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An assessment of mineral concentration of dental enamel neighbouring hypothetical orthodontic brackets using X-ray microtomography

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ABSTRACT

Objectives: Differences in the mineral concentration (MC) level of dental enamel may represent a precursor of white spot lesions adjacent to fixed orthodontic brackets. The aim of the current *in vitro* study was to compare the MC level central, occlusal and cervical to orthodontic attachments.

Methods and Materials: A total of 16 enamel blocks were obtained from sound human premolar samples extracted for orthodontic reasons. The buccal portion of the dental enamel blocks was divided into central, occlusal, and cervical regions and then imaged and measured to calculate the level of MC using quantitative X-ray microtomography methods (XMT) at each site.

Results: There was a substantial variation in the mineral concentration with the lowest level being detected in the cervical region when compared with other regions. The MC of the gingival zone was significantly lower than that of the middle zone ($P<0.05$) and was insignificantly lower than that of the occlusal zone.

Conclusion: Within the limitations of the current study, it can be concluded that the cervical region of the permanent enamel had the lowest mineral concentration using XMT. The cervical region may therefore be more vulnerable to the development of white spot lesions (WSLs) adjacent to a fixed orthodontic appliance during orthodontic treatment.

Clinical significance: Using X-ray microtomography lower mineral concentration in the cervical region of the enamel was observed. This may make these areas particularly susceptible to demineralisation during fixed appliance-based orthodontic treatment and may influence the bond strength of fixed orthodontic attachments.

1. Introduction

Mineralisation can be considered an important property that influencing the mechanical behaviour of mineralised tissues such as teeth [1, 2] and bone [3,4]. Tooth enamel is composed of 95% minerals, 4–5% water and 1% organic compounds by weight [5]. The variation in mineral concentration (MC) levels may predispose to the formation of white spot lesions (WSLs). These lesions may have negative aesthetic connotations while also risking the development of frank cavitation. Previous research has linked the MC level of dental enamel and caries susceptibility [6,7].

The prevalence of visible WSLs on teeth treated with orthodontic appliances ranges from 25% to 28% [8,9], although significant inter-individual variation exists. As a population average, the lowest MC scores were observed in the cervical region of dental enamel which may be associated with the hygiene index [10]. Many studies suggest that MC is inversely correlated with the development of WSLs on the enamel surface [6,7]. In orthodontic practise, WSLs are usually detected on the boundary of the orthodontic bracket, in areas prone to plaque stagnation [11]. Accordingly, remineralisation can be promoted by delivering additional phosphate and calcium ions on the dental enamel surface adjacent to the orthodontic brackets [12].

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X-ray microtomography (XMT) has gained traction within orthopaedic and dental research due to its ability to detect mineral concentration levels at the micron scale allied to its non-invasive and non-destructive nature [12–15]. XMT can provide high-quality analytical data and three-dimensional internal visualisation making it invaluable for longitudinal research [16].

The current *in-vitro* study aimed to evaluate the variations in the mineral concentration profile of sound human enamel tissue neighbouring a hypothetical orthodontic bracket in extracted permanent teeth using a novel non-destructive technique of XMT. We aimed to test the null hypothesis of no difference in the level of MC between three different areas of enamel adjacent to an orthodontic bracket.

2. Materials & methods

2.1. Specimen selection and preparation

The sample size calculation was based on a previous study [17]. Thirty human premolars extracted for orthodontic purposes were randomly selected from an archived cohort with ethical approval from the QMREC Research Ethics Committee (#2011/99). The teeth were selected based on visual estimation utilising an optical stereomicroscope (VWR International, USA) at 40× magnification. Sixteen out of 30 teeth were included in the final grade according to the following inclusion criteria: no obvious carious lesions, no cracks, or any other surface defects. Teeth were stored in 70% volume ethanol solution at 4°C before testing. Separation of the root was performed below the cementoenamel junction. Sixteen enamel blocks of uniform height (roughly 5 mm ± 0.4 in height coronal-apically) were obtained from the non-excluded teeth. The enamel blocks were then polished using a slurry of non-fluoridated pumice and cleaned with water for 60s utilising a slow-speed angle handpiece and prophy brush and subsequently rinsed. Three enamel blocks were fixed together vertically by wax to the floor of a small plastic tube filled with saturated thymol solution to avoid bacterial growth during the experiment and to prevent drying, shrinkage and cracking of the blocks. The enamel blocks were separated from each other while being fixed using two 1 mm thick plastic rings (Fig. 1).

2.2. X-Ray microtomography

Specimens were imaged utilising an ‘in-house’ XMT scanner developed at Queen Mary University of London (QMUL) [18]. The MuCAT2 system, unlike traditional commercial systems, was designed to eliminate ring artefacts and to produce images with high contrast [19], a key factor in producing images for mineral analysis [18,20,21]. Specimens were imaged at the following settings: 90 keV, 180 µA with a 20 µm voxel size. Each specimen was imaged with a typical scan time of 20 hours.

