Micromorphological and hardness analyses of human and bovine sclerotic dentin: a comparative study

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Abstract: The purpose of this study was to test the hypothesis that both human and bovine sclerotic dentin have similar hardness properties, in addition to similar micromorphological characteristics. Sixteen teeth (8 human and 8 bovine) exhibiting exposed dentin in the incisal edge and showing characteristics typical of sclerosis were used. Vickers surface microhardness testing was conducted. Three areas of the dentin surface of each specimen were selected. All teeth were processed for scanning electron microscopy in order to estimate the amount (in percentage) of solid dentin on the sclerotic dentin surface. The data were compared by Student's *t* test ($\alpha = 0.05$). The micromorphological and microhardness data were compared by Pearson's linear correlation test ($\alpha = 0.05$). The mean percentages of solid dentin of human and bovine sclerotic dentin were similar (human 90.71 ± 0.83 and bovine 89.08 ± 0.81, $p = 0.18$). The mean microhardness value (VHN) of human sclerotic dentin was significantly higher than that of bovine sclerotic dentin (human 45.26 ± 2.92 and bovine 29.93 ± 3.83, $p = 0.006$). No correlation was found between the microhardness values and the amount of solid dentin in the sclerotic dentin, irrespective of the species considered (human $R^2 = 0.0240$, $p = 0.714$; bovine $R^2 = 0.0017$, $p = 0.923$; and combined $R^2 = 0.038$, $p = 0.46$). We concluded that although both bovine and human sclerotic dentin present a similar amount of solid tissue, human sclerotic dentin presents higher microhardness than bovine sclerotic dentin.

Descriptors: Dentin; Hardness; Cattle; Aged.

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Introduction

The elderly population, which has increased worldwide, has shown an increasing trend toward preserving natural dentition. Thus, *in vitro* studies using teeth with aging-altered dental tissues are needed to support the oral rehabilitation of elders.[1,2] Altered dentin forms, especially sclerotic or transparent dentin, appear gradually with aging and demand special attention regarding adhesive dentistry procedures. Sclerotic dentin can appear under carious lesions,[3,4] non-carious cervical lesions,[5-7] around the pulp chamber and/or pulp canal,[8] and also in the incisal surface of worn teeth.[1,2] Literature has given special attention to non-carious cervical tooth surface loss in the elderly population;[5-7] however, little is known about non-carious incisal/oclusal tooth surface loss.

In vitro studies using teeth with non-carious incisal/oclusal surface

loss are difficult to perform because of the scarce number of extracted teeth with these characteristics. Extracted human teeth from elders were readily obtainable in the past, especially due to periodontal disease; today, however, owing to the improvement in oral health conditions, the elderly population has maintained its natural dentition for a longer time, increasing its risk for non-carious cervical lesions and incisal/oclusal tooth surface loss. For this reason, bovine teeth have commonly been used as human tooth substitutes for *in vitro* studies.[1,2,9-11]

In a previous study, we examined the morphological similarities between bovine and human sclerotic dentine at the microscopic level. The similarities were confirmed by the density of open tubules in both species[1]. Likewise, bond strength tests comparing human and bovine sclerotic dentine have been carried out to assess whether the morphological similarities would result in similar behavior of these substrates with regard to dental adhesives. This line of research has opened the possibility of conducting research with bovine sclerotic dentine as an alternative to human sclerotic dentine.[2] The advantage of this substitution resides in the greater availability of bovine teeth and in the possibility of improving the standardization of the samples for *in vitro* studies.[1,2,9]

For *in vitro* studies, not only is the micromorphology important, but also the mechanical characteristics of the dentin substrate.[12,13] Microhardness is an important mechanical characteristic that could influence the results of these studies,[14,15] and although both human and bovine sclerotic dentin present similar micromorphology, they may present differences in microhardness or in other mechanical properties.

Based on the paucity of studies comparing these two dentin sources, the aim of the present study was to test the hypothesis that human and bovine sclerotic dentin have similar microhardness properties, in addition to similar micromorphological characteristics.

Methodology

Sample preparation

Eight bovine and eight human incisors were used. These teeth presented exposed dentin on the incisal edge, showing characteristics typical of sclerosis: a brownish, smooth and shiny surface, i.e., a "vitreous appearance." The human teeth were obtained from donors older than 50 years, who needed tooth extractions for clinical reasons, whereas the worn bovine teeth were obtained from animals older than 3 years, slaughtered for meat production. The Ethical Committee of the School of Dentistry from the University of São Paulo approved the project (197/2008).

