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Biochemical composition of carious dentin and different layers of sound dentin

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Abstract: The aim of this study was to evaluate the concentrations of calcium (Ca), inorganic phosphate (P_i) and fluoride (F) in carious dentin and in different layers of sound dentin. The samples examined were 52 permanent teeth (26 sound and 26 carious), which were subjected to two experiments to assess the mineral content of: 1) two layers (internal and external) of sound dentin and 2) sound and carious dentin. Ca and P_i were analyzed using a colorimetric method with arsenazo III (C₂₂H₁₈As₂N₄O₁₄S₂) and molybdate reagents, and F was analyzed using a specific electrode. A non-parametric test, the Mann-Whitney test, was used to verify differences between groups. Sound dentin showed a higher concentration of fluoride in the internal layer than in the external layer ($P = 0.03$), but no inter-layer differences in Ca or P_i concentration were evident. Lower concentrations of Ca, P_i and F were observed in carious dentin than in sound dentin ($P < 0.05$). The results of this study suggest that the internal layer of sound dentin has a higher fluoride content than the external layer, and that carious dentin has lower concentrations of Ca, P_i and F than sound dentin.

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Keywords: dental caries; minerals; dentin; permanent dentition.

Introduction

The process of dental caries results in demineralization of hard dental tissues, leading to a considerable reduction in their mineral content (1-6). Calcium, inorganic phosphate and fluoride are the main constituents of the mineral phase of dentin (2,7-9). The concentrations of these ions play an important role in the processes of demineralization and remineralization of dentin (3-4,10-12).

Several attempts have been made to analyze the mineral composition of different dentin layers (2-4). It is important to study the distribution of minerals in hard tissues and the relationship between the mineral content and pathologic processes. Previous studies of the inorganic phosphate and calcium contents of dentin have involved biochemical analyses (10,13). More recently, however, different methodologies such as energy-dispersive X-ray analysis (2,4,13-14), neutron activation analysis (15,16), atomic absorption (7-8) and laser (17-18) spectrometry have been employed. Biochemical analysis is able to detect small amounts of Ca, P_i and F, and has been widely used in studies of biofilm, dental enamel and biofilm fluid (19-24). Biochemical studies of dentin involved only a few samples and did not analyze the concentrations of Ca, P_i and F together. Accordingly, the results cannot be generalized with confidence to the demineralization of dentin during the process of caries.

The aim of the present study was to evaluate, using biochemical analysis, the concentrations of calcium, inorganic phosphate and fluoride in different mineralized layers of sound dentin, and the mineral content of both sound and carious dentin, in permanent teeth.

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Fig. 1 Schema showing preparation of sound dentin specimens for experiment 1 (mesial halves) and experiment 2 (distal halves). a) Limits of the dentin blocks used in experiment 1: external limit (enamel junction, A-B), internal limit (dentin-pulp junction, C-D), occlusal limit (A-C) and cervical limit (B-D). b) The blocks used in experiment 2 were collected from the occlusal surface 1 mm below the enamel-dentin junction (A).

Materials and Methods

To evaluate the concentrations of calcium, inorganic phosphate and fluoride in dentin (sound and carious), two experiments were carried to assess the mineral content of: sound dentin in different layers (experiment 1) and both sound and carious dentin (experiment 2).

Samples

In both experiments, the samples of sound dentin were obtained from molar teeth supplied by the Human Tooth Bank of the Faculty of Dentistry, Federal University of Santa Maria, RS, Brazil. The teeth had been stored in 2% formalin solution after extraction. The sound molars, without any damage to the crown portion, had been extracted due to periodontal disease, or for orthodontic or prosthetic reasons. The donors were all adults, of both genders, living in the city of Porto Alegre, RS, Brazil.

The carious human dentin samples were obtained from patients treated at the dental clinic of the Faculty of Dentistry, Federal University of Rio Grande do Sul. The samples were collected from teeth before restorative treatment. These teeth were not extracted; only samples of carious dentin were collected.

The total sample comprised 52 permanent molar teeth: 11 first molars, 13 second molars and 28 third molars. This study was approved by the Ethics Committee of the Faculty of Dentistry, Federal University of Rio Grande do Sul, Brazil (protocol number 288/08).