2.3. Mineral concentration measurements

Image reconstruction was carried out using the method outlined in Davis and Mills [21] which is based on a modified Feldkamp cone-beam reconstruction method [21,22]. Tomview (Version 1.1, G.R. Davis, QMUL, 2003-2022), a visualisation software specific to MuCAT2, was used to visualise the 2D slices in one of three orthogonal planes (occlusal, buccolingual, mesiodistal).

Mineral concentration measurements were carried out in Tomview with measurements taken within the enamel subsurface (>60 µm from the enamel surface) on each tooth within three distinct zones: ‘Middle’ representing the area below the middle of the hypothetical orthodontic bracket; ‘Occlusal’ 2 mm above from ‘Middle’, and ‘Cervical’ 2 mm below from ‘Middle’ zone (Fig. 2). Nine points (3-voxel coordinate markers) were positioned in each zone to measure the linear attenuation coefficient (LAC), with three points placed on the middle, occlusal and cervical zones (Fig. 3.B), each slice equating to an approximate size of 20 µm (Fig. 3). A total of 27 points were taken for each tooth (3 points within each zone, 3 zones; 3 × 3 × 3), with each point measuring the mean LAC within a 3 × 3 × 3 voxel volume (27 voxels in total).

The measurements were subsequently exported into Microsoft Excel

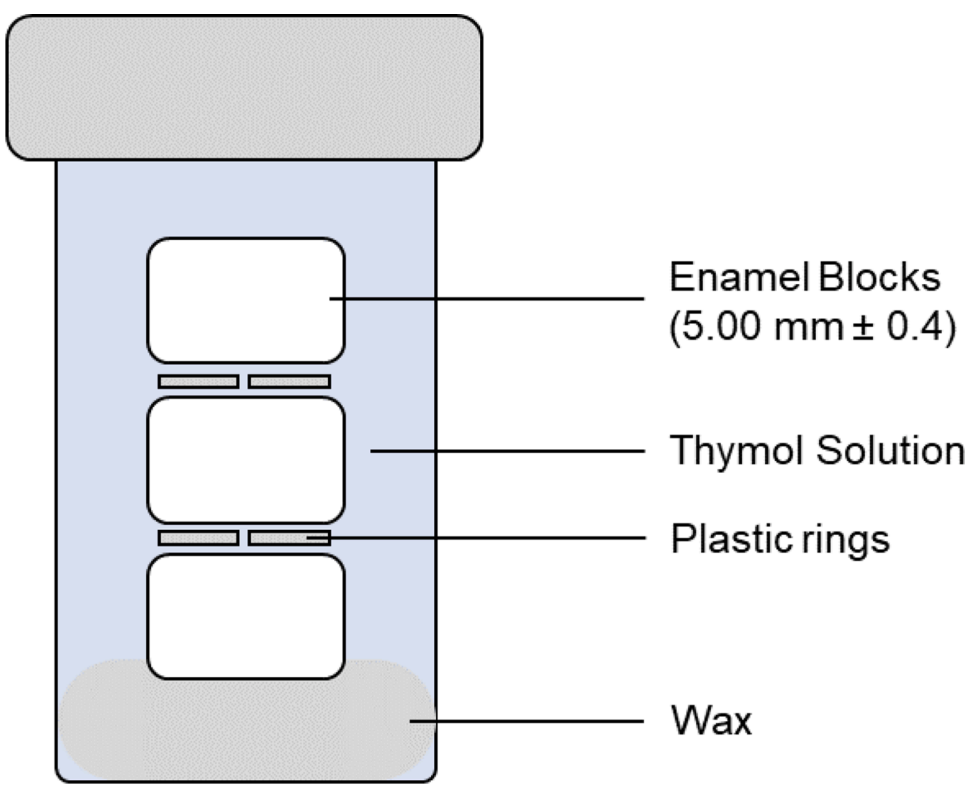

Fig. 1. Specimen set-up for XMT scans.

