Laboratory model to evaluate the influence of stress and corrosion in the formation of non-carious cervical lesions

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ABSTRACT | *Objective:* To conceive and test a laboratory model that simulates the multifactorial etiology of non-carious cervical lesions. The model enables researchers to assess the pathological process with increasing levels of complexity, focusing on tension and corrosion. The model is aimed at clarifying the mechanisms that lead to the development of these lesions. *Methods:* Specimens were manufactured from bovine incisors cut into $18 \times 3 \times 3$ mm sticks, with a notch in the cervical region facing the pulp, in order to concentrate the stresses on the surface of the cementum-enamel junction when fixed at the apical end and loaded for bending on the incisal edge. One group was immersed in distilled water and the other in pH 4.5 acetate buffer for 72 h. Each group was divided into three subgroups: one subgroup without loading, and two subgroups submitted to loading (800 gf) to cause either compression or tensile stress. After the test, 0.05 mm histological lamellae of the specimens were processed and photographed using a light microscope, and the damages were assessed. *Conclusion:* The laboratory model that was developed enabled the precise measurement of the depth of loss and demineralization of tooth tissue in the specimens, whether submitted to stress or not. The formation of other damages, such as cracks and fractures, could also be observed; this made it possible to infer the influence of compressive and tensile stresses on the etiology of non-carious cervical lesions. The model can be further enhanced by making it possible to apply cyclic loads and interspersed abrasive challenges.

DESCRIPTORS | Demineralization; Tensile Strength; Dental Enamel; Dentin.

RESUMO | **Modelo laboratorial para avaliar a influência da tensão e da corrosão na formação de lesões cervicais não cariosas** • *Objetivo:* Idealizar e testar um modelo laboratorial para simulação da etiologia multifatorial de lesões cervicais não cariosas, que permita avaliar o desenvolvimento dessas lesões com níveis crescentes de complexidade, com ênfase na tensão e na corrosão, visando contribuir com o esclarecimento dos mecanismos que conduzem à sua formação. *Métodos:* Os corpos de prova foram construídos com incisivos bovinos cortados na forma de palitos com $18 \times 3 \times 3$ mm, com um entalhe na região cervical da parede voltada para a polpa, a fim de concentrar as tensões na superfície da junção cemento-esmalte quando engastados no extremo apical e carregados em dobramento no extremo incisal. Um grupo foi imerso em água destilada e outro em solução tampão de acetato pH 4,5 durante 72 h. Cada grupo foi dividido em três subgrupos: sem carga e com carga (800 gf) para provocar tração ou para provocar compressão. Após o ensaio, os espécimes foram processados para histologia em lamelas de 0,05 mm, fotografados em microscópio de luz, e os danos foram avaliados. *Conclusão:* O modelo laboratorial desenvolvido permite a medida precisa da profundidade de perda e desmineralização de tecido dentário de corpos de prova submetidos ou não à tensão, bem como observar a ocorrência de outros danos como trincas e fraturas, o que torna possível inferir a influência da tensão de tração e compressão na etiologia das lesões cervicais não cariosas. O modelo poderá ser futuramente enriquecido com a possibilidade de aplicação cíclica de cargas e desafios abrasivos intercalados.

DESCRITORES | Desmineralização; Resistência à Tração; Esmalte Dentário; Dentina.

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INTRODUCTION

Non-carious cervical lesions are characterized by loss of tooth structure near the cementum-enamel junction, not associated with the action of microorganisms. These lesions are usually attributed to a traumatic tooth brushing habit or excessive intake of acidic foods.[1] However, these wedge-shaped lesions, which often present subgingivally or in isolated teeth, cannot be explained satisfactorily by the above-described mechanism.[2,3]

Lee and Eakle[4] suggested that these lesions may be related to occlusal forces that are not aligned with the long axis of the tooth, and that tend to flex it, causing tensile stress on one side and compression on the other side of the tooth. According to the authors, enamel and dentin are highly resistant to compression, and damage to the crystalline structure is less likely to occur under this type of stress. But the two structures have limited resistance to tensile stress, which can break the chemical bonds between the hydroxyapatite crystals. When the union of the crystals is broken, the spaces between the crystals increase and the molecules from the oral environment can penetrate this region, making the breakage irreversible.

