, classified according to the North Carolina visual scale, were evaluated by nanoscratch tests by fusing a module coupled to the Nanoindenter XP equipment. The scratch was carried out by a spherical indenter (4.9 μm in radius) applying a constant load of 100 mN during a total horizontal displacement of 2000 μm. During the test, the spherical tip slides on the specimen surface producing a characteristic groove (Fig. 1). Then the indenter goes back to the initial position sliding at an opposite direction applying a very light load of 20 μN, drawing the residual surface profile of the permanent groove (Fig. 4a). When the total scratch distance has been achieved and the material has recovered elastically, the width of the residual groove can be measured.

Morphological analysis - The tested specimens were prepared for SEM observation to establish a relationship between mechanical properties and occlusal lesion morphology. The degree of occlusion of the dentin tubules was used to define the degree of sclerosis.

After mechanical testing, dentin surfaces were cleaned with water spray and compressed air. The specimens were dried and covered with a gold layer for SEM observation (JEOL-JSM-6400°).

Statistics - The elastic moduli and hardness values for the normal and sclerosed teeth were compared using a one-way ANOVA seeking to determine if there were any significant differences among the groups. When differences were found, they were identified and compared using Tukey's multiple comparison test with alpha preset at 0.05.

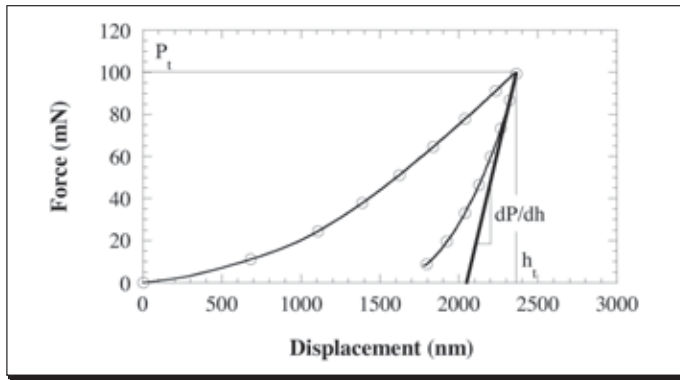


Fig.2. Load-displacement curves in dentin used to calculate elastic modulus and hardness. Experimental record is shown without hold time.

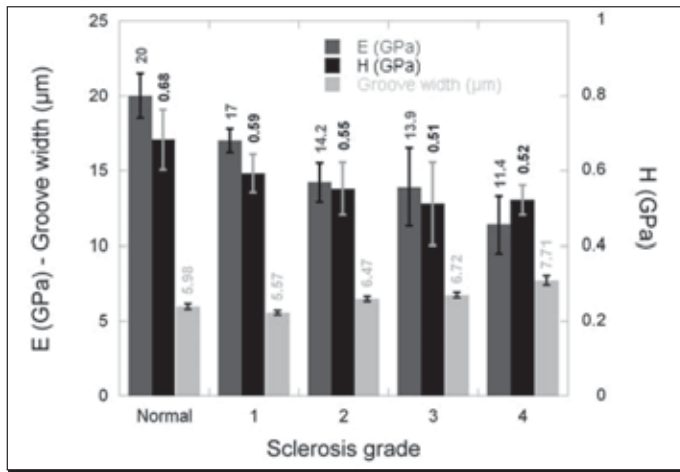


Fig. 3. Elastic modulus, hardness and nanoscratch groove width data versus sclerosis grade.

Results

Nanoindentation - Hardness and elastic modulus were determined from the experimental force-displacement curves. An example is shown in Fig. 2. Table 2 shows the results obtained after 30 indentations on occlusal lesions with different degrees of sclerosis. In general, an inverse relation is observed between the degrees of dentin sclerosis and the values of elastic modulus and hardness, decreasing when the degree of sclerosis on the occlusal surface increases (Fig. 3). The differences observed between undamaged dentin and severe sclerotic lesions are significant ($P < 0.05$). Hardness followed a similar tendency as did elastic modulus when comparing undamaged dentin with severe sclerosis. The results are not conclusive at intermediate degrees of sclerosis (Table 2). These results show that the mechanical properties of the analyzed sclerotic dentin are clearly lower than those of the corresponding normal dentin.