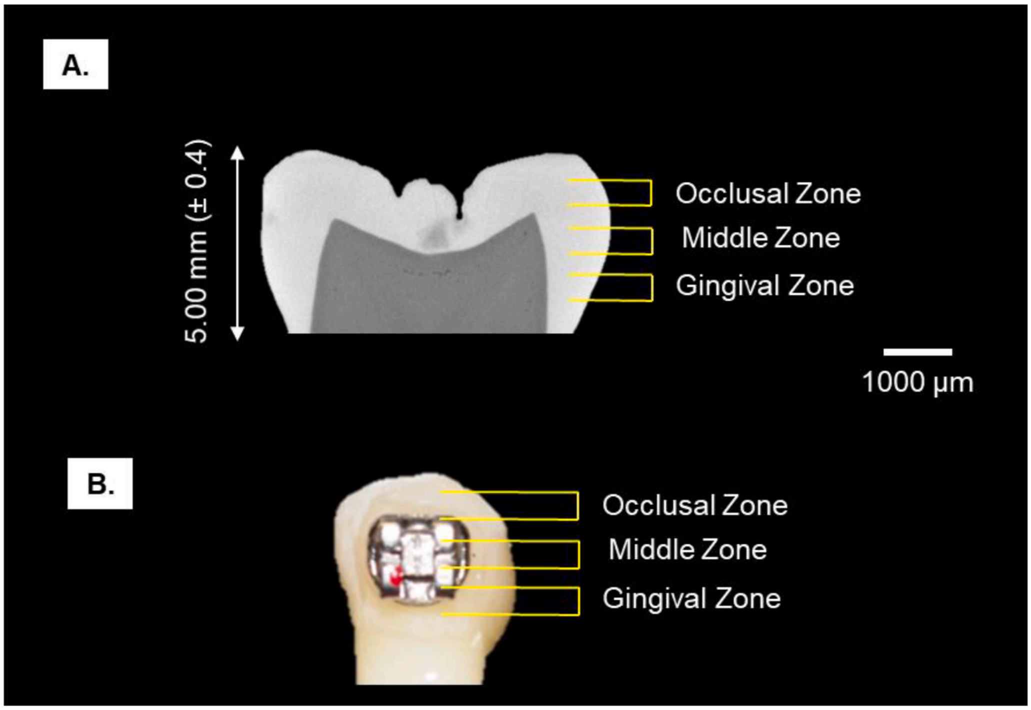

Fig. 2. A: An example of a reconstructed tooth with the three distinct zones labelled; 'Occlusal', 'Middle' and 'Cervical'; B: Location of the orthodontic bracket in relation to the three zones.

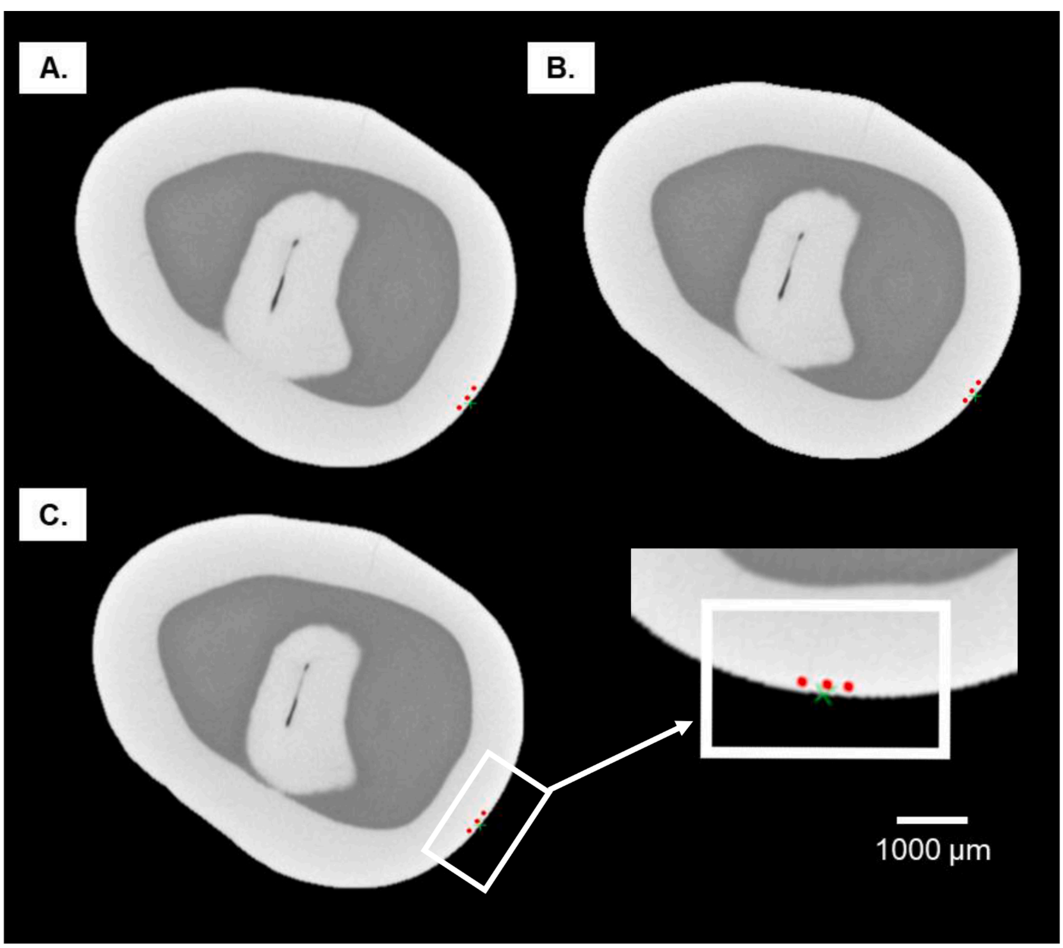

Fig. 3. Locations of the measurements, in a representative tooth within the 'Middle' zone. A. Slice 151/266. B. 149/266. C. 153/266.

(Version 1909, 2019; Microsoft, USA) for further analysis. To convert LAC measurements to mineral concentration, the following formula was used [21]:

$$C = \frac{\mu - \mu_S}{\mu_m - \mu_s} \rho m$$

Where:

μ is the measured LAC.

μ_m is the LAC of 100% mineral (3.12 cm^{-1} at 40 keV [23]).

μ_s is the mean measured LAC of the solution.

ρ_m is the known density of 100% mineral (3.16 gcm^{-3}).