Teeth from both experimental groups (human and bovine) were embedded into acrylic resin (Jet Clássico, Campo Limpo Paulista, Brazil), leaving only the incisal edges exposed. Next, the specimens were sectioned 5 mm below and parallel to the incisal edge, to obtain 16 discs of superficial dentin (5-mm thick). The incisal surfaces of all samples were then polished with #1200-, 2000- and 4000-grit sandpaper discs (Buehler, Lake Bluff, USA), under water cooling, for 60 s each. The polishing was finished with felt discs impregnated with Metadi II diamond paste (Buehler, Lake Bluff, USA). The specimens were then analyzed under optical microscopy to check the quality of the polish and to ensure that the sclerotic dentin characteristics of the polished surfaces were maintained. Next, the specimens were cleaned in an ultrasound cube (Kondortech, São Carlos, Brazil) (3 times for 10 minutes each), and stored in distilled water for 7 days.

Microhardness measurements

The 16 specimens were submitted to the Vickers microhardness test. The microhardness of each specimen was measured using a microindentation hardness tester (Shimadzu Corporation, Kyoto, Japan), under a load of 50 g for 45 s. Three pre-determined areas per specimen were selected: in the center and in two areas equidistant from the center to the proximal and distal sides of the incisal edge.[1] In each selected area, 3 Vickers indentations (100 μm apart) were performed, resulting in 9 indentions per tooth. The data were expressed in Vickers Hardness Number (VHN). The mean VHN value of the 9 measurements obtained per tooth represented the VHN of the tooth for the statistical analysis.

At the end of the microhardness testing, the

specimens were prepared for further scanning electron microscopy (SEM) investigation.

Scanning Electron Microscopy (SEM)

Preparation of the samples ready for SEM processing included washing in a detergent solution (Biodinâmica, Ibiporã, Brazil), sterilization in an autoclave (Dabi Atlante, Ribeirão Preto, Brazil) and fixation with 2.5% glutaraldehyde in a 0.1 M phosphate buffer (Electron Microscopy Sciences, Hatfield, USA). The same three pre-determined areas used for the microhardness test (center, proximal and distal) were electron-micrographed. The electron micrographs were taken at the same working distance, using a magnification of 2000x and operating at 20 kV. Electron micrographs were obtained with a Philips XL-30 scanning electron microscope (FEI Company, Hillsboro, USA).

An image analyzer program (Imagelab, São Paulo, Brazil) was used to measure the percentage of solid dentin on the incisal edges of all teeth. By excluding the percentage of the surface area occupied by empty dentinal tubules, the program calculates the percentage of solid dentin – both peri- and intertubular – on the sclerotic dentin surface.

Statistical analysis

The data obtained is presented as mean, standard deviation (SD) and standard error of the mean (SE) of both the microhardness (in VHN) and the solid dentin measurements (% of the total area) for the 8 specimens per group. The data from bovine and human dentin were compared by Student's *t*-test using the BioStat 5.0 statistical program (Analyst Soft, Belém, Brazil). Correlation between amount of solid dentin and microhardness was analyzed by the Pearson linear test using the same statistical program. The significance level was set at $\alpha = 5\%$ ($p \leq 0.05$).

Results

Figure 1 illustrates the micromorphology of the superficial sclerotic dentin of the incisal edges of human and bovine teeth. The scanning electron micrographs showed a similar number of dentinal tubules distributed homogenously throughout the dentin surface in both groups (human, Figure 1A, and bovine, Figure 1B). Most of the tubules were totally or partially closed, leaving apertures of different dimensions and shapes.

Table 1 shows the descriptive statistics for the data obtained in the morphological and microhardness analyses for the two groups assessed. The mean percentages of solid dentin on the surface of the sclerotic dentin at the incisal edges of human and bovine teeth were similar ($p = 0.18$). The mean VHN value of human sclerotic dentin was significantly higher than that of bovine sclerotic dentin ($p = 0.006$).

