Archives of Oral Biology 82 (2017) 134–140

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Quantitative study of the proportion of the pore volume of human fluorotic enamel filled by resin infiltrant

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ARTICLE INFO

Keywords:
Infiltrant
Dental enamel
Fluorosis
Capillarity
Histopathology

ABSTRACT

Aim: Capillarity theory predicts that the pore volume infiltrated by a liquid in a body with tubular capillaries is directly proportional to the capillary radius. The expected volume available for infiltration is the loosely bound water volume, which can be related to the capillary radii. We tested the hypothesis that the proportion of the pore volume infiltrated by resin infiltrant (V_{ratio}^{resin}) is correlated and agrees with the proportion of the pore volume with loosely bound water (V_{ratio}^{a2}).

Design: Seven human fluorotic third molars (4 unerupted and 3 erupted; TF scores 4 to 7; fluoride content of inner coronal dentin ranged from 143 to 934 μg Fluoride/g) were prepared and resin infiltration was performed during 10 min in fluorotic enamel ground sections. Penetration depths were measured (polarizing microscopy and CLSM) and mineral volume and non-mineral volumes were measured at histological points (n = 92) along transversal lines traced from the enamel surface to the enamel-dentin junction.

Results: No well-mineralized surface layer was found. Infiltration depths ranged from 250 μm to 900 μm. V_{ratio}^{resin} ranged from 1.8 to 17.7% (mean of 10.13% ± 4.1%), was lower than V_{ratio}^{a2} ($p < 0.00001$ Hedge's g = 1.51, 95% CI: 1.18/1.83), and correlated positively with V_{ratio}^{a2} (R = 0.684; 95% CI: 0.557/0.780) and negatively with the air volume remained after infiltration (R = −0.79; 95% CI: −0.698/−0.780). V_{ratio}^{a2} exceeded V_{ratio}^{resin} in 5% (1/4 of V_{ratio}^{a2}) on average.

Conclusion: V_{ratio}^{a2} and V_{ratio}^{resin} correlated well, but lacked good agreement. Organic matter, firmly bound water and air remained in enamel pores after resin infiltration.

1. Introduction

Resin infiltration is a technique that results in the filling (by capillarity) of enamel pores with a cured resin, reducing both permeability and light scattering of enamel (Kielbassa, Müller, & Gernhardt, 2009; Meyer-Lueckel & Paris, 2008; Paris, Hopfenmuller, & Meyer-Lueckel, 2010; Torres & Borges, 2015). The prediction that the penetration coefficient of infiltrants correlates with their penetration depth (as predicted by theory; Washburn, 1921) has been tested and confirmed in carious enamel (Paris, Meyer-Lueckel, Cölfen, & Kielbassa, 2007), but there are still some gaps on the nature of resin infiltration into enamel pores that are related to confronting experimental data with some theoretical predictions. Capillarity theory predicts that the pore volume infiltrated by a liquid in a body with tubular capillaries is directly proportional to the capillary radius (Washburn, 1921), but such a prediction has yet to be tested with resin infiltration in enamel with low mineral content (carious, fluorotic, softened; poorly mineralized in general).

Resin infiltration in enamel is performed after enamel is dried at room temperature in order to create tubular capillaries filled with air (Meyer-Lueckel et al., 2013). The expected volume available for infiltration in enamel is the loosely bound water volume, i.e. the enamel component volume replaced by air during drying at room temperature, which can be related to the radius available for infiltration. Data on both the resin-infiltrated pore volume and on the correlation between infiltrated pore volume and pore radius in poorly mineralized enamel

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http://dx.doi.org/10.1016/j.archoralbio.2017.06.017
Received 22 September 2016; Received in revised form 5 May 2017; Accepted 13 June 2017

are lacking. Thus, the aim of this study was to test the hypothesis that the proportion of the pore volume infiltrated by resin infiltrant is correlated and agrees with the proportion of the pore volume with loosely bound water in human fluorotic enamel.

2. Material and methods

2.1. Sample size calculation

The independent datum was the histological site/point (area of 15 μm × 15 μm), following previous studies on the quantification of component volumes of enamel caries (Angmar, Carlstrom, & Glas, 1963; Barbosa de Sousa, Soares, & Vianna, 2013; Theuns, Shellis, Groeneveld, Van Dijk, & Poole, 1993). Sample size (number of histological sites) calculation requires a predicted effect size. As a reference, we used the recently reported correlation coefficient "r" of 0.55 between the pore volume infiltrated by Thoulet's solution with refractive index (RI) 1.47 and the loosely bound water volume in natural carious enamel (Meira, de Mattos Britto, & de Sousa, 2015) as the predicted effect size. From what is currently available in the literature, the penetration coefficient of Thoulet's 1.47 (2297 cm/s) (Meira et al., 2015) is the closest one to the penetration coefficient of the resin infiltrant (204 cm/s) (Meyer-Lueckel & Paris, 2010). Using a two-tailed type II error of 1%, power of 99%, and an effect size r of 0.5, the calculated sample size (equation 12.3.5 of Cohen, 1988) was 76. Considering possible sample size loss of 25% during the experiment, the total calculated sample size was *95 histological points*. The independent datum was the histological point (not the tooth) because each histological point has its particular loosely bound water volume, which is the effective volume available for infiltration of foreign liquids into carious enamel (Meira et al., 2015).

