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ORIGINAL ARTICLE

Evaluation of dentin permeability of fluorotic permanent teeth

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ABSTRACT

Objective: The *in vitro* permeability characteristics of dentin have been studied extensively and used to evaluate the efficacy of various preventative and restorative procedures. The aim of this *in vitro* study was to precisely determine the dentin permeability of fluorotic premolar teeth using an electronic hydraulic conductance measurement system with photosensors and to compare the data with healthy premolars.

Methods: In total, 40 fluorotic and healthy premolar teeth with complete root formation that were extracted for orthodontic purposes and had no caries, restoration, fractures, or cracks were selected for this study. Teeth were classified according to a modified form of the dental fluorosis index of Thylstrup and Fejerskov. The dentin discs were placed in an electronic hydraulic conductance measurement system equipped with photosensors, which was designed for measurements of dentin permeability. The amount of distilled water passed through each dentin disc (μL/min) under a constant pressure was determined. Dentin permeability data of the fluorotic and healthy teeth were recorded and analyzed statistically.

Results: The present study showed that fluorosis influenced the volume of fluid that passed through the dentin and the dentin permeability was decreased, whereas dental fluorosis severity was increased in permanent teeth.

Conclusion: The number of teeth with fluorosis is increasing, depending on fluorine sources, so more appropriate treatments will need to be evaluated by standardizing the methods employed in related studies.

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Introduction

Dentin permeability can be evaluated by calculating its hydraulic conductance (Lp) by fluid filtration methods. Changes in the hydraulic conductance of dentin have been used to test the efficacy of various restorative and preventative procedures [1,2].

Excessive ingestion of fluoride during the period of pre-eruptive tooth formation may cause dental fluorosis. The prevalence of dental fluorosis has increased in both optimally fluoridated and non-fluoridated areas of the world [3,4].

Isparta City, which is located in a volcanic area, is an area of endemic fluorosis in Turkey. Gölcük Crater Lake and the Andık River are the water sources for Isparta. The fluoride levels in these sources increase with increased temperature, especially in the spring and summer. The annual minimum and maximum fluoride levels of Gölcük Crater Lake and the Andık River are 0.79 and 1.55 mg/L (mean ± SD: 1.12 ± 0.29), and 2.55 and 3.40 mg/L (mean ± SD: 2.96 ± 0.31), respectively [5]. Dental fluorosis of varying severity can be observed in the teeth of children living in Isparta. Fluorotic teeth exhibit problems in bonding with and pulp responses to materials [6]. To the best of our knowledge, no reported study has examined dentin permeability in fluorotic permanent teeth.

The aim of this study was to determine the dentin permeability of fluorotic premolar teeth using an electronic hydraulic conductance measurement system equipped with photosensors, and to compare the data with healthy permanent teeth.

Materials and methods

In total, 40 fluorotic and healthy maxillary and mandibular premolar teeth with complete root formation extracted from different individuals aged between 13 and 15 years for orthodontic purposes were selected and used in this study. Prior to the study, patients and their parents were informed that the extracted teeth would be used in an *in vitro* study, and their informed consent was obtained under a protocol approved by the Süleyman Demirel University Ethics Committee.

The Thylstrup and Fejerskov Index (TFI) is the only index that attempts to correlate the clinical appearance of fluorosis with the pathologic changes in human enamel and it uses a 10-point scale, where 0 represents the non-affected tooth and 9 the most severely affected tooth [7]. Fluorotic and healthy permanent teeth were obtained from patients with normal past medical histories who had been living in

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neighbourhoods with high water fluoride levels (>0.79 mg/L) in Isparta since birth and others who had been living in centres of population with lower water fluoride levels (<0.3 ppm) since birth, respectively. Because of the difficulties in adequately identifying, grading, and evaluating fluorosis in permanent teeth in epidemiological studies, the teeth without caries, restorations, factures, or cracks were used for the experimental parts and their degree of fluorosis was classified according to a modified TFI as shown in Table 1 (Figure 1(a–d)).