The sample size was based on a pilot study considering a statistical power of 0.8 and a level of significance of 0.05. A total of 24 samples for calcium and 13 samples

for analysis of fluoride and inorganic phosphate were found. Considering a possible loss of 8%, the sample size was 26 molar teeth for each analysis.

Specimen preparation

The crowns of 26 sound molar teeth were divided into two halves (mesial and distal) with a low-speed diamond saw machine (Buehler, Lake Bluff, IL, USA). The mesial halves were used in experiment 1 and the distal halves in experiment 2 (Fig. 1).

In experiment 1, blocks of dentin were obtained from the proximal surface within the following limits: the enamel-dentin junction and dentin-pulp junction (lateral limits), where two lines were drawn perpendicular to the lateral limits – a line from the pulp horn in the direction of the enamel-dentin junction (occlusal limit) and a line 2 mm below the occlusal limit (cervical limit) (Fig. 1a). The blocks were cut into two halves (external and internal blocks) with a low-speed diamond saw machine.

In experiment 2, blocks of sound dentin (1 mm^3) were obtained from the occlusal surface, 1 mm below the enamel-dentin junction (Fig. 1b). The blocks were collected using a low-speed diamond saw machine and a wafering blade. Prior to cutting, a caliper rule was used to measure the dimensions for cutting the dentin block ($1 \times 1 \times 1 \text{ mm}$).

Carious dentin samples were obtained from occlusal caries lesions before restorative treatment. Each treated tooth was isolated with a rubber dam. Before collection of carious dentin, the superficial necrotic disorganized soft dentin was removed employing a visual and tactile approach. The cavity was washed with saline and dried with sterile swabs, and material was sampled for biochemical analysis. An endodontic file was used as a reference to measure the depth of the carious cavity. Sound and carious dentin samples were obtained at a similar distance from the pulp.

After sectioning and collection, all samples were frozen at -20°C for subsequent analysis.

Biochemical analysis

All the samples (sound and carious dentin) were subjected to the same biochemical procedures. The samples were dehydrated for 24 h at 37°C , manually ground, and then weighed (Sartorius, Göttingen, Germany). For Ca, P_i and F extraction from the dentin, 0.5 M hydrochloric acid (HCl) was added to the microtubes (0.5 mL HCl/mg dry weight dentin) containing the samples. After 3 h of constant agitation at room temperature, the samples were centrifuged for 10 min at 14,000 rpm (Eppendorf, Hamburg, Germany) and the supernatant was retained

Table 1 Concentrations of calcium, inorganic phosphate and fluoride in the internal and external layers of dentin

	<i>n</i>	Internal layer			External layer			<i>P</i>
		Percentiles			Percentiles			
		25	50	75	25	50	75	
Calcium (μmol Ca/mg dentin)	26	0.20	0.28	1.11	0.19	0.28	0.86	0.52
Inorganic phosphate (μmol P _i /mg dentin)	23*	0.27	0.50	1.02	0.25	0.30	0.97	0.64
Fluoride (ppmF)	26	2299.00	5379.00	12777.00	1218.00	2879.00	7196.00	0.03

*In three samples the amount of P_i could not be determined
Mann-Whitney non-parametric test

Table 2 Concentrations of calcium, inorganic phosphate and fluoride in carious and sound dentin

	<i>n</i>	Carious dentin			Sound dentin			<i>P</i>
		Percentiles			Percentiles			
		25	50	75	25	50	75	
Calcium (μmol Ca/mg dentin)	25*	0.09	0.18	0.53	0.16	0.32	0.62	0.04
Inorganic phosphate (μmol P _i /mg dentin)	26	0.04	0.07	0.13	0.10	0.14	0.25	0.00
Fluoride (ppmF)	26	289.00	967.00	2283.00	1197.00	2727.00	5232.00	0.01

*In one sample the amount of Ca could not be determined
Mann-Whitney non-parametric test

for Ca, P_i and F determination. Inorganic phosphate and calcium were determined colorimetrically using the reagents arsenazo III and molybdate, respectively (17,19-21). Fluoride measurement was performed using a fluoride-sensitive electrode (Orion 9609, Orion Research Inc., Beverly, MA, USA) connected to an ion analyzer (Procyon Instrumentos Científicos, São Paulo, São Paulo, Brazil) (17,19,21-22). The results were expressed as micromoles per milligram of dentin ($\mu\text{mol/mg dentin}$) for Ca and P_i , and in parts per million (ppm) for F.

