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Relative fluoride response of caries lesions created in fluorotic and sound teeth studied under remineralizing conditions

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ABSTRACT

Objectives: The present in vitro pH cycling study investigated potential differences between caries lesions created in fluorosed and sound enamel with regards to their responsiveness to fluoride under remineralizing conditions.

Methods: 360 human first molars (sound and fluorosed) were divided into four groups based on their Thylstrup–Fejerskov score (TF0-3). Each group was further divided into two treatment groups ($n = 45$): deionized water or 383 ppm fluoride. Artificial enamel caries lesions were created and pH cycled for 20 d using an established net remineralization model. Quantitative light-induced fluorescence was used throughout the study to investigate lesion severity and changes thereof. Data were analyzed using two-way ANOVA.

Results: There were no differences in lesion severity between all groups after lesion creation ($p_{lesion} = 0.1934$). The TF score vs. treatment interaction was significant at all other time points ($p_{10\ d} = 0.0280$; $p_{20\ d} \leq 0.0001$; $p_{secdemin} = 0.0411$). Relative differences in responsiveness to fluoride vs. deionized water increased with increasing TF scores. In comparison to lesions created in sound enamel, lesions created in enamel with moderate fluorosis (TF 2/3) were more prone to remineralization in the presence than in the absence of fluoride. Furthermore, lesions created in enamel with moderate fluorosis exhibited more remineralization in the presence of fluoride than lesions created in sound teeth, whereas the opposite was true for deionized water.

Conclusion: Bearing in mind the limitations of laboratory research, the extent of enamel fluorosis severity may directly impact subsequent lesion re- and progression as well as the lesion's responsiveness to fluoride.

Clinical relevance: Caries lesions in fluorotic teeth are more vulnerable to progression but respond more strongly to fluoride than those in non-impacted teeth.

1. Introduction

Enamel fluorosis is a hypomineralization of dental enamel caused by prolonged ingestion of excessive amounts of fluoride during tooth development.[1] The severity of enamel fluorosis depends on the amount of fluoride exposure, the age of the child, their individual response, as well as other factors including nutritional status. The association between enamel fluorosis and fluoride exposure has been reported since the early 1900s.[2]

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There is evidence that the prevalence of enamel fluorosis in many countries has increased over the last three decades along with a noted decrease of dental caries.[3] As a consequence of the reported enamel fluorosis prevalence increase, the U.S. Department of Health and Human Services (HHS) and the U.S. Environmental Protection Agency (EPA) recently changed their recommendations on the optimal level of fluoride in water to maximize the protective effects of fluoride while limiting the occurrence of enamel fluorosis. The level has now been set at 0.7 mg of fluoride per litre of water; instead of the previously recommended range of 0.7–1.2 mg/l. The HHS based this changed recommendation on an observed increase in the prevalence of enamel fluorosis as a result of increased fluoride intake from multiple sources.

Fluorosed enamel is characterized by outer hypermineralization and subsurface hypomineralization. The higher concentration of fluoride is believed to affect cell/matrix interactions as the teeth are forming.[4] Hypomineralization of enamel is caused by the retention of amelogenins in the early maturation stage of tooth development.[5] As a result, the affected enamel does not mature and has surface and subsurface porosities. The tooth becomes more porous than sound teeth, with porosity increasing relative to the severity of fluorosis; the degree and extent of porosity increases in a dose-related manner relative to the tissue/fluid concentrations of fluoride.[6]