This mechanism of pathological loss of substance in the cementum-enamel junction has been called "abfraction" to differentiate it from other mechanisms that may be involved in the formation of non-carious cervical lesions.[5]

It is difficult to determine the etiology of certain non-carious cervical lesions, since the loss of tooth substance occurs over a prolonged period, and it is therefore difficult to ascertain whether the etiological factor present during the examination was present during the entire process.[6]

According to Grippo,[7] the mechanisms that contribute to the formation of non-carious cervical lesions can act individually or in synergistic combinations with other mechanisms. They may act simultaneously, sequentially or alternatively, leading to the loss of dental mineral tissues.

Although it is accepted that non-carious cervical lesions have multifactorial etiology, the relative contributions of various possible mechanisms remain unclear. For many authors, the most widely accepted causes of these lesions are abrasion and corrosion; however, abfraction is one of the most debated and controversial theories, which still needs to be proved.[8-10]

The objective of this study was to present a laboratory model that simulates the multifactorial etiology of non-carious cervical lesions. This model enables researchers to assess the pathological process with increasing levels of complexity, focusing on stress and corrosion, in order to clarify the mechanisms that lead to the development of these lesions.

TECHNIQUE EMPLOYED
Specimen preparation

Bovine incisors were used for this laboratory model. They were cleaned and examined with a magnifying glass in order to discard those with cracks and structural defects. The teeth were stored in a 1% thymol solution at 4°C. The teeth were rinsed in running water and then stored in distilled water at 4°C two days before beginning their preparation.

The teeth were mounted in a cutting machine (Isomet; Buehler, Lake Bluff, IL, USA) and fixed with sticky wax (Kota, São Paulo, SP, Brazil). Two transverse cuts were made with slowly rotating double-sided diamond discs (Extec, Enfield, CT, USA), under refrigeration, to remove the apical third of the root and occlusal third of the crown, thus obtaining 18 mm specimens (8 mm crown and 10 mm root). The teeth were then cut axially to obtain $18.0 \times 3.0 \times 3.0$ mm sticks of the buccal surface.

