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# Predicting infiltration of the surface layer of natural enamel caries

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ABSTRACT

*Aim:* To test the hypothesis that the water volume more easily available for diffusion ($\alpha_d$) is the best predictor among all major components of the proportion of pore volume infiltrated by a liquid in the surface layer of dry natural enamel caries (NEC).

*Materials and method:* Two aqueous solutions of mercuric and potassium iodide (Thoulet's solutions) with different refractive indexes (1.4 and 1.47) and penetration coefficients (3212 cm/s and 2297 cm/s) were tested at histological points ($n = 63$) of ground sections of NEC lesions. Component volumes were measured with microradiography and interpretation of birefringence. Real-time 2D mapping of capillary flow was performed with orientation-independent polarizing microscopy.

*Results:* $\alpha_d$ was a good predictor for both liquids (T1.40: $R^2 = 0.413$; T1.47: $R^2 = 0.505$), but was similar to the water and air volumes for Thoulet's 1.47, and to the mineral and organic volumes for Thoulet's 1.40. From real-time 2D mapping, infiltration in ground sections occurred in two propagation directions, perpendicularly to the prism paths (at the centre of the lesion bodies) and axially to the prism paths (at all parts of the lesions), with two penetration rates, the faster related to prisms sheaths and the slower related to intraprismatic pores, affecting penetration length and air displacement.

*Conclusions:* $\alpha$d was a good predictor for both liquids, but was similar to the water and air volumes for T1.47 and to the mineral and organic volumes for T1.40. Both flow mechanics and component volumes are required to interpret infiltration of liquids into NEC.



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## 1. Introduction

The early stages of the carious process result in a complex network of nano-scaled larger-than-normal pores in dental enamel. Options for treating natural enamel caries (NEC) lesions have long been investigated by arresting further development and reducing pore size. Reduction of pore size can be achieved by remineralization[1] or infiltration of the pores with fluid resins (known as infiltrants).[2] The outcomes of such treatments depend, among other factors, on the ease with which remineralizing agents and infiltrants penetrate into NEC. Data indicated that the surface layer (SL) of NEC reduces the penetration of materials into NEC. Its removal has been shown to be important for proper penetration of resin adhesives and infiltrants into NEC,[3–6] and increased penetration of remineralizing materials has been achieved by procedures involving demineralization (for more information see review by Cochrane et al.)[1] and removal of organic matter.[7] As was recently highlighted in a study, development of procedures to increase penetration of materials through the SL of NEC will represent significant advance in clinical remineralization[1] as well as infiltration. A recent report showed that infiltrants penetrate deeper into NEC when they are active (probably because their SL is more porous)[8] or when inactive lesions are pretreated with acid (to enhance pore sizes).[9] Compared to sound enamel and inactive NEC lesions, active NEC lesions more frequently present pH $\leq$ 5.5 in the pores of their lesion bodies, which might favour a more porous SL.[10] To preserve more tissue over time, it was emphasized that preserving the SL of NEC is preferable to acid pretreatment,[9] and a more conservative pre-treatment of the SL of active NEC was recently reported.[11]

Resin infiltrants are transported into enamel pores by capillary forces when carious enamel is dry,[12,13] filling the air volume of enamel pores. Such air volume is the volume of air that replaces the water pore volume removed at room temperature (known as the loosely bound water volume), which, in turn, is a fraction of the water pore volume,[14] and the total pore volume of carious enamel is the sum of the organic and water volumes.[15] The volume of certain foreign liquids infiltrated into the pores of carious enamel has been previously quantified: quinoline and alcohols in the dark zone of NEC, using interpretation of enamel birefringence[16]; and a resorcinol–formaldehyde resin in the whole enamel caries lesion (including both natural and artificial lesions, but with no data at any particular histological layer) by measuring the mass of chloronaphthalene imbibed into the air pore volume created by drying in vacuum at room temperature.[17] Those measurements neglected the organic volume and the volume of water that remains after drying at room temperature (known as the firmly bound water volume). There are no quantitative data on the volume filled by liquids (including infiltrants) in carious enamel taking into account all constituents of the pore volume, and such data are important for studying the permeability of the SL of NEC. Recent methodological developments with ground sections of NEC have enabled spatially resolved measurement of the water volume more easily available for diffusion,[15] which is expected to be directly proportional to the air volume in dry carious enamel. By testing this parameter, the influence of the components of enamel (mineral, water, and organic matter) on the permeability of the SL can be further investigated and contribute to improve infiltration of materials into NEC.

The aim of this study was to test the hypothesis that the water volume more easily available (compared to the total water, firmly bound water and loosely bound water volumes) for diffusion is, among all enamel component volumes, the best predictor of the proportion of total pore volume infiltrated by a liquid in the SL of dry (at room temperature) NEC. The liquids tested were two aqueous solutions with high refractive indexes and high penetration coefficients (Thoulet's solution), which were previously used to define histological layers of NEC under polarizing microscopy.[18]

## 2. Materials and methods

### 2.1. Samples

Sixty-three histological points were selected from 21 non-cavitated approximal inactive (opaque enamel with shiny surface) NEC lesions from human third molars and premolars, which were extracted for oral health reasons. Lesion activity was determined by a consensus of calibrated examiners (intra-examiners kappa 0.739 and 0.856; inter-examiner kappa 0.812) with regard to the NYVAD system[19] applied to a pool (30 lesions) of non-cavitated NEC lesions that were analyzed under the stereomicroscope after removal of organic coating (dental plaque) on the tooth surface (1% sodium hypochlorite for 30 s), washing with water (30 s), and air drying with compressed air (10 s). This study was approved by the Ethical Committee on Research in Humans of the Lauro Wanderley University Hospital (Federal University of Paraiba, Brazil; protocol number 09285912.3.0000.5188), and adult volunteers donated all teeth with signed consent. Teeth and ground sections were kept in 0.02% aqueous sodium azide solution before and after infiltration experiments with test solutions. Longitudinal sections were obtained from the approximal surfaces with NEC using a diamond disc under water irrigation. Then, each section was ground to a thickness of 50–90 μm using a lapping jig as recently described.[20] Sample thickness at the selected histological points were measured with the sections positioned edge-on in a transmitted light polarizing microscope equipped with a reticle and a 20× objective (resolution of 0.7 μm).

### 2.2. Quantification of mineral volume

Glass plates sensitive to X-rays (resolution of 2000 lines/mm; AGHD, Microchrome Tech., USA) were covered by all ground sections and an aluminium step-wedge calibration standard (10 sheets, each with a thickness of 20 μm and purity of 0.999%; ESPI Chemicals, USA); subsequently, they were exposed to X-rays in a PCBA Inspector (General Electric, Germany; Tungsten anode filtered by a 0.254 mm thick beryllium window) operating at 40 kV and 0.25 mA for 25 min. The corresponding emission peak is 24 keV,[21] which was used to calculate linear attenuation coefficients of enamel mineral (using density of 2.99 g $cm^{-3}$)[22] and aluminium (using