Very few studies have evaluated the effectiveness of fluoride on lesion progression in fluorosed teeth and compared remineralization patterns between fluorosed and non-fluorosed, sound teeth. Their findings were contradictory; Suma et al.[7] showed that dental caries increased and enamel thickness decreased with increased severity of enamel fluorosis in fluoride endemic areas. Waidyasekera et al.[8] found that moderately fluorosed enamel showed a significant caries resistance. In contrast, mild and moderately fluorosed dentine was significantly more susceptible to caries in vitro. Driscoll and colleagues[9] found a higher proportion of teeth with severe fluorosis were decayed or filled, and attributed it to pitting of the teeth, staining or both. On the other hand, a study conducted in the US on schoolchildren showed that molars with moderate-to-severe enamel fluorosis had lower caries prevalence than those without enamel fluorosis.[10] The chemical, morphological and histologic characteristics of fluorosed teeth may explain the reported variations in caries experience and also partially explain differences between fluorosed and sound enamel with regards to lesion progression and patterns of demineralization and remineralization. However, our understanding of the consequences of enamel fluorosis on lesion formation and remineralization is still poor and deserves further attention by the research community. Furthermore, the extent of enamel fluorosis severity in particular and related consequences on de- and remineralization in the absence or presence of fluoride has not been addressed adequately in the past. Consequently, the aims of the present in vitro study were: (1) to investigate the relative fluoride response of caries lesions created in sound and fluorotic teeth of varying severities of fluorosis under remineralizing conditions in vitro and (2) to investigate the impact of the presence and severity of enamel fluorosis on caries lesion formation. The null hypothesis for the present study was: there is no significant difference in the effectiveness of fluoride to enhance caries lesion remineralization, defined as change in enamel fluorescence relative to lesion baseline, between fluorosed teeth and non-fluorosed, sound teeth.

2. Material and methods

2.1. Specimen collection

A total of 360 extracted permanent human first molar teeth (90 non-impacted, sound and 270 non-impacted, fluorotic teeth) were divided into four groups ($n = 90$ in each group, one specimen per tooth) depending on their TF score (0, 1, 2, 3), teeth with visual signs of caries, cracks, extrinsic and/or intrinsic staining were unsuitable for this study and discarded. The extracted teeth were stored in 0.1% thymol solution (Sigma–Aldrich, MO, USA) until used.

2.2. Diagnosis of enamel fluorosis severity

Enamel fluorosis severity in extracted teeth was visually assessed using Thylstrup–Fejerskov Index (TFI)[11,12] by two examiners. Overall kappa statistic was 0.92 and the weighted kappa was 0.95 that indicates good agreement between the examiners.

2.3. Specimen preparation

Before sectioning, all teeth were gently cleaned using a polishing wheel but without abrasive to remove only any debris and other surface contaminants that could potentially interfere with the study aims. Then, the teeth were cut in half coronally–apically and mesially–distally leaving buccal and lingual tooth halves from which the roots were removed. As the susceptibility to fluorosis varies between tooth surfaces[13] and to eliminate surface-specific differences in natural fluoride concentration[5] which would have been confounding factors, only the buccal half was used in the present study; the lingual half and all other tooth sections were discarded. Subsequently, a sound enamel window, approx. 200 μm deep and measuring approx. 3 × 2 mm, measured by using a digital calliper) was created in the cervical part of each buccal half (using a Sof-Lex disc 3 M, MN, USA) and slow speed (NSK Nakanishi Inc., Kanuma, Japan). This procedure removed the outer layer that was affected by fluorosis to expose a standardized sound enamel surface. This window served as the sound enamel reference area for QLF measurements. The crown halves were then mounted individually onto 1 in. square acrylic blocks using non-fluorescent dental impression wax (Alminax, Kemdent, UK) to facilitate repeat QLF measurements. The entire tooth surface, apart from an experimental window, measuring approx. 3 × 3 mm in the centre of the specimen, and the sound enamel window were covered with red-coloured nail varnish (Sally Hansen Advanced, Hard as Nails Nail Polish, NY, USA). The previously created sound enamel window was covered with colourless, clear nail varnish (Sally Hansen Advanced, Hard as Nails Nail Polish, NY, USA). The specimens were balanced into four main groups of 90 specimens each (TF scores 0, 1, 2, 3) and each main group was then divided into two subgroups of 45 specimens