One stick was prepared and used as a standard for making the others. The buccal surface of the standard stick was worn down along its entire length with a cylindrical diamond bur (Reference
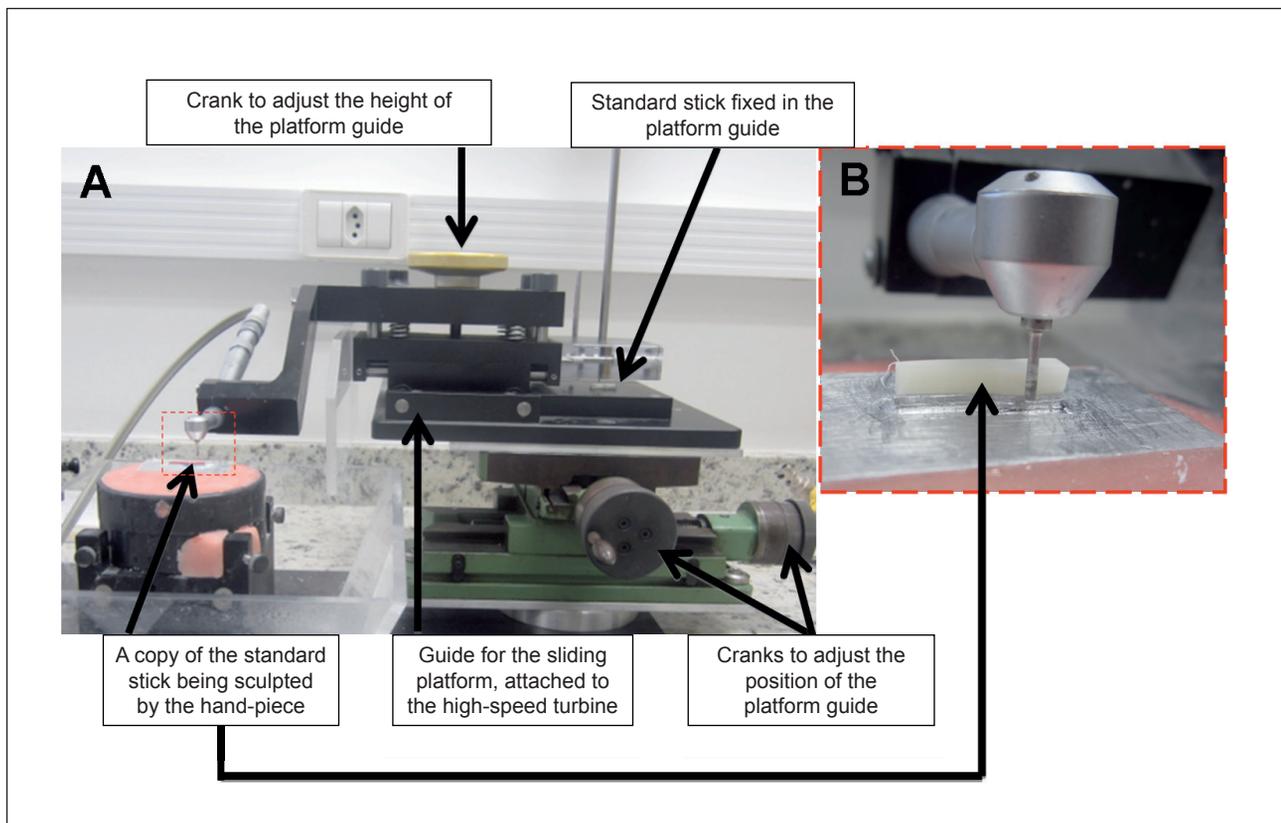
Figure 1 | Device used for fabrication of the specimens. **A:** overview; **B:** detail of the support table and stick support.

1093 FF; KG Sorensen, São Paulo, Brazil) at high speed to smooth and plane the surface, and to eliminate traces of cementum, because the enamel and dentin of bovine teeth are normally covered with irregular cementum formations. Afterwards, a cylindrical transverse notch (1.6 mm in diameter in the axial wall of the dentin of the cervical region) was made using a cylindrical bur (Reference 3101; KG Sorensen) at high speed. The purpose of the notch was to concentrate the traction or compression stresses in the cervical region of the buccal surface.

The standard stick was fixed onto a device to make exact replicas (in shape and size) out of bovine incisors (Figure 1). The buccal surfaces of the sticks were polished with felt discs and diamond paste (Diamond Gloss; KG Sorensen) in order to obtain a standardized smooth surface.

A small cavity was made using a 1/2 spherical carbide bur at high speed on the incisal edge of the sticks. In the cavity, 0.5 mm orthodontic wire, folded into an L-shape, was bonded using acid etching, dentin adhesive and composite resin. The reason for this procedure was to establish a precise point to apply the load. In the case of the sticks subjected to compressive stress, the free end of the wire was directed towards the lingual side of the stick, whereas, in the case of the sticks subjected to tensile stress, the free end was directed towards the buccal surface.

