Comparison of surface roughness of root cementum and orthodontically induced root resorption craters from high- and low-fluoridation areas: a 3D confocal microscopy study

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Abstract

**Background:** Fluoride has a major role in strengthening the structure of enamel against acids. Despite differences between caries and root resorption processes, both events inherently involve acidic dissolution of dental tissues. The aim of the present study was to investigate the effects of water fluoridation levels on the surface roughness of root cementum and resorption craters. The findings provided more insight into the influence of fluoride on the surfaces of intact cementum surface and resorption craters.

**Methods:** Twenty-eight orthodontic patients were recruited from two cities in Turkey, with high (≥ 2 ppm) and low (≤ 0.05 ppm) water fluoridation. These patients needed bilateral maxillary first premolar extraction as part of their orthodontic treatment and were allocated into two study groups (n = 14 in each group) based on water fluoridation exposure level: the high-fluoride group (HF) and low-fluoride group (LF). 150 g of buccal tipping forces was applied to all maxillary first premolar teeth for 12 weeks with a beta-titanium spring which was reactivated every 4 weeks. All maxillary premolars were removed at the end of the experiment for surface roughness assessment using three-dimensional confocal microscopy and the associated software. The buccal root surface and the largest buccal resorption crater were investigated.

**Results:** Resorption craters were significantly rougher in LF group compared to HF group (p = 0.002). Craters were rougher than the intact root surfaces (p = 0.000). Cervical and apical regions were significantly rougher than the middle region (p = 0.000 and p = 0.024, respectively).

**Conclusions:** Higher water fluoridation level of ≥ 2 ppm resulted in significantly smoother root resorption craters than low water fluoridation level of ≤ 0.05 ppm when the teeth were subjected to 150 g of buccal tipping force. Fluoride seems to have a protective role at the interface of root resorption, and further mineral or histological studies may shed light on the exact protective process against root resorption.

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Background

Some degree of root resorption can be considered physiological as resorption craters have been reported in roots of unerupted third molar teeth [1]. Most (90.5%) non-orthodontic patients have some extent of root resorption [2]. The application of orthodontic forces can cause transient inflammatory surface resorption, otherwise known as orthodontic-induced inflammatory root resorption (OIIRR) [3]. Studies have reported an increase in root resorption in orthodontic patients from 15 to 73%, with moderate and severe root resorption increasing from 2 to 24.5% [4].

A decrease in susceptibility to OIRR where cementum is more mineralised has been suggested [5, 6]. Fluoride has a major role in the prevention of dental caries by inducing the conversion of hydroxyapatite into fluorapatite and strengthening the structure of enamel against acids [7]. Despite differences between caries and root resorption processes, both events inherently involve acidic dissolution of dental tissues. In dental caries, the acid is produced externally by biofilm, whilst osteoclasts degrade bone and cementum by sealing a particular area with the ruffled border, delivering acid secretions as well as proteinases [8]. The acidic environment demineralises the inorganic components whilst providing an acidic environment for the optimal activity of the proteinases to degrade the organic material. The potential role of incorporating fluoride into cementum and its possibility of reducing root resorption are of interest. A few studies have investigated the effect of fluoride on OIRR in rats [9–11] and humans [12, 13]. These studies demonstrated an inverse relationship between systemic fluoride exposure and root resorption crater size. One rat study also reported a decrease in the surface roughness of resorption craters and a reduction in the amount of root movement [14]. There are only two human studies that compared OIRR in individuals from high and low water fluoridation areas after 28 days of light or heavy (25 g or 225 g) buccal orthodontic force [15, 16]. These studies reported significantly reduced volume of OIRR with higher fluoride concentration (≥ 2 ppm) but only in the high force (225 g) group [15]. However, this effect on OIRR was not found after 12 weeks of passive retention with multi-stranded stainless steel wire [16]. Studies relating fluoride to OIRR is scarce, especially the lack of human studies reported in the literature.