After removal of the soft tissues, teeth were stored at 4 °C in 10% formalin solution until use, within 6–8 weeks after extraction. Prior to the preparation of dentin discs, radiographs were taken to determine the height of the pulpal horns, which was indicated on the teeth with a water-resistant pencil. Using a fissure bur and a high-speed bur under water cooling, two guide grooves were prepared in the centre of the mesial and buccal surfaces of the crowns to prevent possible confusion between pulpal and occlusal surfaces and to standardize the measurement area of the dentin discs. Roots were cut, and then mandibular teeth were embedded in colourless and maxillary teeth in red

Table 1. Modified form of Dental Fluorosis Index of Thylstrup and Fejerskov for the study.

0 TFI score → healthy tooth tissue
1–2 TFI scores → mild fluorosis
4–5 TFI scores → moderate fluorosis
7–8 TFI scores → severe fluorosis

translucent orthodontic acrylic resin in standardized moulds (Figure 2). Teeth were embedded in different colours of transparent acrylic resin to provide a standardization in experimental measurements while exposing the sections of buccal, mesial, and occlusal surfaces of upper and lower teeth.

Parallel-plane dentin sections were cut parallel to the occlusal surface of the teeth using a low-speed diamond saw (Isomet, Buehler, Ltd., Lake Bluff, IL) with water cooling. The dentin sections closest to the pulp chamber, but lacking any evidence of a pulpal horn, were chosen. Both sides of the dentin discs were further ground with 600-grit silicon carbide abrasive discs (Buehler, Monheim, Germany) and placed on a polishing device (Ecomet 5, Buehler, Monheim, Germany) under running water for 15 s to create a standardized smear layer. The final thickness (1.00 ± 0.05 mm) of the dentin slices was measured to an accuracy of 0.001 mm using a digital micrometer (40 EX Digital Micrometer, Mahr Federal, Inc., Providence, RI).

To create a constant dentin surface area and to prevent deformation of the dentin discs under pressure, pulpal sides of all discs were attached to standardized Plexiglas rings of 1 mm thickness, with a hole in the centre exposing a 0.125 cm^2 dentin area, using cyanoacrylate adhesive (Zapit, Dental Ventures of America, Inc., Anaheim Hills, CA; Figure 3(a,b)).

Hydraulic conductance of the discs was measured using an electronic hydraulic conductance measurement system with an accuracy of 0.001 s; Figure 4 [8]. The dentin discs