2.4. Statistical analysis

IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL, USA) was used to analyse the sample data, including descriptive statistics (mean, standard deviation). One-way analysis of variance (ANOVA) was used to explore any significant difference in the mineral concentration among the three regions neighbouring the hypothetical bracket. Tukey's post hoc test was performed to identify any significant difference between the two regions. A *P*-value less than 0.05 was considered statistically significant. The normality of data was confirmed using Shapiro–Wilks's test ($P > 0.05$).

3. Results

Using one-way ANOVA, a significant difference was found in the mean mineral concentration between the three zones ($P<0.05$, Table 1). Tukey's post hoc test demonstrated that the middle zone (A1) had the highest enamel mineral concentration (MC) when compared with other zones (3.07 g cm^{-3}, Table 2). The mean difference in enamel mineral concentration between middle, occlusal and cervical zones is illustrated in Table 2. The MC of the gingival zone (A3) was significantly lower than that of the middle zone (A1) ($P<0.05$) and was insignificantly lower than that of the occlusal zone (A2). In addition, the occlusal zone (A2) had lower MC than the middle zone (A1); however, this variation was not significant. The MC profile of the three measurement zones is illustrated in Fig. 4.

4. Discussion

The MC of enamel tissue varied between regions adjacent to the hypothetical orthodontic bracket. The cervical region was found to have the lowest mineral concentration compared to the middle and occlusal regions. Conversely, the middle region was found to have the highest MC level. This might be due to the thickness of the enamel in this region. Similarly, Wong et al. measured the MC level of deciduous teeth using the XMT method and found a large difference in the amount and the gradients of MC in posterior primary teeth [17]. In addition, the current study highlights the potential impact of the low mineral concentration in the cervical region of the dental enamel on potential WSL development during fixed appliance-based treatment. Moreover, there may be potential implications in terms of the bond strength of orthodontic attachments.

The formation of WSLs or enamel decalcification is a major problem during fixed appliance-based treatment. Dental plaque tends to accumulate in the cervical area underneath orthodontic brackets. The highly acidic nature of dental plaque and decreased pH allow for the colonisation of aciduric bacteria and may culminate in demineralisation of the enamel tissue [24]. Accordingly, enamel releases calcium and phosphate toward saliva to buffer the environment leading to the development of WSLs [25]. Using XMT, Cochrane et al. found that enamel with WSLs in molars has a lower mineral concentration than that of sound enamel, ranging approximately from 1.73 to 2.48 g/cm^3 and 2.57 to 2.67 48 g/cm^3 respectively [26]. The finding of lower baseline values in the cervical region is therefore of clinical relevance.

Table 1
Descriptive statistics and ANOVA test of mineral concentration of three measurement zones.

Measurement Zones	Samples No.	Mean(g/cm^{-3})	SD	*P-value*
Middle Zone (A1)	16	3.07	± 0.11	
Occlusal Zone (A2)	16	3.03	± 0.14	**0.03***
Cervical Zone (A3)	16	2.95	± 0.12	

SD = standard deviation
* Significant at $P < 0.05$

Table 2
Differences in the level of the mineral concentration in the three measurement zones (Sample *t*-test).

Pairwise comparisons	Mean(g/cm^{-3})	Mean Difference(g/cm^{-3})	*P* value
A1:A2	A1= 3.07 A2= 3.03	0.04	0.56
A1:A3	A1= 3.07 A3= 2.95	0.12	**0.03***
A2:A3	A2= 3.03 A3= 2.95	0.08	0.23

A1: Middle Zone; A2: Occlusal Zone; A3: Cervical Zone.
* = Significant ($P < 0.05$);

Gorelick et al. found that the cervical area of the buccal surface of the crown with a bonded or bonded fixed attachment was more susceptible to WSLs [27]. Similarly, in a further study the enamel gingival to the attachments in both the maxillary and mandibular dental arches was most affected by WSLs with male gender, poor oral health, high sugar diet and lengthy orthodontic treatment increasing the risk of deleterious change [28]. The present study helps to explain this pattern the cervical region most susceptible, while the central zone is less prone to demineralisation. MC is directionally proportional to the structural integrity and the mechanical properties of the enamel tissue [29]. Based on the observed MC, preventative protocols for WSLs could be refined in order to better address the risk of cervically-located plaque accumulation.

A strength of the current study was the use of a highly-sensitive and reliable method for quantification of the MC level of permanent enamel without the need to surface stain specimens [12-14,30]. Several studies have discussed enamel mineral concentration [31–33] using different characterisation techniques in the context of demineralisation-remineralisation processes [34,35], disease [36] and age [37]. Micro-computed tomography was utilised to measure the elemental content as well as the mineral density in different layers of tooth enamel depending on the individual age [31,38]. To our knowledge, this is the first study to evaluate the mineral concentration in the permanent dentition neighbouring a hypothetical orthodontic bracket using XMT measurement.