Adhesive tape (1.5 mm wide, 18 mm long) was bonded to the labial surface of the sticks, occupying a central longitudinal band of the surface. Two coats of an acid-resistant clear varnish (Procosa, São Paulo, SP, Brazil) were then applied to all surfaces of the sticks. The varnish was allowed to dry for 24 h. The tape was then removed, leaving a cen-
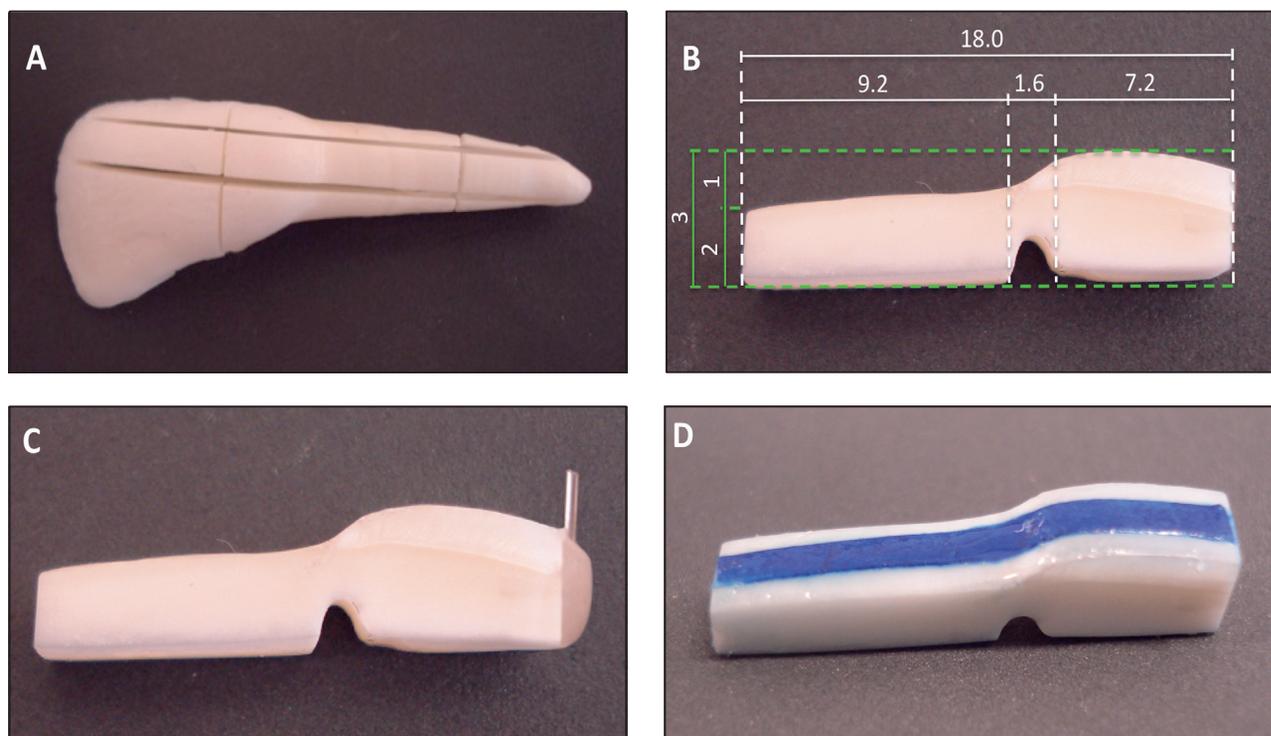
Figure 2 | Preparation of test specimens. **A:** Bovine tooth with cuts made for the manufacturing of dental sticks; **B**: Specimen after making the cervical notch, with dimensions in millimeters (mm); **C:** Specimen with the wire attached to the incisal end for load application; **D:** Specimen with a central longitudinal band covered with tape, and remaining areas protected with acid-resistant varnish.

tral band on the buccal surface of the sticks, which were exposed to the acid challenge, while the adjacent areas were protected by the varnish. The protected lateral buccal regions served as a reference for comparison between the areas of enamel and dentin exposed (or not) to the demineralizing solution (Figure 2).