The aim of the present study was to investigate the effects of water fluoridation levels on the surface roughness of root cementum and resorption craters in a clinically relevant human orthodontic model. The findings provided more information regarding the influence of fluoride on the interface at which root resorption occurs, the cementum surface and the resorption craters themselves. The null hypothesis was that there was no difference in surface roughness on root cementum and root resorption craters between subjects exposed to higher water fluoridation of ≥ 2 ppm and lower water fluoridation of ≤ 0.05 ppm.

Materials and methods

The sample and selection criteria have been described in our earlier study [17]. Briefly, the sample comprised of 5 males and 23 females (average age 15 years, range 11–21 years) who required orthodontic treatment involving bilateral maxillary first premolar extraction. The subjects were recruited from two Turkish cities, including Isparta (high-fluoridation level of ≥ 2 ppm) and Samsun (low-fluoridation level of ≤ 0.05 ppm). The selection criteria were no significant medical history or craniofacial anomaly, no previous dental or orthodontic treatment on the maxillary first premolars (with complete apexification), no past or present signs or symptoms of periodontal disease or bruxism and residence in the cities from birth (without migration). Ethics approval (2008/166) was granted by Ondokuz Mayis University in Turkey for this study, and specimens were analysed at the University of Sydney, Australia.

The subjects were divided into high-fluoride group (HF) and low-fluoride group (LF) (Fig. 1). The buccal surfaces of maxillary first premolar and first molars were bonded with Speed brackets 0.022" slot (Strite Industries, Cambridge, Ontario Canada). The ligation method was standardised by the nature of self-ligating brackets. To minimise occlusal forces to the maxillary first premolars, a transpalatal arch with posterior acrylic bite ramps was placed (Transbond Plus Light Cure Band Adhesive, 3 M Unitek, Monrovia, California, USA) (Fig. 2). A buccal tipping force of 150 g was applied for 12 weeks by using 0.017" × 0.025" beta-titanium-molybdenum alloy (TMA) cantilever springs (Beta III Titanium, 3 M Unitek, Monrovia, California, USA) from the maxillary first molars to the maxillary first premolars (Fig. 2). The springs were then reactivated every 4 weeks, followed by removal of the appliance and extraction of maxillary first premolars (using the extraction forceps on tooth crowns only) at 12 weeks. The teeth were stored in deionised water (Milli-Q) at ambient room temperature. Prior to analysis, the root surfaces were cleaned with a sterile gauze soaked...
in deionised water (Milli-Q) and periodontal fibres were removed with tweezers.

Surface roughness was determined using a high definition three-dimensional confocal microscopy (Leica DCM8, Leica Microsystems, Germany) (Fig. 3). All roots were scanned at the largest buccal cervical root resorption crater and on the intact root surface cementum at the buccal cervical, buccal middle and buccal apical regions (Fig. 4). The largest buccal cervical root resorption crater was utilised as this was the most consistently present and identifiable crater. An exception was made of one sample in the LF group as there was no identifiable crater on the buccal cervical region and substitution was made with the largest palatal cervical crater. An optical lens of 10× magnification was used with no digital magnification to collect data from an area of 1.75 mm by 1.32 mm. The optical resolution was 0.46 microns. Specific software was used to extract surface roughness (Sa) values from the raw data (Mountains Map 7, Digital Surf, France). Curvature and spikes in the raw data determined to be more than 85 degrees to the flattened surface were removed prior to roughness calculations. Surface roughness values for craters were determined by two areas of 35 µm × 35 µm. The same two arbitrarily selected areas of 35 µm × 35 µm were used to calculate average surface roughness values. Exceptions were made in cases where resorption craters, cracks or obvious debris were present.
on the surface, whereby the nearest unaffected area was selected.