The outcomes of the current *ex-vivo* study demonstrated that a significant difference in the MC of enamel tissue was found between the middle and cervical parts with the lowest level of MC in the cervical region when compared with the middle and occlusal regions. Similarly, Barnhart et al. [39] found that the density of the cervical region was significantly lower than the middle and incisal regions. Wilson et al. [40] suggested that the occlusal enamel region is more mineralised than the cervical area as it forms early with more time for maturation and crown formation than the cervical area of enamel tissue. In addition, the cervical region is thinner than the occlusal and the middle region, which inherently makes the enamel less dense in cervically compared to other enamel regions [41]. Scanning electron microscopy has suggested that the cervical region tends to be formed from the "prismless" enamel of permanent human teeth [42,43]. Akkus et al. [10], utilising Raman spectroscopy, reported no significant difference in the MC level between the incisal and middle region; however, MC content in the cervical region was lower than in other regions mirroring the current study. The difference in MC gradient may be related to the difference between the

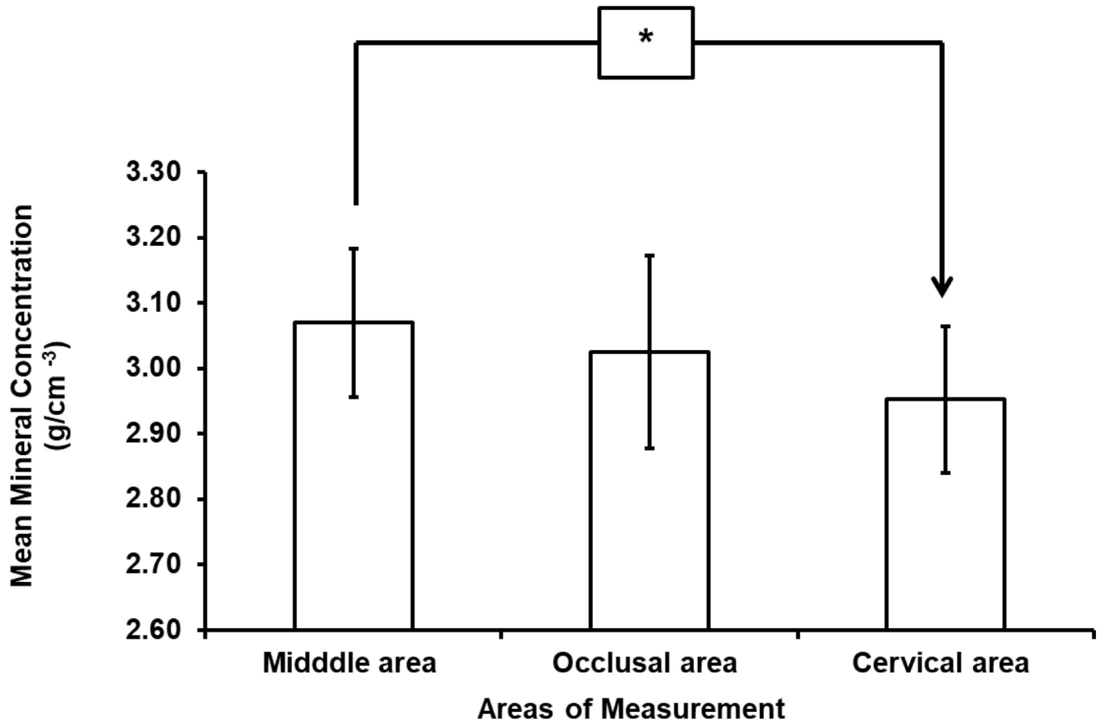

Fig. 4. The mean of mineral concentration (g/cm^{-3}) by tooth area (Error bars indicate the range of mineral concentration measured). * Significant ($P<0.05$).

methods of imaging used.

The current study suggested that the low level of MC in the cervical area neighbouring the orthodontic bracket in permanent teeth could be linked to the higher susceptibility of developing WSLs in this region [6] potentially due to increased porosity. Dijk et al., [44] suggested that MC is an important factor in determining the rate of demineralisation. In addition, the level of MC can be associated with the susceptibility to caries development, having an inverse relationship between the MC level and the prevalence of caries [45]. Several studies [6,45] assumed that the MC can be considered a critical factor in determining both remineralisation and demineralisation. Therefore, the presence of WSLs in the cervical area adjacent to the orthodontic bracket may not only be related to higher plaque retention but also the low level of MC along the cervical region. It is worth noting, that this statistical uncertainty (error bars) obtained through data analysis and seen in Fig. 4 most likely reflects noise in the image acquisition, as opposed to biological variation. However, the XMT system used for this study, has been specifically designed to eliminate artefacts produced from noise typically seen in X-ray systems [19]. Therefore, any noise affecting the statistical uncertainty is likely to be minimal.