SIMULATION OF ETIOLOGIC FACTORS
Mechanical factor

The sticks were divided into two groups: one group was immersed in distilled water and the other in pH 4.5 acetate buffer for 72 h. Each group was divided into three subgroups: two of the subgroups were submitted to a static load (one for tensile stress and the other for compression), while the third subgroup was not subjected to any loading. A device was constructed to carry out the loading, consisting of a support for twelve weights. Each weight

was machined from a PVC pipe and the load could be adjusted by the placement of lead shot (the type used for hunting). A metal rod was attached at each end of the pipe. The rods ran through guide holes that kept the weight in position. The lower rod was supported at the tip of the orthodontic wire bonded to the end of the stick, in order to concentrate tension in the cervical region of the specimens.

The load applied to the sticks was 800 gf. This load was defined in a pilot study, and corresponded to two thirds of the mean fracture load of 12 sticks tested using a universal testing machine (Kratos, São Paulo, Brazil).

The loading device contained a tank for the demineralizing solution. The inside of this tank had a holder for fixation of the sticks, a thermostat to keep the temperature constant at 37°C and a minipump to agitate the solution. Figure 3 shows the device used for the static load.
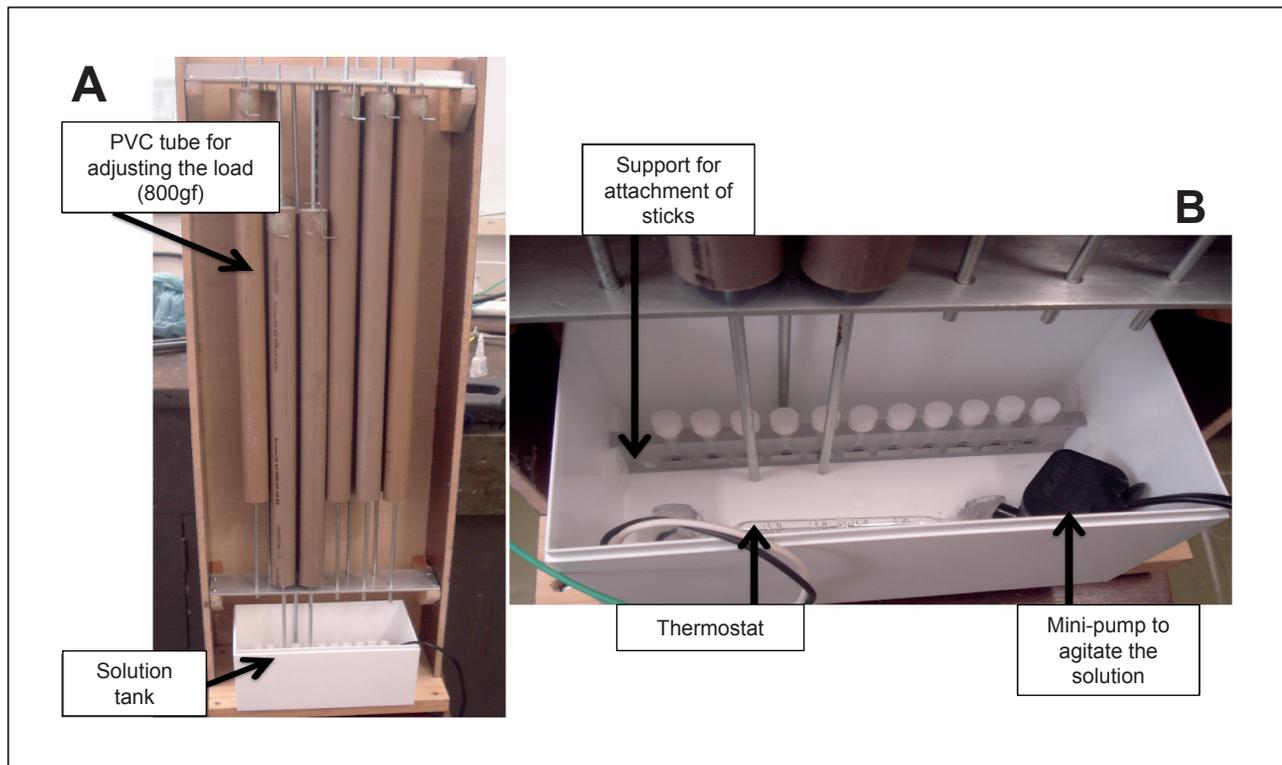
Figure 3 | Device used to carry out the static loading. **A:** overview; **B:** detail of the inside of the solution tank.

