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Characterisation of developmentally hypomineralised human enamel

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Short title: Developmentally hypomineralised enamel

Key words: Molar incisor hypomineralisation, carbonate and mineral contents, enamel ultrastructure.

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ABSTRACT

Objectives: To investigate and clarify physical and chemical properties of enamel affected by Molar Incisor Hypomineralisation (MIH).

Methods: A series of *in vitro* studies were performed on extracted molars affected by MIH and sound teeth for controls. Tooth sections underwent Vickers microhardness testing before lapping and subsequent transverse microradiographic analysis and examination under polarised light microscopy. Carbonate content was determined by CO₂ release from acid digestion. Unprepared and fractured surfaces were examined under scanning electron microscopy.

Results: MIH-affected molars demonstrated a severe degree of hypomineralisation with an average mineral content of only 58.8% vol% mineral. Vickers microhardness was significantly reduced in MIH compared with controls (1.8 ± 1.1 v 4.4 ± 1.0 GPa, $p < 0.05$) and polarised light microscopy revealed the bulk of MIH lesions had a porosity of $\leq 5\%$ but also substantial areas of $\geq 10\%$ and smaller areas exceeding 25% porosity. A surface layer was frequently observed on both intact and broken-down lesions and cervical regions of MIH teeth were typically spared. Carbonate content of MIH enamel was higher than control samples (6.6 ± 2.1 v 4.4 ± 1.1 wt%, $p < 0.05$). Scanning electron microscopy showed that both the enamel rod and surface ultrastructure were defective. Clinical characteristics did not consistently correlate with all properties.

Conclusions: The properties of MIH-affected enamel significantly differ from those of normal enamel and were highly variable, however some common characteristics were observed. Implications for aetiology and clinical management are discussed.

Clinical Significance Statement

This study establishes a qualitative as well as the quantitative nature of developmental hypomineralisation in MIH-affected enamel with implications for caries risk. The enamel features of affected teeth, particularly identification of a surface layer, sparing of cervical regions and significant surface porosities, will help inform better management strategies.

1. Introduction

Molar-Incisor Hypomineralisation (MIH) is a condition characterised by demarcated, qualitative defects of enamel of systemic origin, affecting one or more permanent molars, with or without involvement of the incisor teeth. There has been growing recognition and interest in MIH since its proposal as a distinct condition in 2001.¹ The poor long-term prognosis, rapid physical deterioration and poor restorative outcomes, coupled with difficulties achieving patient comfort for normal function, oral hygiene and adequate local analgesia for treatment, can make MIH a particularly challenging and costly condition with potential for adverse outcomes beyond oral health.²⁻⁴ Establishing the nature of the defects is critical to inform effective management strategies and may also provide insights to the currently undetermined aetiology.^{5,6}

Research has focused primarily on enamel, though there is some suggestion the underlying dentine may also be abnormal.⁷ Hardness studies have identified a significant reduction in affected enamel;⁸⁻¹⁰ polarised light microscopy (PLM) demonstrated increased porosity;¹¹ back-scatter scanning electron microscopy (SEM) determined mineral content reduction was 5%, however x-ray microtomography studies indicated mineral deficits of up to 19% and 20%^{10, 12, 13} and protein content has been shown to be significantly increased.^{14, 15} Reports on the calcium phosphate ratio (Ca:P) of MIH affected enamel are not consistent; a single sample x-ray diffraction study observed no substitution peak shifts, however a secondary mass ion spectroscopy study reported both an overall increase in carbon content and an association between higher carbon levels and hypomineralisation severity.^{10, 16} SEM examination revealed increased disorganisation of crystals, hollowed out prism cores and abnormal etching patterns.^{8, 10, 17} Pulpal changes have been observed using histological and biomarker methods.¹⁸⁻²⁰

Despite the presumption of hypomineralisation inherent in nomenclature, as yet no analysis of the mineral content of MIH-affected enamel has been reported using the gold standard of transverse microradiography (TMR). Given the reported 5% reduction in mineral content was associated with an unexpectedly dramatic reduction in nanohardness it would be prudent to re-assess any correlation between these two properties.¹⁰ Porosity has only been examined with intact lesions, however studies of other properties, particularly clinical severity and protein content, suggest breakdown is a determining factor.^{15, 21} Similarly, lesions have only been observed in air and water, but more detailed characterisation is possible using higher refractive indices (RI) imbibing media.¹¹ The conflicting reports regarding Ca:P and lattice substitutions also warrant further investigation; higher levels of carbon could reflect the increased organic content, or higher carbonate levels which have been implicated in caries susceptibility.^{10, 16, 22} This susceptibility may explain the abnormal caries patterns to which MIH-affected teeth are recognised as prone, but enamel surface characteristics may also contribute and these have not been examined as SEM investigations used either etched or fractured samples.^{8, 10, 17} To address these knowledge gaps, in the present study we examined the physical and chemical properties of MIH-affected enamel from extracted first permanent molar teeth using Vickers microhardness, TMR, PLM, SEM and carbonate determination.