The height of the clinical crown of premolars and molars is shorter than maxillary canines and central incisors. Thus, orthodontic brackets on premolars and buccal tubes may be bonded near the cervical part of the crown enamel, which has the least amount of mineral concentration and therefore increases the susceptibility to bonding failure. Several studies have confirmed significant failure of bonding in the posterior teeth [46–49]. Moreover, previous studies demonstrated the substandard etch patterns in the cervical enamel compared with middle and incisal regions [42,43] which could be related to the low amount of MC in the cervical region. The findings may suggest the necessity for a modified etching protocol for the cervical region of the enamel crown. This would create a good environment for successful bracket bonding. In addition, we suggest the demand for fluoride application particularly in the cervical region to reduce the susceptibility of development of WSLs around orthodontic brackets during orthodontic treatment. The current study highlighted the importance of the XMT technique in the estimation of MC level and its substantial potential for future scientific research in assessing the relationship between WSLs development, mineral concentration, and orthodontic treatment. The limitations of the study include: that the sample was randomly selected from a specific cohort; thus, the result cannot be generalised. In addition, the possible presence of potential confounders such as previous exposure to fluoride could change the actual mineral concentration of the enamel. After controlling various risk factors for WSL formation, further *in vitro* research to assess which area of the labial/buccal surface of the crown are more prone to WSL development after immersion of the whole crown in demineralised solution.

5. Conclusion

The XMT method offers a viable non-destructive measurement, permitting detailed analysis of the mineralisation of enamel. Within the limitations of the current study, our findings provide a baseline quantitative measurement of the mineral concentration of permanent enamel, which might help to understand the relationship between white spot lesions and fixed appliance-based orthodontics. Quantifiable differences in mineral concentration were detected between different regions of permanent dental enamel. In particular, the cervical enamel was found to be of significantly lower mineral concentration than the middle zone of the buccal surface of premolars. The cervical portion may therefore be more vulnerable to demineralisation. This might contribute to the development of WSLs in the cervical area of the teeth bonded with fixed orthodontic brackets while also influencing the bond strength with fixed orthodontic attachments.

CRediT authorship contribution statement

Thaer Jaber Al-Khafaji: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Visualization. **Bahn Agha:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization. **Almustafa Alhumadi:** Writing – review & editing. **Wisam W. Alhamadi:** Writing – review & editing. **David Mills:** Methodology. **Graham Roy Davis:** Methodology, Writing – review & editing.

Alexander J. Cresswell-Boyes: Methodology, Investigation, Resources. **Padhraig S. Fleming:** Conceptualization, Validation, Writing – review & editing, Visualization.