Corrosive factor

In the group of specimens subjected to the acid challenge, both the subgroups of specimens not subjected to loading, as well as those subjected to loading, were immersed in a 900 mL of an acetate buffer demineralizing solution containing 2.2 mM of calcium chloride ($CaCl_2$), 2.2 mM of sodium phosphate (NaH_2PO_4), and 0.05 M of acetic acid, at pH 4.5 adjusted with potassium hydroxide (KOH). The solution contained 95 mg/L of thymol to prevent the growth of microorganisms. The length of time of acid challenge exposure, determined by the pilot study, was 72 h.

EVALUATION OF THE EFFECTS

Histological slides of specimens impregnated with acrylic resin were prepared to evaluate the effects on the sticks. Initially, the sticks were rinsed for 24 h, and then the varnish was removed from the stick surfaces with a cotton swab soaked in acetone, gently applied on its surface to prevent the wearing of deteriorated regions. The specimens were dehydrated in an ascending alcohol sequence, and then soaked in methyl methacrylate using standard methodology for embedment, cutting, wear and polishing of the plates to obtain slides that were approximately 0.05 mm thick.

The specimens were divided into two groups to obtain the lamellae; one was sectioned transversely and the other, longitudinally. Two plates were obtained from the transversely cut specimens; one in enamel and the other in dentin, both at a distance of 0.5 mm from the dentin-enamel junction of the stick. An acid-exposed lamella from the central region was obtained for the longitudinal sections, including dentin and enamel regions.

The histological slides were observed and photographed at 40, 100 and 200 times magnification

Figure 4 | Microscopic image of the stick showing the measurements of depth of dentin loss (Dl) and dentin demineralization (Dd).
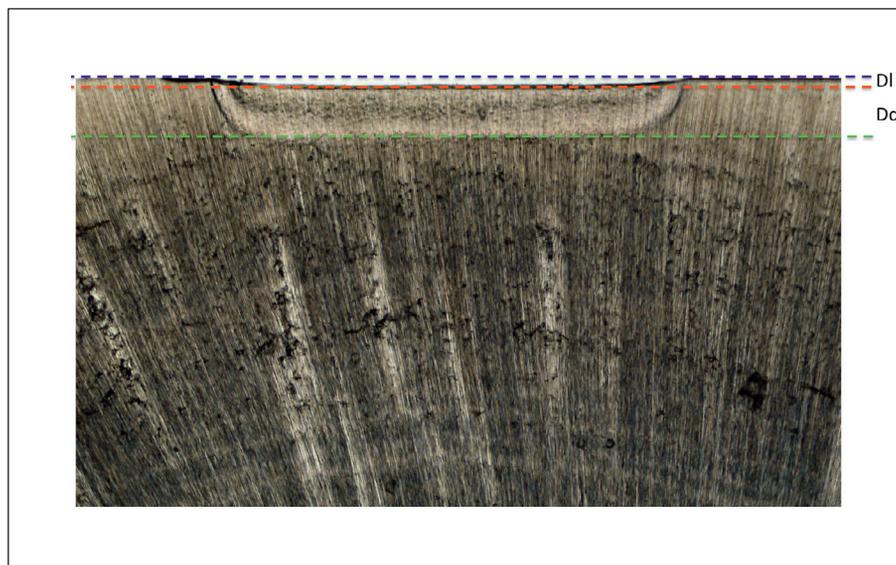
Figure 5 | Microscopic image of the stick showing the measurement of the depth of enamel demineralization (Ed).