No correlation was found between the microhardness and the amount of solid dentin on the surface of the sclerotic dentin at the incisal edge, irrespective of the species considered (human $R^2 = 0.0240$, $p = 0.714$; bovine $R^2 = 0.0017$,

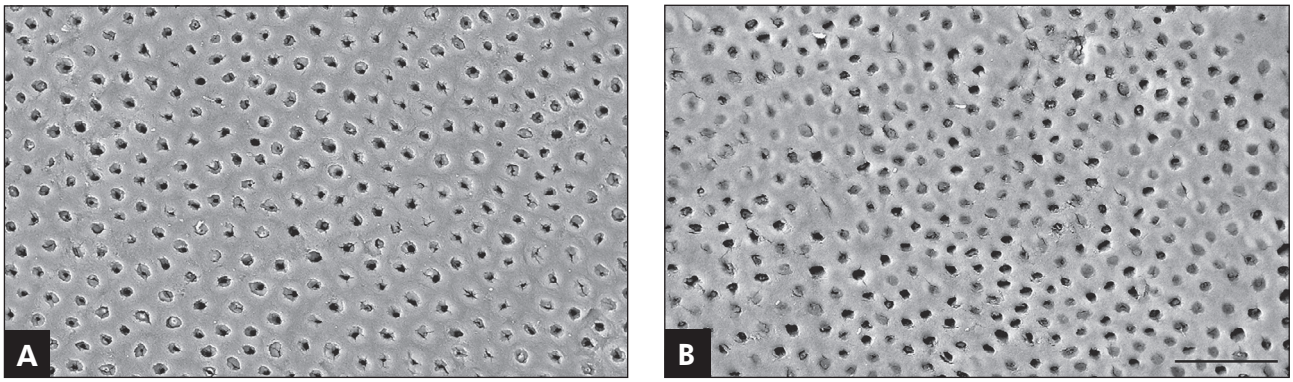

Figure 1 - Illustrative scanning electron micrographs of human (A) and bovine (B) sclerotic dentin. Note that although the images are not clearly similar, the amount of solid dentin (i.e. dentin area with no open tubules) are similar according to the image program (both images are in the same magnification; bar = 20 μm).

Table 1 - Descriptive statistics for the results of morphology and microhardness of the two groups assessed.

Parameters	Solid Dentin (%)		Microhardness (VHN)	
	Human	Bovine	Human	Bovine
n	8	8	8	8
Mean	90.71	89.08	45.26	29.93
SD	2.36	2.30	8.25	10.84
SE	0.83	0.81	2.92	3.83

SD: standard deviation; SE: standard error of the mean.

$p = 0.923$; and combined $R^2 = 0.038$, $p = 0.46$).

Discussion

Bovine sclerotic dentin may be considered a good substitute for human sclerotic dentin in adhesion studies, for many reasons. This alternative substrate has advantages over the human substrate, especially because samples are easier to acquire and standardize. The first advantage to using bovine substrate to substitute human sclerotic dentin refers to the micromorphological similarities between the two dentin forms. The tubule density in the superficial sclerotic dentin at the incisal edges of bovine and human teeth is also similar.[1] However, it is known that not only are the morphological characteristics of the substrate important for adhesion effectiveness, but also its mechanical properties.

The mechanical properties of dentin are particularly important for restorative treatment.[15] Among the relevant mechanical properties of dentin, microhardness has a strong relationship with dentin bond strength.[16] As a result, microhardness is an initial and important factor in predicting the behavior of dentin/restoration interfaces.[17] For this reason, this study aimed at comparing the microhardness of human and bovine sclerotic dentin, as well as the micromorphology of the two dentin forms. Dentin tissue from the incisal edges of aged human teeth, with exposed dentin presenting macroscopical characteristics of sclerotic dentin, and that of bovine teeth with the same characteristics were compared for micromorphology and microhardness. As expected, the micromorphology of both substrates was similar, showing equal amounts of superficial solid dentin. Nevertheless, the microhardness (VHN) of the human sclerotic dentin was significantly higher than that of the bovine tissue. Turssi *et al.*[18] reported that the Knoop microhardness of sound human root dentin was higher than that of bovine root dentine. On the other hand, other authors, also using Knoop microhardness, have shown similar values for sound human and bovine dentine.[19] When using bovine sclerotic dentin to substitute human sclerotic dentin in adhesion studies, this possible mechanical difference should be taken into consideration. Another interesting result of the present study was the absence of correlation between micromorphology and microhardness in sclerotic dentin.