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References

[1] J.H. Kinney, M. Balooch, S.J. Marshall, Jr.G.W. Marshall, T.P. Weihs, Hardness, Young's modulus of human peritubular and intertubular dentine, Arch. Oral Biol. 41 (1996) 9–13, https://doi.org/10.1016/0003-9969(95)00109-3.
[2] T. Kodaka, K. Debari, M. Yamada, M. Kuroiwa, Correlation between microhardness and mineral content in sound human enamel (short communication), Caries Res. 26 (1992) 139–141, https://doi.org/10.1159/000261498.
[3] I. Leichter, J.Y. Margulies, J.Mizrahi A.Weinreb, G.C. Robin, B. Conforty, M. Makin, B. Bloch, The relationship between bone density, mineral content, and mechanical strength in the femoral neck, Clin. Orthop. Relat. Res. 163 (1982) 272–281. PMID: 7067262.
[4] R. Hodgskinson, J.D. Currey, G.P. Evans, Hardness, an indicator of the mechanical competence of cancellous bone, J. Orthop. Res. 7 (1989) 754–758, https://doi.org/10.1002/jor.1100070518.
[5] M. Baldassarri, H.C. Margolis, E. Beniash, Compositional determinants of mechanical properties of enamel, J. Dent. Res. 87 (2008) 645–649, https://doi.org/10.1177/154405910808700711.
[6] R.P. Shellis, Relationship between human enamel structure and the formation of caries-like lesions in vitro, Arch. Oral Biol. 29 (1984) 975–981, https://doi.org/10.1016/0003-9969(84)90144-4.
[7] A.G. Targino, A. Rosenblatt, A.F Oliveira, A.M. Chaves, V.E. Santos, The relationship of enamel defects and caries: a cohort study, Oral Dis. 17 (2011) 420–426, https://doi.org/10.1111/j.1601-0825.2010.01770.x.
[8] K.C. Julien, P.H. Buschang, P.M. Campbell, Prevalence of white spot lesion formation during orthodontic treatment, Angle Orthod. 83 (2013) 641–647, https://doi.org/10.2319/071712-584.1.
[9] M.D. Brown, P.M. Campbell, E.D. Schneiderman, P.H. Buschang, A practice-based evaluation of the prevalence and predisposing etiology of white spot lesions, Angle Orthod. 86 (2016) 181–186, https://doi.org/10.2319/041515-249.1.
[10] A. Akkus, A. Akkus, R. Roperto, O. Akkus, T. Porto, S. Teich, L. Lang, Evaluation of mineral content in healthy permanent human enamel by Raman spectroscopy, J. Clin. Exp. Denty 8 (2016) e546–e549, https://doi.org/10.4317/jced.53057.
[11] S.E. Bishara, A.W. Ostby, White spot lesions: formation, prevention, and treatment, Semin. Orthod. 14 (2008) 174–182, https://doi.org/10.1053/j.sodo.2008.03.002.
[12] T.J. Al-Khafaji, F. Wong, P.S. Fleming, N. Karpukhina, R. Hill, Novel fluoride and strontium-containing bioactive glasses for dental varnishes-design and bioactivity in Tris buffer solution, J. Non-Cryst. Solids 503 (2019) 120–130, https://doi.org/10.1016/j.jnoncrysol.2018.09.037.
[13] S.K. Hahn, J.W. Kim, S.H. Lee, C.C. Kim, S.H. Hahn, K.T. Jang, Microcomputed tomographic assessment of chemomechanical caries removal, Caries Res. 38 (2004) 75–78, https://doi.org/10.1159/000073924.
[14] T.N. Clementino-Luedemann, A. Dabanoglu, N. Ilie, R. Hickel, K.H. Kunzelmann, Micro-computed tomographic evaluation of a new enzyme solution for caries removal in deciduous teeth, Dent. Mater J. 25 (2006) 675–683, https://doi.org/10.4012/dmj.25.675.
[15] R. Müller, H. Van Campenhout, B. Damme, G. Van Der Perre, J. Dequeker, T. Hildebrand, P. Rüegsegger, Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography, Bone 23 (1998) 59–66, https://doi.org/10.1016/S8756-3282(98)00068-4.
[16] T.T. Huang, A.S. Jones, L.H. He, M.A. Darendeliler, M.V. Swain, Characterisation of enamel white spot lesions using X-ray micro-tomography, J. Dent. 35 (2007) 737–743, https://doi.org/10.1016/j.jdent.2007.06.001.
[17] F.S. L.Wong, P. Anderson, H. Fan, G.R. Davis, X-ray microtomographic study of mineral concentration distribution in deciduous enamel, Arch. Oral Biol. 49 (2004) 937–944, https://doi.org/10.1016/j.archoralbio.2004.05.011.
[18] G.R. Davis, A.N Evershed, D. Mills, Quantitative high contrast X-ray microtomography for dental research, J. Dent. 41 (2013) 475–482, https://doi.org/10.1016/j.jdent.2013.01.010.
[19] G.R. Davis, J.C. Elliott, X-ray microtomography scanner using time-delay integration for elimination of ring artefacts in the reconstructed image, Nucl. Instrum. Methods Phys. Res. A 394 (1997) 157–162, https://doi.org/10.1016/S0168-9002(97)00566-4.
[20] G.R. Davis, J.C Elliott, High definition X-ray microtomography using a conventional impact X-ray source, J. Phys. IV 104 (2003) 131–134, https://doi.org/10.1051/jp4:200300045.
[21] G.R. Davis, D. Mills, P. Anderson, Real-time observations of tooth demineralization in 3 dimensions using X-ray microtomography, J. Dent. 69 (2018) 88–92, https://doi.org/10.1016/j.jdent.2017.11.010.
[22] L.A. Feldkamp, L.C. Davis, J.W. Kress, Practical cone-beam algorithm, J. Opt. Soc. Am. A: Opt. Image Sci. Vis. 1 (1984) 612–619, https://doi.org/10.1364/JOSAA.1.000612.
[23] M. J. Berger, J. H. Hubbell, S. M. Seltzer, J. Chang, J. S. Coursey, R. Sukumar, D. S. Zucker, K. Olsen, XCOM: Photon Cross Section Database (version 1.5), National Institute of Standards and Technology, Gaithersburg, MD, 2010. Available on http://physics.nist.gov/xcom (25 July, 2022, date last accessed), doi: https://doi.org/10.18434/T48G6X.
[24] M.W. Beerens, M.H. Van Der Veen, H. Van Beek, J.M. Ten Cate, Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up, Eur. J. Oral Sci. 118 (2010) 610–617, https://doi.org/10.1111/j.1600-0722.2010.00780.x.