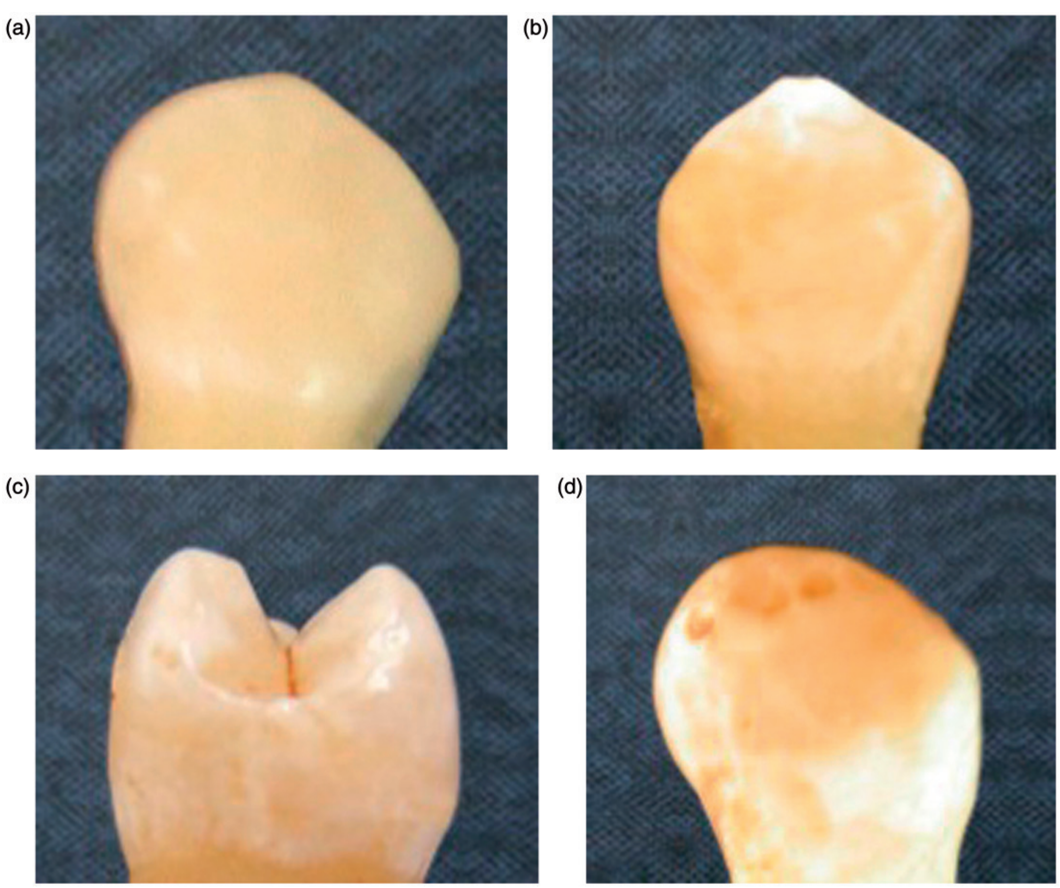

Figure 1. (a–d) Views of teeth with different flurosis level, a: healthy, b: mild, c: moderate, and d: severe.

Figure 2. Samples of the teeth embedded in two colours of translucent orthodontic acrylic resin.

(a) (b)

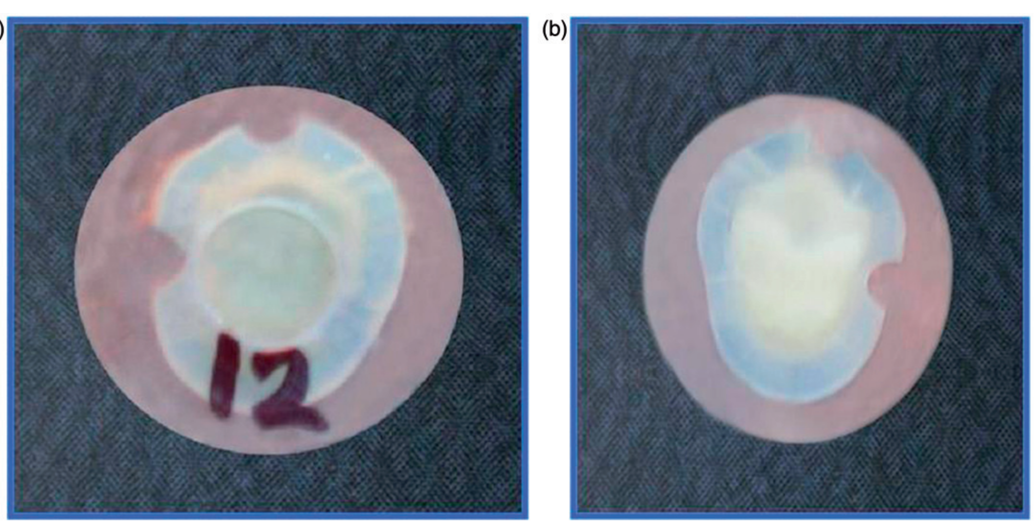

Figure 3. (a, b) Pulpal and occlusal sides of the dentin discs sticked on plexiglass rings.