2. Methods

Extracted molars diagnosed clinically as affected by MIH by the treating dental clinician were collected and placed in 0.2% clear chlorhexidine for transportation. Informed consent was obtained and ethics approval granted by the Human Research Ethics Committee of the University of Melbourne Ethics ID 0719683. The diagnosis of MIH was confirmed by the investigators then received teeth were placed in 4% neutral buffered formaldehyde for at least

two weeks, rinsed thrice with double de-ionised water then stored at 4°C and 100% humidity until use. Whole teeth were embedded in methyl-methacrylate resin (Paladur, Heraeus, Germany) and cut bucco-lingually into 600µm thick sections using a water cooled diamond embedded circular saw (Minitom, Struers, Denmark). Sections were polished with successively finer grade silicone carbide paper (600 - 4000 grit) and stored as previously described. Colour and surface characteristics were assigned based on the appearance of the intact tooth to improve clinical relevance.

Sections were placed on the stage of a light microscope (Leica, Germany) with a microhardness testing device (MHT-10, Anton Paar, Austria) and kept hydrated throughout. Tests using a Vickers indenter ($F = 0.3\text{N}$, $T = 5\text{secs}$) were performed for each specimen with indentation paths oriented parallel and perpendicular to the DEJ. Images of indentations were captured using the 50x lens objective and hardness calculated according to the Vickers Hardness equation.

Following microhardness testing, samples were mounted with nail varnish on metal stubs and lapped as described above to ~100µm with the exact thickness measured using a bench mounted micrometer. Transverse microradiographs were produced by radiographing the sections alongside an aluminium stepwedge, microradiographs were digitised and grey values converted to volume % mineral (vol%min) as described previously²³ using the equation and constants described by Angmar.²⁴ Line profiles were taken through the region of interest from outside the enamel though to the DEJ as well as lesion area analysis, defined by the extent of enamel with greater than 1% porosity.

Sections were also examined using the 5x objective of a polarised light microscope (Leica, Germany) with camera attached in the following media: air (RI 1.00); water (RI 1.33) and Thoulet's solution (RI 1.41 and 1.47). Superimposition of acquired images was used to

determine the hardness, mineral content and porosity of the enamel to allow comparison of the properties.

Additional teeth were sectioned with the saw creating a block with a natural surface on one face or partially sectioned then fractured through the hypomineralised lesion. Specimens were mounted on sample holders, gold sputter-coated and examined in an environmental SEM (FEI, Quanta, Oregon, USA) at 15kV under low vacuum.

For enamel carbonate measurements powdered enamel samples were weighed dry in an Eppendorf tube before placement in a sealed chamber connected to a pressure transducer containing 3mL 5M hydrochloric acid pre-saturated with respect to CO₂. Real time data were recorded using a laptop computer as the acid was allowed to contact the enamel and the observed voltage change was used to calculate the pressure change and subsequently the amount of carbon dioxide released.

Images were analysed using Image Tool™ (UTHSCSA, USA) software where appropriate. Data were entered into Excel (Microsoft Corp, Washington, USA) software for analysis: descriptive statistics, graphs, line-of-best-fit and, for microhardness data ANOVA analysis with the critical level for significance set at $p < 0.05$, were undertaken using the same software.

3. Results

The properties of MIH-affected enamel were highly variable, both within and between lesions, defying simple description however some common patterns and features were observed. Visual, hardness, TMR and PLM examinations revealed that the lesions affected typically the full enamel thickness (see Figures 1-4). The microhardness of hypomineralised enamel was significantly reduced compared with controls (1.8 ± 1.1 v 4.4 ± 1.0 GPa respectively, $p < 0.05$). The mineral content determined using TMR was significantly lower

than control enamel with an average of 58.8 vol% min down to a minimum of 31.7 vol% min. The porosity of the bulk of the lesions as measured using PLM was $\leq 5\%$ but areas with $> 25\%$ porosity were present and the carbonate content was significantly higher than that of the control samples (6.6 ± 2.1 v 4.1 ± 1.1 wt% respectively, $p < 0.0005$) (see Table 1). White/cream and intact lesions tended to be less severely affected than yellow/brown and broken down lesions respectively but this difference was not consistently statistically significant (see Table 1). An approximately $25\mu\text{m}$ wide surface layer was often apparent and demonstrated an increased hardness (even reaching normal values), increased mineral content and decreased porosity relative to the underlying lesion (see Figure 2).