**Statistical analysis**

Statistical Package for Social Sciences software (IBM SPSS Statistics for Windows, version 25.0, IBM, Armonk, NY) was used for statistical analysis. As the data and the residuals of the models were not normally distributed, log transformation was performed to obtain normality. One-way ANOVA was used to investigate the effect of fluoride, location and their interaction on surface roughness. Significance was set at the $p<0.05$ level. Effect sizes using Cohen's $f$ were also calculated using the formula: $f = \sqrt{(\eta^2 / (1 - \eta^2))}$. An effect size ($f$) of 0.10 is mild, 0.25 is moderate, and 0.40 is high.

**Results**

Statistically significant differences were found between the locations and the interaction between fluoride and location. The root resorption craters were significantly rougher than the intact root surfaces ($p=0.000$) (Fig. 5). The cervical and apical regions of the intact root surfaces were significantly rougher than the middle region ($p=0.000$ and $p=0.024$, respectively) (Fig. 5). The effect size was high (Table 2).

At the resorption crater, the LF group was significantly rougher than the HF group ($p=0.002$) (Table 1, Fig. 6). The effect size was high (Table 2).

**Discussion**

The protective effect of fluoride on dental caries has been well established. Water fluoridation has been implemented in most major cities within the optimal range of 1 ppm for the beneficial effects of caries prevention without the consequence of significant fluorosis [17]. The electronegativity of fluoride readily converts hydroxyapatite to fluorapatite when fluoride is present in mineralised tissues. The deposition of fluoride has been shown to be greater in teeth than bone, and especially high in cementum [18].

In addition, the presence of fluoride favours bone formation by having an anabolic effect on osteoblasts [19] as well as significantly reducing the number of osteoclasts at the pressure regions of the periodontal ligament [20].

Previous studies have shown that fluoride could reduce the size of root resorption craters [14, 15, 21]. The volumetric root resorption of the same sample of the current study has also been assessed [22]. Significantly less root resorption was found on the palatal, palatal apical and pressure zones of the root. The presence of more systemic fluoride in the HF group likely resulted in the formation of more fluorapatite which is more structurally stable than hydroxyapatite. As fluorapatite was more stable and acid resistant, this may be responsible for less resorption and smoother surfaces at resorption craters in the HF group. Similar results were found in a previous rat study [14] that displayed a decrease in the surface
roughness of root resorption craters in the higher fluoridated group.

An assessment of the resorption process as described histologically by Brudvik and Rygh [23–25] provides an explanation for the findings in the current study. Creation of root resorption craters is initially at the periphery of necrotic periodontal ligament by mononucleated macrophage-like and fibroblast-like cells. After a few days, tartrate-resistant acid phosphatase (TRAP)-positive giant cells without a ruffled border arrive and continue to remove the central part of the necrotic tissue. These cells remove pre cementum underneath and nearby the necrotic area to expose mineralised cementum. Whilst the removal of necrotic periodontal ligament appears to be via phagocytosis, the resorption of cementum appears to be extracellular only as phagocyted cementum is not found inside these cells. Areas subjected to more forces such as the cervical and apical regions may have had some degree of surface resorption. The inspection of these intact areas had shown increased surface roughness. Thus, it is logical that trends in the data showed increased surface roughness in cervical and apical regions which had increased force compared to middle region, and increased risk of some microscopic

| Location   | Surface roughness (Sa) | Mean difference (95% CI) | P value* |
|------------|------------------------|--------------------------|----------|
|            | High fluoride          | Low fluoride             |          |
|            | Mean  | SD    | Mean  | SD    | Mean  | SD    |          |
| Crater     | 13.90 | 4.55  | 24.74 | 9.32  | 10.84 | (5.14, 16.54) | 0.002    |
| Cervical   | 10.58 | 5.37  | 11.01 | 6.37  | 0.42  | (−4.15, 5.00) | 0.268    |
| Middle     | 7.838 | 9.72  | 4.17  | 1.35  | −3.67 | (−9.06, 1.71) | 0.967    |
| Apical     | 8.69  | 3.57  | 6.32  | 2.32  | −2.37 | (−4.70, −0.03) | 0.108    |
| Total      | 10.25 | 6.52  | 11.56 | 9.85  | 1.31  | (−0.955, 3.568) | 0.255    |

*p values from one-way ANOVA with log transformation of Sa values (logSa)
surface resorptions despite the assessed cementum sur-
faces being ‘intact’.