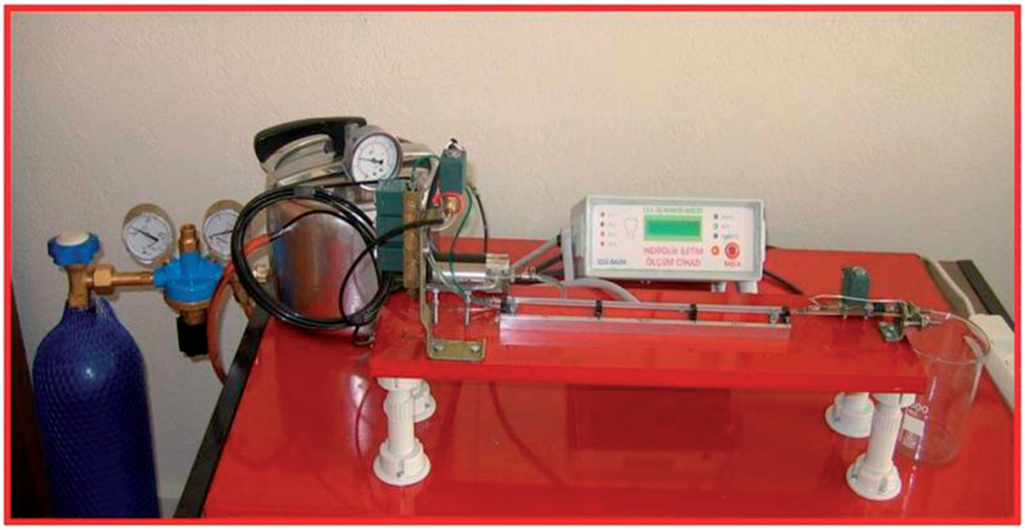

Figure 4. Electronic hydraulic conductance measurement system with photosensors and experiment mechanism.

were placed in the split-chamber device between two rubber O-rings with a 0.4 cm-diameter hole in the center. Distilled water, filtered through a 0.2 μm membrane (Schleicher & Schuell FP30/0,2 CAS, Dassel, Germany), was passed through the system. A pressure tank produced 10 psi air pressure, which was constant throughout the experiment, using two air pressure regulators added to the pressure tank and the reservoir. The solenoid valves produced air bubbles automatically, and the movement of air bubbles was detected by the photosensors. The electronic hydraulic conductance measurement system determined the amount of distilled water passing through each dentin disc (μL/min) under constant pressure.

Hydraulic conductance values were determined by the method of Reeder et al. [9]. Three consecutive measurements were made for each dentin disc, and the mean of the measurements gave the amount of fluid passed per minute. The first Lp (Lp1) value was calculated by the volumetric fluid flow dividing the water pressure (690 cm H_2O) and the surface area of dentin (0.125 cm^2). After then the first measurements phosphoric acid (34.5% concentration; Vococid, Voco, Cuxhaven, Germany) was used for 15 s to remove the smear layer on both sides of the discs. Next, upon rinsing discs with distilled water for 30 s, three consecutive measurements were made for each dentin disc again. The second Lp (Lp2) value was calculated by the same formula.

The hydraulic conductance for each dentin disc was calculated [10]:

$$\mathrm{Lp} = \frac{\mathrm{Jv}}{\mathrm{A.}\ \Delta\mathrm{P}},$$

where Lp is the hydraulic conductance ($\mu L\, cm^{-2}\, min^{-1}$ cm H_2O), Jv is the volumetric fluid flow ($\mu L\, min^{-1}$), A is the dentin surface area (cm^2), and ΔP is the hydrostatic pressure (cm H_2O).

For calibration of the electronic hydraulic conductance measurement system with photosensors, a 0.45-μm cellulose acetate filter (Sartorius) with known permeability was used. Prior to the experiment, a Plexiglas disc without a hole was placed in the split-chamber device, and the system was tested for leakage. Between measurements, the dentin discs were stored at 4 °C in distilled water filtered through a 0.2-μm membrane.

All measurements were performed in the laboratory under constant temperature and standardized illumination conditions. After three measurements were made for each disc, the discs were removed from the system, and then replaced again for the last measurement to verify the consistency of the measurements and to observe that the positioning of the discs in the split-chamber device was correct.

SPSS software (ver. 10.0, SPSS, Chicago, IL) was used to analyze the hydraulic conductance (Lp) of the fluorotic and healthy teeth. Data were analyzed using Kruskal–Wallis and Mann–Whitney U tests. Statistical significance was set at $p < .05$.

Results

Dentin permeability was decreased when dental fluorosis severity increased in permanent teeth (Table 2). Comparisons of the groups showed that fluorosis influenced the volume of fluid that passed through the dentin. First hydraulic conductance (Lp1) results (mean ± SD) of the control, mild fluorosis, moderate fluorosis, and severe fluorosis groups were 0.007 ± 0.001, 0.006 ± 0.001, 0.004 ± 0.000, and 0.003 ± 0.000 $\mu L\, cm^{-2}\, min^{-1}$. cm H_2O, respectively. After etching hydraulic conductance (Lp2) results (mean ± SD) of the control, mild fluorosis, moderate fluorosis, and severe fluorosis groups were 0.050 ± 0.012, 0.047 ± 0.013, 0.028 ± 0.006, and 0.020 ± 0.005 $\mu L\, cm^{-2}\, min^{-1}$ cm H_2O, respectively.