Fusayama *et al.*[20] stated that the microhardness of dentin decreased with depth, based on the decreasing fraction of solid dentin. In accordance with this statement, Pashley *et al.*[21] observed an inverse correlation between microhardness of dentin and tubule density (i.e., increased tubule density near the pulp corresponded to reduced hardness). However, we have found no correlation between micromorphology and microhardness in the sclerotic dentin specimens analyzed in this study. In fact, although both bovine and human sclerotic dentin exhibited similar tubule density[1] as well as similar fractions of solid dentin, the microhardness of the bovine dentin was significantly smaller than that of the human dentin.

Marshal Jr. *et al.*[17] reviewed the structure and properties of dentin in an attempt to assess the methods used for dentin hardness measurement. According to these authors, because tubule density also correlates with the position between the pulp and the dentin-enamel junction (DEJ), it is not possible to determine from microindenter methods whether the decreased hardness was due to dentin morphology (increased tubule density) or differences in the constituent material properties of the dentin. Using atomic force microscopy (AFM), which can give more detailed information about the hardness and the microstructure of dentin, Kinney *et al.*[12,13] have found evidence that could explain the lack of correlation between the micromorphology and microhardness of the sclerotic dentin analyzed in our study. These authors observed that the peritubular dentin hardness did not depend on location. Thus, most – if not all – of the decrease in hardness ac-

cording to location can be attributed to changes in the hardness of the intertubular dentin, and not to an increase in the number of tubules. Whereas the number of tubules in non-pathological human dentin is not relevant for determining the hardness of this tissue, based on our results, we could infer that this also could be true for sclerotic dentin.

Although the composition of the intertubular dentin has not been assessed in this study, two differences between bovine and human sclerotic dentin would indicate the importance of this parameter in determining microhardness. First of all, the time involved in the sclerotic process of human dentin (over 50 years) is significantly higher than that involved in the sclerotic process of bovine dentin (3 years). The second difference resides in the oral environment conditions of cattle and humans. Cattle regurgitate incompletely-chewed food and re-chew it. This food is fermented by microorganisms that release fatty acids.[22] Although the acidity of these substances is buffered by sodium bicarbonate from the saliva, the substances can exert a demineralization effect on the sclerotic dentin, thus creating a substrate that, although similar in overall morphology, would differ in inorganic content. In fact, acid substances in contact with dentin surfaces promote a decrease in indentation resistance.[23] On the other hand, the rapid sclerosis of bovine dentin in comparison to that of human dentine at the incisal edges of worn teeth could also be the result of differences in the mechanisms of dentin formation and tubule closing. One possibility would be the deposition of crystals resulting from two sources: attrition or as a result of remineralization following the action of acids produced during bovine digestion. The morphology and the mineral content of these crystals in the tubule lumen and in the intertubular dentin could be responsible for the lower microhardness values found for the bovine dentin. Besides the mineral content, the organic fraction of the intertubular dentin in worn bovine teeth could also be different from that of human dentin, which could account for differences in the mechanical properties of the two dentin forms. Thus, the intertubular dentin of human and bovine sclerotic substrates must be further investigated in terms of the organic and inorganic composition of each dentin form. The collagen content and the chemical composition of the crystals deposited on these fibers in the intertubular sclerotic dentin should be studied.

Adhesion mechanisms are strongly based on the penetration of resin into the dentinal tubules for the formation of resin tags and microtags, and on their hybridization with intertubular dentin.[5] Thus, putative differences in amount and composition of intertubular dentin could contraindicate the use of bovine substrate as a substitute for human sclerotic dentin. However, in regard to bovine dentin, micromorphology was found to be more relevant than microhardness in determining bond strength. In fact, we have already shown that the bond strength of an adhesive system to bovine sclerotic dentin is influenced significantly by superficial treatment.[2] Alteration of superficial micromorphology by applying a diamond bur or diamond paste was responsible for improving bonding to bovine sclerotic dentin, to the point where values similar to those of bonding to sound dentin are reached.[2]

Bovine sclerotic dentin could substitute human sclerotic dentin in *in vitro* studies; however, further investigation is necessary to better understand how the differences and similarities between these biological substrates influence bond strength. More than just validating bovine dentin as a substitute for human sclerotic dentin, such studies could certainly contribute to improving the outcome of adhesive procedures in worn teeth; in turn, this improvement would help maintain tooth function and aesthetics in aging patients.

Conclusions

Based on the conditions of this study, we concluded that although bovine and human sclerotic dentin present similar micromorphology, human sclerotic dentin presents higher microhardness than bovine sclerotic dentin.

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