An example of the results of the additional tests carried out to determine the mechanical properties of areas with different degrees of sclerosis in the same tooth is shown in Fig. 4. In the occlusal sclerotic lesion, hardness and elastic modulus are lower than those of undamaged tissue.

Nanoscratch - Table 2 shows the results of the nanoscratch tests. In general, the width of the grooves increases with the degree of sclerosis. The groove width measured in undamaged dentin is clearly lower than those of moderate or severe sclerosis. Figure 1 shows an example of the results of nanoscratch tests.

Table 2. Correlation of elastic modulus, hardness and nanoscratch groove width with degree of sclerosis.

Degree of sclerosis	E (GPa)	H (GPa)	Groove width (µm)
Normal	20.0 (1.5) ^a	0.68 (0.08) ^A	5.98 (0.19) ^I
1	17.0 (0.8) ^b	0.59 (0.05) ^B	5.57 (0.14) ^{II}
2	14.2 (1.3) ^c	0.55 (0.07) ^B	6.47 (0.15) ^{III}
3	13.9 (2.6) ^c	0.51 (0.11) ^C	6.72 (0.18) ^{IV}
4	11.4 (1.9) ^d	0.52 (0.04) ^{B,C}	7.71 (0.31) ^V

Values are means (SD), (n = 30). Values identified by different superscript letters are significantly different ($P < 0.05$).

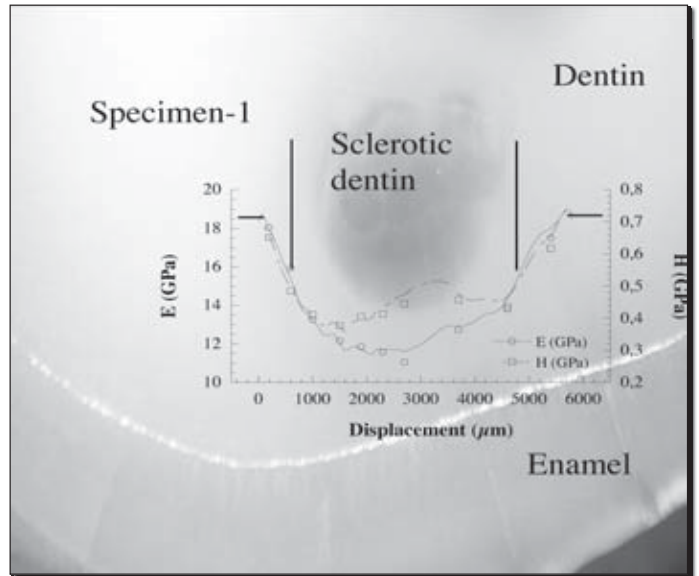


Fig. 4. Comparison of elastic moduli (E) and hardness (H) when measured in normal dentin (black horizontal lines) or across a large, sclerotic, occlusal lesion in a single tooth (grade 4) delineated by the two black vertical lines.

SEM - Both normal and affected dentins were observed by SEM to characterize the tissue morphology of the different specimens. The occlusal wear lesions correspond ultrastructurally to sclerosis in different degrees, showing changes in their structure and mineralization: the diameter of the tubules is reduced in different degrees depending on the severity of the sclerosis, with an increase in thickness of peritubular dentin, and complete tubular obliteration by sclerotic casts in the most severe lesions. Also, the morphological irregularity of the tubules, seen in the surface of the lesion, increased with the sclerosis level. A great variability in all the parameters studied was observed within the same lesion for the intermediate degrees of sclerosis. There was an acceptable correlation between the dentin degree of sclerosis according to the sclerosis scale, and the morphological characteristics when comparing unaffected and severe sclerotic lesions. When considering intermediate degrees of sclerosis, such a correlation was not evident. (Figs. 5-7).