Because the Kruskal–Wallis test showed significant differences ($p < .001$), the Mann–Whitney U test was performed for binary comparisons for the Lp1 and Lp2 values of the study groups. Although there was no statistically significant difference between the healthy and mild fluorosis groups ($p = .940$); significant differences were observed between healthy and moderate fluorosis ($p = .002$), healthy and severe fluorosis ($p < .001$), mild and moderate fluorosis ($p = .001$),

Table 2. Hydraulic conductance data in relation to different dental fluorosis severity for Lp1 and Lp2 values and increasing rate after etch (μl cm^{-2} min^{-1}cm H_2O).

Degree of flourosis	Lp1 (mean ± SD)	Lp2 (mean ± SD)	Increase rate after etching
Flurosis (−)	0.007 ± 0.001	0.050 ± 0.012	7.12 ± 0.97
Mild fluorosis	0.006 ± 0.001	0.047 ± 0.013	6.91 ± 1.18
Moderate fluorosis	0.004 ± 0.000	0.028 ± 0.006	6.55 ± 0.84
Severe fluorosis	0.003 ± 0.000	0.020 ± 0.005	6.61 ± 0.88

mild and severe fluorosis ($p < .001$), and moderate and severe fluorosis groups ($p = .007$) for Lp1 values. There was no statistically significant difference between the healthy and mild fluorosis groups ($p = .597$), while significant differences were observed between healthy and moderate ($p = .001$), healthy and severe fluorosis ($p < .001$), mild and moderate fluorosis ($p < .0002$), mild and severe fluorosis ($p < .001$), and moderate and severe fluorosis groups ($p = .010$) for Lp2 values.

Discussion

A modified Thylstrup and Fejerskov index (TFI) was used since there were too few permanent teeth for comparison with each fluorosis group. Categorizing the teeth with equal ranges of TFI scores and assigning approximately equal numbers of teeth to each group, results showed that the mild, moderate, and severe fluorosis groups had TFI scores of 1 and 2, 4 and 5, and 7 and 8, respectively. TFI was determined based on variability in the enamel tissue, similar to the entire fluorosis index. Because no fluorosis index based on clinical signs has been defined for dentin tissue, the degree of fluorosis was classified according to the modified TFI.

Evaluation of hydraulic conductance can help to elucidate the permeability characteristics of dentin and factors that affect them, making it possible to determine the mechanism(s) responsible for fluorosis-related complications, such as pain, sensitivity, restorative failure, and pulp damage. The permeability of dentin also plays an important role in microleakage problems. Changes in the hydraulic conductance of dentin can be used to determine the efficacy of restorative agents, binding agents, base agents, bleaching agents, and agents used to remove the smear layer. The permeability of dentin is influenced by the presence of secondary, sclerotic, and tertiary dentin, as well as by the presence of necrotic tissue or a smear layer. It is related to the numbers and diameters of tubules and inversely proportionally related with distance to the pulp [11]. Thus, age appear as another important factor in these studies and in present study where the pool of teeth were provided from a narrow age range for the measurements of hydraulic conductance.

The electronic photosensor hydraulic conductance measuring device used to determine the permeability of dentin discs is a light-sensitive system, including its photosensors. Thus, measurements were performed under constant-light conditions in present study.

The hydraulic conductance of dentin is directly influenced by variability in the thickness of the dentin section, the pressure that is applied, the open surface of the dentin, heat, the presence of a smear layer, and the dentin region from which the section of dentin was taken. In the presence of a smear layer [9,12], the hydraulic conductance of dentin decreases, whereas with decreased thickness of the dentin section [9,10] or an increase in the open surface [9] the hydraulic conductance of dentin increases. An increase in applied pressure causes an increase in hydraulic conductance [13]. While the distance to the pulp is increased, depending on the number and the diameter of tubules, hydraulic conductance decreases [10,14]. Additionally, developmental stage of teeth, storage medium used, and their storage time also might have affected the measurements of hydraulic conductance [14,15]. In present study, all these parameters were considered prior to the handling of the teeth samples. Therefore, previous findings and fundamental knowledge were used to construct methodology from the beginning of the storage to the evaluation of hydraulic conductance.