Part of the difference in roughness may also be related
to the distribution of periodontal fibre insertion points.
The cervical and apical regions of cementum typically
have more embedded periodontal ligament supporting
(Sharpey’s) fibres in order to support loading of the tooth
in the longitudinal direction [26]. Cervical regions also
have further gingival fibre insertions. Thus, the trend for
increased roughness in these areas may be attributed to
this.

When complete removal of the thickness of miner-
alised cementum has occurred, multi-nucleated odon-
toclast cells with a ruffled border are found adjacent to
underlying dentine [25]. As we assessed the largest buc-
cal crater in the buccal cervical region of each root, it
is most likely that the resorption craters have entered
dentine. Once resorption is into dentine, the process of
resorption is like bone. The ruffled border of odontoclast
cells seals off a particular area and produces mass acidic
secretions and proteinases to resorb mineralised tissue
and the organic matrix [8]. This process of resorption can
be likened to dental erosion whereby differential rates of
resorption of organic and inorganic components result in
increased surface roughness [27]. Thus, it is logical that
resorption craters were rougher than intact root surfaces.

This study contributed to the gap in the current litera-
ture regarding fluoride and orthodontic root resorption.
The surface roughness which results from the resorption
crater indicated that fluoride may have a protective role
at the structural level. However, the exact mechanism is
still unknown. It may be due to the incorporation of fluo-
ride leading to stronger fluorapatite, or possibly enhanced
recruitment of cementoblasts leading to thicker cemen-
tum and better repair of root resorption craters. Further
investigation on the mineral contents and histological
analysis may shed light on the process by which fluoride
can protect teeth against OIIRR.

There may be a potential role for fluoride supple-
ments during orthodontic treatment to increase systemic
fluoride in low water fluoridation areas of <0.05 ppm.
However, the risk and benefit and the correct dosage
of fluoride supplements require further investigation.
Knowing systemic fluoride exposure influences root
resorption and helps clinicians to identify a potential
adjunctive preventive method for OIIRR.

Fig. 6 Box plot of surface roughness for high- and low-fluoride groups

| Table 2 Effect size for fluoride, location and their interaction |
|---------------------------------|-----------------|-----------------|
|                                 | Cohen’s $f$     | Interpretation  |
| Location                        | 0.89            | High            |
| Fluoride                        | 0.11            | Mild            |
| Location and fluoride           | 0.49            | High            |
Conclusion

Exposure to higher water fluoridation seems to reduce the surface roughness orthodontic root resorption catalysts. Fluoride has a protective role at the interface of root resorption, but the exact mechanisms of action are still unknown. Further investigation on the mineral contents and histological analysis may shed light on the process by which fluoride can protect teeth against OIIRR. Furthermore, water fluoridation within the therapeutic level for dental caries with less risk of fluorosis needs to be tested.

Abbreviations

OIIRR: Orthodontic-induced inflammatory root resorption; HF: High-fluoride group; LF: Low-fluoride group; TMA: Beta-titanium-molybdenum alloy; Sa: Surface roughness; TRAP: Tartrate-resistant acid phosphatase.

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Author contributions

MAD designed the study. SET and TT participated in methodology and investigation. CM acquired all data for the confocal microscopy and wrote the manuscript. CM, MAD, LLC and SR interpreted the data. LLC, SET, TT and SR revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All participants provided informed consent. Ethics approval (2008/166) was granted by Ondokuz Medip University in Turkey where clinical specimens were collected.

Consent for publication

We have obtained institutional informed consent for all our patients so that their data can be published.

Competing interests

The authors declare that they have no competing interests.

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