Discussion

The results of this study show an inverse relation between the different degrees of dentin sclerosis and the values of elastic modulus and hardness. These results are clear when comparing sound and severely sclerotic tissues. Furthermore, if intermediate grades are analyzed, the high variability of the sclerotic

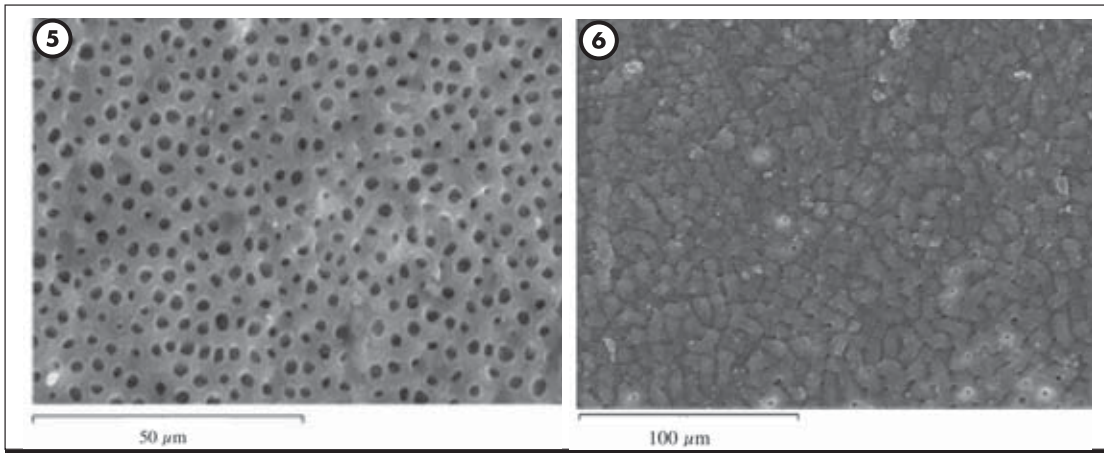


Fig. 5. SEM image of the normal dentin. The appearance is uniform and the dentin tubules are open with a homogeneous boundary. The peritubular dentin is apparent in some but not all tubules as a faint halo of lighter dentin around the tubule orifice.

Fig. 6. Image of occlusal sclerotic lesion graded 4. Total occlusion of the dentin tubules in the central area is observed, while open tubules with irregular boundary is observed in other zones. The image of the dentin area analyzed shows that most tubule orifices are occluded with solid, irregular material.

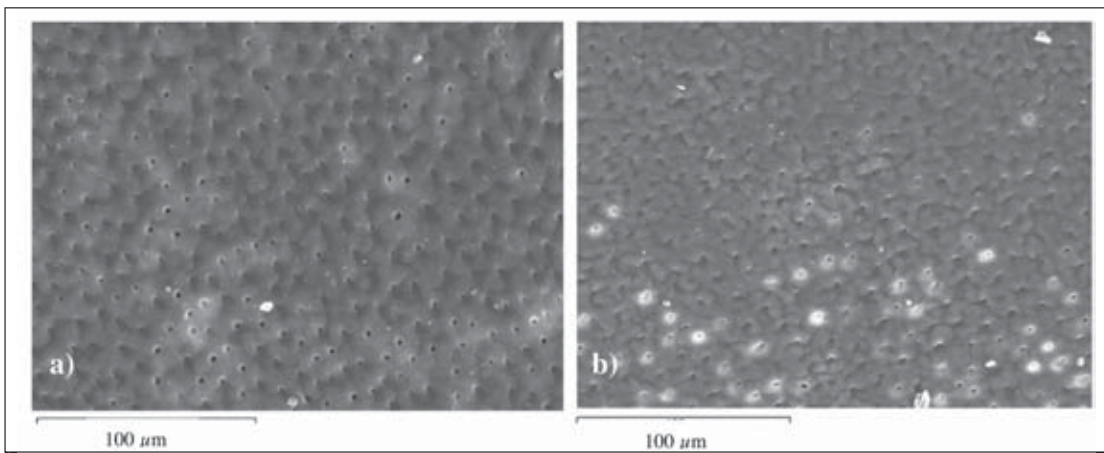


Fig. 7. Images of sclerosis scales 1 (a) and 3 (b). Great variability is observed with an irregular distribution of open tubules. Partial and total obliteration of the tubules are also found. It was not possible to distinguish the different grades of sclerosis, by their SEM morphology.