[25] O.Y. Yu, I.S. Zhao, M.L. Mei, E.C.M. Lo, C.H. Chu, A review of the common models used in mechanistic studies on demineralization-remineralization for cariology research, Dent. J. 5 (2017) 20, https://doi.org/10.3390/dj5020020.
[26] N.J. Cochrane, P. Anderson, G.R. Davis, G.G. Adams, M.A. Stacey, E.C. Reynolds, An X-ray microtomographic study of natural white-spot enamel lesions, J. Dent. Res. 91 (2012) 185–191, https://doi.org/10.1177/0022034511429570.
[27] L. Gorelick, A.M. Geiger, A.J. Gwinnett, Incidence of white spot formation after bonding and banding, Am. J. Orthod. 81 (1982) 93–98, https://doi.org/10.1016/0002-9416(82)90032-X.
[28] K. Khalaf, Factors affecting the formation, severity and location of white spot lesions during orthodontic treatment with fixed appliances, J. Oral Maxillofac. Res. 5 (2014), https://doi.org/10.5037/jomr.2014.5104 e4.
[29] F. Lippert, R.J.M. Lynch, Comparison of Knoop and Vickers surface microhardness and transverse microradiography for the study of early caries lesion formation in human and bovine enamel, Arch. Oral Biol. 59 (2014) 704–710, https://doi.org/10.1016/j.archoralbio.2014.04.005.
[30] F.S. Wong, J.C. Elliott, G.R. Davis, P. Anderson, X-ray microtomographic study of mineral distribution in enamel of mandibular rat incisors, J. Anat. 196 (2000) 405–413, https://doi.org/10.1046/j.1469-7580.2000.19630405.x.
[31] B. He, S. Huang, C. Zhang, J. Jing, Y. Hao, L. Xiao, X. Zhou, Mineral densities and elemental content in different layers of healthy human enamel with varying teeth age, Arch. Oral Biol. 56 (2011) 997–1004, https://doi.org/10.1016/j.archoralbio.2011.02.015.
[32] Y.R. Jeng, T.T. Lin, T. Y.Wong, H.J. Chang, D.B. Shieh, Nano-mechanical properties of fluoride-treated enamel surfaces, J. Dent. Res. 87 (2008) 381–385, https://doi.org/10.1177/154405910808700414.
[33] R.A. Farah, M.V. Swain, B.K. Drummond, R. Cook, M. Atieh, Mineral density of hypomineralised enamel, J. Dent. 38 (2010) 50–58, https://doi.org/10.1016/j.jdent.2009.09.002.
[34] N. Efeoglu, C.Efeoglu D.Wood, Microcomputerised tomography evaluation of 10% carbamide peroxide applied to enamel, J. Dent. 33 (2005) 561–567, https://doi.org/10.1016/j.jdent.2004.12.001.
[35] N. Efeoglu, D.J. Wood, C. Efeoglu, Thirty-five percent carbamide peroxide application causes in vitro demineralization of enamel, Dent. Mater. J. 23 (2007) 900–904, https://doi.org/10.1016/j.dental.2006.06.032.
[36] T. Koehne, R.P. Marshall, A. Jeschke, B. Kahl-Nieke, T. Schinke, M. Amling, Osteopetrosis, osteopetrorickets and hypophosphatemic rickets differentially affect dentin and enamel mineralization, Bone 53 (2013) 25–33, https://doi.org/10.1016/j.bone.2012.11.009.
[37] Q. Zheng, H. Xu, F. Song, L. Zhang, X. Zhou, D.Huang Y.Shao, Spatial distribution of the human enamel fracture toughness with aging, J. Mech. Behav. Biomed. Mater. 26 (2013) 148–154, https://doi.org/10.1016/j.jmbbm.2013.04.025.
[38] B. He, S. Huang, J. Jing, Y. Hao, Measurement of hydroxyapatite density and Knoop hardness in sound human enamel and a correlational analysis between them, Arch. Oral Biol. 55 (2010) 134–141, https://doi.org/10.1016/j.archoralbio.2009.12.005.
[39] E.C. Barnhart, P.M. Campbell, A. Noureldin, K. Julien, P.H. Buschang, The quality of etched enamel in different regions and tooth types and its significance in bonding and the development of white spot lesions, Angle Orthod. 91 (2021) 576–582, https://doi.org/10.2319/090120-761.1.
[40] P. R.Wilson, A.D. Beynon, Mineralization differences between human deciduous and permanent enamel measured by quantitative microradiography, Arch. Oral Biol. 34 (1989) 85–88, https://doi.org/10.1016/0003-9969(89)90130-1.
[41] N.X. West, A. Joiner, Enamel mineral loss, J. Dent. 42 (2014) S2–S11, https://doi.org/10.1016/S0300-5712(14)50002-4.
[42] Y.Takahashi Y.Arakawa, M. Sebata, The effect of acid etching on the cervical region of the buccal surface of the human premolar, with special reference to direct bonding techniques, Am. J. Orthod. 76 (1979) 201–208, https://doi.org/10.1016/0002-9416(79)90121-0.
[43] K.A. Galil, G. Z.Wright, Acid etching patterns on buccal surfaces of permanent teeth, Pediatr. Dent. 1 (1979) 230–234. PMID: 399680.
[44] J. W.Van Dijk, J.M. Borggreven, F.C. Driessens, Chemical and mathematical simulation of caries, Caries Res. 13 (1979) 169–180, https://doi.org/10.1159/000260398.
[45] P.O. Gerdin, K. Astrand, Enamel roentgen density. I. Enamel roentgen density in deciduous teeth from six-year-old children with and without caries prevalence, Sven. Tandlak. Tidskr. 62 (1969) 805–814. PMID: 5267063.
[46] C.R. Mattick, R.S. Hobson, A comparative micro-topographic study of the buccal enamel of different tooth types, Eur. J. Orthod. 27 (2000) 143–149, https://doi.org/10.1093/ortho/27.2.143.

[47] R.A. Linklater, P.H. Gordon, Bond failure patterns in vivo, Am. J. Orthod. 123 (2003) 534–539, https://doi.org/10.1067/mod.2003.S0889540602000252.
[48] R.S. Hobson, J.F. McCabe, A.J. Rugg-Gunn, The relationship between acid-etch patterns and bond survival in vivo, Am. J. Orthod. 121 (2002) 502–509, https://doi.org/10.1067/mod.2002.122239.
[49] U. Adolfsson, E. Larsson, B. Ogaard, Bond failure of a no-mix adhesive during orthodontic treatment, Am. J. Orthod. 122 (2002) 277–281, https://doi.org/10.1067/mod.2002.126401.