2.2. Tooth extractions

This study was approved by the Ethics Committee for Human Research (protocol number 2003.1.1329.58.2). The fluorotic third molars were extracted in the city of Venâncio Aires, State of Rio Grande do Sul, Brazil, in a district where severe fluorosis was described, which was due to the use of water containing approximately 8 ppm of fluoride (Marimon, Knöller, & Roisenberg, 2007). Another seven extracted non-fluorotic third molars were collected from volunteers who attended the oral surgery clinic of the Dental School of Ribeirão Preto of the University of São Paulo, and lived in the city of Ribeirão Preto, State of São Paulo, Brazil, for dentine fluoride analysis. The patients were informed both verbally and in writing about the purposes of the research, and signed an informed consent document and a tooth donation term. Teeth were stored at −20 °C until processing.

2.3. Macroscopic analyses

The teeth had the roots removed with a diamond disk in a sectioning machine and the crowns were photographed using a Macro 100EF Lens (Canon, Japan). Teeth were scored for fluorosis degree using the Thylstrup & Fejerskov index (Thylstrup & Fejerskov, 1978) and for root formation stage.

2.4. Determination of fluoride ion

Additional seven non-fluorotic erupted teeth with completed root were also collected to comprise a control group for fluoride. A 100 μm thick longitudinal section of each tooth crown (seven fluorotic and seven control teeth) was used for fluoride determination. Dentine was separated from the enamel, and a piece of dentine near the pulp was removed, weighed and transferred to a plastic test tube, to which 15.55N nitric acid (HNO_3: Sigma Chemical Co. USA) was added in a proportion of 0.1 mL acid/1 mg of dentine for complete dissolution of the dentine. Thereafter the acid was neutralized with 15.55 N NaOH and the mixture was buffered with TISAB II (Total Ionic Strength Adjustment Buffer II). The fluoride concentration was determined by means of a specific electrode (Orion Research Inc., Model 96-09; Boston, United States) coupled to an ion analyzer (Orion Research Inc., Model EA 940, Boston, United States). Standard solutions (Orion #940907) in triplicates, at concentrations from 0.05 to 0.5 μgF/mL, were prepared in the same way as the samples. The results were expressed in μgF/g of dentine. Dentine fluoride concentration was used because its correlation with dental fluorosis is higher than the correlation between enamel fluoride concentration and dental fluorosis (Vieira, Hancock, Limeback, Mara, & Grynpas, 2004).

2.5. Measurement of mineral volume

Another undemineralized ground section, now cut longitudinally to the tooth axis, was cut with a diamond disc mounted in a low speed handpiece under water irrigation and reduced manually to 100 μm using a lapping jig. The slices were placed on a microradiography film (High Resolution HD Plate, HTA Enterprises Microchrome Technology Products, CA, EUA) and submitted to an energy of 40 KeV for 25 min in a PCBA X-ray Inspector equipment (General Electric, Germany) equipped with a Beryllium window. An aluminum stepwedge of 10 steps (22 μm each) was also used to obtain a calibration curve between gray levels and aluminum thickness. Films were developed and photographed under a light microscope using a 5X eyepiece (Axioskop 40, Carl Zeiss, Germany) and a digital camera. Technical procedures required to measure mineral volume were based on a mineral density of 2.99 gcm^{-3} (Elliott, 1997) using the Angmar equation (Angmar et al., 1963), and followed a methodology described recently (Macena et al., 2014). The microradiography was obtained before resin infiltration and the thickness of each sample was measured after resin infiltration in a light microscope as described before (Medeiros, Soares, & Sousa, 2012). Mineral volume was measured at selected histological points (15 × 15 mm) located along a transversal line in the buccal surface of the enamel layer and at the following distances from the enamel surface: 20 μm, 40 μm, 60 μm, 80 μm, 100 μm, 150 μm, 200 μm and then at 50 μm intervals up to close (40 μm away) from the enamel-dentin junction. Those points were the sum of infiltrated and non-infiltrated histological points. Only the resin-infiltrated points were computed to satisfy the required sample size calculated above.

2.6. Non-mineral volumes before resin infiltration

The optical phase retardances measurements under water immersion required to measure non-mineral volumes from the interpretation of enamel birefringence (Sousa, Vianna, & Santos-Magalhães, 2006) were obtained (at the same histological points described before) using the orientation-independent polarizing microscopy technique with a single liquid crystal (single polscope) (Shribak, 2011). The single polscope configuration was the same as that described recently (Meira et al., 2015). The advantage of the single polscope over the traditional phase retardance performed manually with compensators is that measurements at all points of interest in the digital image file are obtained fast and simultaneously, mapped data are stored and can be checked subsequently, and real-time measurements can be performed. Using sample thickness data obtained after resin infiltration, enamel birefringence was computed and total water and organic volumes were calculated as described elsewhere (Sousa et al., 2006). The samples were also analyzed with the single polscope after air-drying at room temperature (25° C and 50% of air humidity) for 48 h, but most of the lesion area was opaque (no phase retardance), so that birefringence of dried enamel needed to quantify loosely and firmly bound water volumes could not be obtained. In order to solve this problem, we used the recently developed approximation (Macena et al., 2014) to calculate the loosely bound water volume (α_2):