In our study, teeth were used after storing for 6–8 weeks following extraction. According to a previous study the permeability of dentin was found to increase substantially between 1 h and 1 d following tooth extraction [14]. This increase in the *in vitro* permeability of dentin was caused by the separation of weakly bound organic components of tubules that had already begun to decrease following tooth extraction. There was no significant change during the following 3–4 weeks, indicating that stable pH was reached. In studies on the *in vitro* permeability of dentin, problems in methodology may occur if measurements are performed prior to the mentioned stable phase to be reached. Therefore, it is recommended that teeth should be stored for some time, depending on the thickness of the sample, in order to pass the stage of rapid increase in permeability [14].

The dentin disc is a useful model for assessing the permeability and the sensitivity of dentin [16]. To standardize the region of dentin that is measured and to prevent possible complications in the process of taking sections, in the current study, teeth were prepared as described previously [8]. Using this method, the surface of dentin to be measured was standardized, and the deformation of dentin tissue that might occur under pressure was minimized in present study.

Our literature review revealed that different agents have been used for different durations and at differing concentrations to remove the smear layer from dentin tissue [1,10,13–15,17–19]. In our study, smear layer was removed according to the procedure used clinically. Permeability of dentin before removing the smear layer was found to be similar to the permeability shown in previous studies [9,14].

In healthy permanent teeth, the mean hydraulic conductance was found to be much smaller in the presence of the smear layer while it was increased after etching.

In present study, initial increase (after etching) in hydraulic conductance of teeth with fluorosis decreased with increasing severity of fluorosis, but this decrease was not statistically significant. In permanent teeth, Zavala-Alonso et al. [20] reported the nanostructure of human dentin surfaces affected by dental fluorosis (DF) before and after phosphoric acid etching by atomic force microscopy (AFM) and they conclude that effective etching times were 15 s for healthy and mild dentin fluorosis, 30-s for moderately fluorotic dentin, and 45–60 s for severe fluorotic dentin.

Several studies found that whereas enamel fluorine content was not correlated with the content of fluorine in dentin, the TFI score, or the diameter or density of tubules in the dentin, the fluorine content of dentin was significantly correlated with TFI score, the diameter of the tubules in dentin, and the density of those tubules [21,22]. Furthermore, with increasing fluorosis severity, the diameter of tubules is increased, but the density of tubules is decreased; thus, because fluorine affects the mineralization of dentin negatively, an increase in the severity of fluorosis decreased the 'quality' of the teeth. These results differ from those of our study. Along with the increase in severity of fluorosis, the reported increase in the diameter of tubules would be expected to cause an increase in the permeability of the dentin, as the diameter of tubules increases directly proportionally to an increase in hydraulic conductance. However, in our study, we found that the permeability of dentin of permanent teeth decreased with the increase in fluorosis. This finding, i.e. the decreasing permeability of dentin in permanent teeth with increasing severity of fluorosis, may occur by a mechanism: while the reduction in permeability is affecting the progression of caries, presence of fluorine delays and eventually ends it.

The reduced permeability of dentin in teeth with fluorosis can be suggested as a positive feature in protecting pulp. The appropriate fluorine dose while reducing the permeability of dentin without causing aesthetic problems must be determined in future studies.

Currently, the number of teeth with fluorosis is increasing depending on fluorine sources; so, standardized treatments are needed as much as preventive methods. We believe that our findings will be useful to improve restorative treatment approaches of fluorotic teeth.

In conclusion, the hydraulic conductance of dentin can be used not only to determine the performance of restorative agents but also to test conditioning agents in order to remove the smear layer. In clinically the conventional etching time might be insufficient for restorative procedures in which dental fluorosis is involved. Instead, longer etching time is needed to remove the smear layer from fluorotic dentin.

Disclosure statement

Authors declared that any potential financial and non-financial conflicts of interest.

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