specimens leads to overlapping between close sclerosis grades, and clear conclusions cannot be reached. This can be explained because the North Carolina dentin sclerosis scale seems to have little specificity for slight or moderate sclerotic lesions and then, different grades of actual sclerosis could be easily linked to the same grade of the visual scale. Nevertheless, it is a simple way to classify lesions and acceptable correlations between the visual degrees of sclerosis and morphological characteristics of sclerotic dentin are obtained when undamaged and severe sclerotic specimens are compared.

The results obtained by nanoscratch are especially interesting because they offer a direct measurement of the wear resistance that seems to be lower in the affected than in the sound dentin, and indicates that the sclerotic process involved in the occlusal wear lesions leads to a faster wear.

Morphological changes produced in sclerotic dentin have been widely studied,^{5,11,24-26} although some aspects are yet unknown. The most significant circumstance is the obturation of the dentin tubules by cubic or rhombohedral crystals of withlockite, which are responsible for the transparency of sclerotic dentin. A hypermineralized superficial layer has been reported

in the wedge-shaped cervical lesions related to bacterium deposits,¹¹ but not in other locations. The changes produced in the intertubular dentin, like changes in mineralization or collagen matrix alterations are less known, but some chemical changes lead to high demineralization resistance.^{24,26} Sclerotic dentin also shows higher mineralization in peritubular zones.

There is no agreement in the literature about the mechanical properties of sclerotic dentin measured in different zones. Traditionally, the mineral content is related to mechanical properties. The higher mineralization grade, the higher hardness, elastic modulus and wear resistance. Therefore, sclerotic dentin is considered harder than undamaged dentin due to its higher mineralization.²⁷ However, recent studies have reported hardness values of transparent dentin under carious lesions less or equal to undamaged dentin, in both primary¹² and permanent teeth.^{13,25} Similarly, Zheng *et al*,¹⁴ studying the transparent region beneath moderately active and arrested carious lesions, obtained lower mechanical properties than in apparently normal dentin. The influence of the different dentin components on the mechanical properties should be considered; Kinney *et al*¹⁷ concluded that the intertubular dentin is the main factor, while

the dentin tubules have minimum influence. According to that, sclerotic dentin should show similar mechanical properties to normal dentin, in spite of its higher mineralization grade on peritubular areas and the crystalline deposits in the dentin tubules. This tendency will be altered only if changes in the mineral composition of the intertubular dentin are expected. Balooch *et al*¹⁸ compared the mechanical properties of normal and transparent root dentin by nanoindentation tests, showing similar hardness and elastic modulus in both peritubular and intertubular regions. Besides, there are circumstances (altered forms of dentin) where the expected linear relationship between mineral concentration and mechanical properties vanishes, and dentin can display low elastic moduli and abrasion resistance in spite of near-normal (90%) levels of mineral concentration.²⁸

In the present study, the morphological characteristics of occlusal wear lesions, according to SEM images, are examples of sclerotic dentin and similar to those described in other locations. These findings are in agreement with previous studies on attrited human dentin.¹⁵ However, the mechanical properties are lower than those of the corresponding normal dentin, and similar to that of the transparent zones beneath caries. This would suggest that these lesions might be less mineralized than normal dentin. Transparent and sclerotic dentin are commonly used as similar terms, but in fact, transparency is only an optical phenomenon produced by crystalline deposits in the dentin tubules. These deposits have little influence if any on mechanical properties and they have no relation with the intertubular mineralization grade. Other factors should also be taken into account: the lesion production mechanism, the degree of hydration of the specimens,^{29,30} and the lesion location, that determines the density, distribution and orientation of the tubules;^{4,31-34} Marshall *et al*¹³ showed that nanomechanical properties fall in deeper dentin. In the present study with horizontally prepared crown segments, the distance from the transparent lesions to the pulp was not determined. Additional research will be needed to correlate the nanomechanical properties and the mineral density of the transparent occlusal lesions produced by wear.

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