Statistical analysis

A non-parametric test, the Mann-Whitney test, was used to verify differences in calcium, inorganic phosphate and fluoride concentrations in different layers of dentin and for comparison between sound and carious dentin. The statistical analyses were done using the SPSS software package, version 17.0, for Windows (SPSS, Chicago, IL, USA) at a significance level of 0.05.

Results

Table 1 shows the concentrations of Ca, P_i and F in different layers of dentin. For calcium and inorganic phosphate, no differences in concentration were observed between the internal and external layers. Only for fluoride

did the internal layer show a higher concentration than the external layer ($P = 0.03$).

The concentrations of calcium, inorganic phosphate and fluoride in sound and carious dentin are expressed in Table 2. Carious dentin had lower concentrations of calcium, inorganic phosphate and fluoride than sound dentin ($P < 0.05$).

Discussion

The present study assessed the inorganic composition of sound and carious dentin of permanent human teeth using biochemical analysis.

The concentrations of Ca and P_i were similar in the internal and external layers of dentin, while a higher concentration of fluoride was observed in the internal layer than in the external layer. There was considerable variation in the concentrations of calcium, inorganic phosphate and fluoride among the analyzed samples. Previous studies have reported the composition of minerals in sound dentin (1,15,25). The concentrations of calcium and inorganic phosphate may vary among individuals according to age, gender, location and environmental factors (7,15,24,25). The variation of mineral concentrations observed in the present study was similar to that already noted in other studies (7,13,15).

Angker et al. (3), using back-scattered electron (BSE) imaging, showed that the deep circumpulpal dentin (accounting for around 15% of the circumpulpal dentin) had a decreased mineral content. However, this small difference in mineral content observed by Angker et al. (3) was not noted by Levine (13), or in the present study. This difference in results could have been attributable to sample collection methodology. In the present study, each dentin sample was divided into two halves, whereas in the study by Angker et al. (3) the measurements were done continuously with a working distance of 10 mm. The small portion (15% of the circumpulpal dentin) with a lower mineral content observed by BSE imaging accounted for only a small part of the internal layer we observed. Therefore, such small differences in mineral content could not be measured in the present study.

The great variation in the amount of fluoride observed in sound dentin among the samples has also been noted by others. However, the concentration of fluoride observed in the present study was higher than that in other studies done in the 1970s (7-8,15), perhaps due to the increased availability of fluoride. Furthermore, our samples were obtained from an area with a fluoridated water supply where there was access to fluoride dentifrice, whereas the samples used in other studies (7-8,15) were collected from non-fluoridated areas where access to fluoride dentifrice was probably limited. Higher concentrations of fluoride were observed in the internal dentin layer than in the external layer. Weatherell et al. (26) also observed an increase in fluoride concentration from the dentin-enamel junction toward the pulp chamber.

Cariou dentin has a lower mineral content than sound dentin (1-4,13). In the present study, significantly lower amounts of calcium and inorganic phosphate were detected in carious dentin than in sound dentin. These results are consistent with the pathology of dental caries, which is characterized by mineral loss (1-4,13).

In dental enamel the concentrations of fluoride in carious teeth are higher than in sound teeth, due to a remineralization process that allows the formation of fluorapatite (27,28). In the present study, carious dentin had a lower concentration of fluoride than sound dentin. The carious dentin was collected from carious cavities with intact walls, which showed no destruction of the proximal wall, and thus were not fully exposed to the oral environment. These environmental conditions could explain the lower amount of fluoride due to the predominant demineralization process occurring in the carious dentin located at the bottom of the cavity.

The results of this study suggest that the internal layer of sound dentin has a higher fluoride content than

the external layer, and that carious dentin has lower concentrations of Ca, P_i and F than sound dentin. Further studies examining the effects of different treatments on carious dentin using the same methodology should be conducted. The present results are considered to provide useful reference data.

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