using a light microscope (Olympus BX 60; Olympus Corporation, Shinjuku, Tokyo, Japan) equipped with a camera (Olympus DP 72; Olympus Corporation).
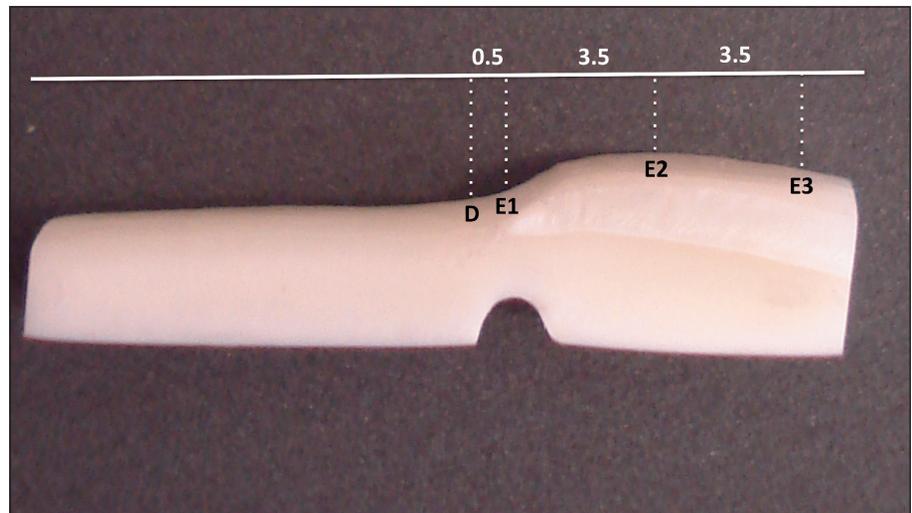
The depth of demineralization and/or the loss of dentin and enamel were measured in the photographs taken from the slides. The presence of cracks and fractures of the enamel was also assessed, as well as the formation of gaps in the junction between enamel and dentin, for comparison between groups.

Cell F software (Olympus Soft Imaging Solutions GmbH, Olympus Corporation) was used to mark the images and measure the depth of demineralization and tooth tissue loss.

In the transversely sectioned lamellae, the depth of dentin loss (Dl) was considered as the distance between two parallel lines that were drawn, one close to the buccal tooth surface that was protected by the

Figure 6 | Sites where measurements were made of the depth of demineralization of enamel (E) and dentin (D) in longitudinally sectioned specimens.

varnish (blue line, Figure 4) and the other passing through the central region at the bottom of the cavity (red line, Figure 4). The depth of dentin demineralization (Dd) was measured by calculating the distance between two parallel lines, one drawn close to the bottom of the cavity (red line), and the other subjacent to the demineralized region, which had a lighter color than the sound dentin (green line).

The depth of enamel demineralization (Ed) was measured in the same manner, i.e., by measuring the distance between two parallel lines, one line tangent to the buccal surface of the enamel, which had been protected by varnish (blue line, Figure 5), and the other subjacent to the demineralized region, which was darker than the sound enamel (green line, Figure 5).

Four measurements of the demineralization depth were made in the longitudinal section lamellae: one measurement in the dentin located apically, at a distance of 0.5 mm from the cervical region (junction of the enamel and dentin on the buccal surface; D), and three measurements in the enamel (Figure 6):

- the first in the cervical region (E1),
- the second in the middle third of the buccal surface of the stick, 3.5 mm from the cervical region (E2), and
- the third in the incisal third of the buccal surface of the stick, measured at 7.0 mm from the cervical region (E3).

The objective was to compare areas subject to higher or lower tension.

CONCLUSION

The laboratory model that was developed enabled the precise measurement of the depth of loss and demineralization of tooth tissue of specimens that were or were not submitted to stress. It also made it possible to observe the occurrence of cracks and other damage, such as fractures, and, therefore, to infer the influence of tensile and compressive stresses on the etiology of non-carious cervical lesions. The model can be further enhanced by making it possible to apply cyclic loads and interspersed abrasive challenges.

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