Hardness and mineral content profiles generally showed a sharp decrease immediately beneath the surface layer if present then a gradual increase towards the DEJ (see Figures 3 & 4). The cervical areas were spared consistently and the properties of visually unaffected areas were consistent with normal enamel in terms of hardness and mineral content profiles, hardness values, porosity, and carbonate content (see Figures 2 & 4). Visually the lesion viewed in water under PLM most closely corresponded to TMR and there was a strong inverse linear relationship between the properties of porosity and mineral content ($R^2 = 0.99$). A weaker inverse linear relationship was found between microhardness and porosity ($R^2 = 0.77$) but microhardness and mineral content demonstrated only a weak positive correlation ($R^2 = 0.37$).

SEM imaging revealed that clinically intact enamel surfaces demonstrated frequent surface depressions, abrasions and porosities (see Figure 5). Broken-down surfaces were highly irregular displaying anything from an amorphous “smear” layer with functional markings, to large porosities, to frank exposure of rod structure. Fractured specimens were also abnormal with prominent inter-rod spaces, disorganised crystals, rod dislocations and deficient or hollow rod cores in cross-section (see Figure 5).

4. Discussion

The current study offers insights into the nature of MIH with important implications for aetiology, natural history and management. Hardness, TMR, PLM and carbonate measurements all confirm previous reports that the cervical areas tend to be spared with the implication that the behaviour of enamel in these areas, perhaps most particularly important in terms of restorative material bonding, is likely to be predictable.^{9, 11} It has been suggested that yellow/brown and broken down lesions constitute a more severe form of MIH, with yellow/brown lesions more likely to suffer post-eruptive breakdown (PEB). Our results suggest this proposed categorisation is appropriate, however it requires further investigation as, whilst hardness data provided a statistically significant difference, only a trend was noted for mineral and carbonate contents. This finding is particularly noteworthy for the intact/PEB comparison; given that the areas of enamel lost were likely the most severely affected. For the lesions with PEB to still demonstrate poorer properties despite this loss, implies the original differences before breakdown may have been even greater. TMR results lend some support to this supposition with lower minimum mineral content values found in intact lesions but higher mean lesion values compared with that of those samples with PEB. Despite this it should be noted that even clinically “mild” lesions had significantly reduced hardness and mineral content and sometimes showed extensive enamel involvement.

Reported patterns regarding gradual change in hardness parallel to the DEJ, aberrant mineral content gradient perpendicular to the DEJ and porosity exceeding 5% were confirmed, but also expanded to establish hardness perpendicular to the DEJ also displayed an abnormal gradient consistent with mineral content, and that porosity was greater than 10% for substantial areas and could exceed 25%. Although the degree of hypomineralisation varied considerably it was always marked compared with normal enamel. Only the mineral

content of the milder lesions were comparable with previously published data.^{12, 13} However, these previous studies did not use the gold standard for mineral content determination, TMR. Differences may also be attributed to natural sample variation and/or limitations of the newer techniques. The present results are consistent with the dramatic reduction in physical properties and more so than previous combined mineral content and hardness reports using backscatter SEM which is susceptible to other influences.¹⁰ Even so, mineralisation and hardness did not correlate strongly in this investigation, however the severity of the hypomineralisation established may provide an explanation as authors of an artificial caries study noted poor correlation once mineral content dropped below 45 vol% min.²⁵ Consequently study design using one method as a proxy for the other should be avoided or interpreted with caution when investigating MIH.

Mineralisation and porosity correlated well, and in a manner similar to caries suggesting that PLM more readily demonstrates minor changes. While the porosity of hypomineralised enamel is undoubtedly increased, it must be borne in mind that birefringence can be affected by increased carbonate substitutions and organic content.²⁶ Both these components have now been demonstrated in MIH-affected enamel in abnormal quantities and therefore PLM may be somewhat inaccurate in representing the true porosity of the tissue.^{14, 15} Nanohardness is reportedly also susceptible to the influence of organic content and MIH-affected enamel has been demonstrated to contain a higher protein content^{14, 15}.

The discovery of a surface layer implies a potential for developmentally hypomineralised enamel to undergo supplemental mineralisation post-eruptively (“re”-mineralisation) and indicates the processes of de- and re-mineralisation in developmentally defective and normal enamel may share some fundamentally comparable mechanisms. Unfortunately the layer may pose a similar challenge to caries management in terms of

restricting deeper mineralisation; however the observation that the layer forms in deeper tissue layers following PEB indicates that all affected enamel is capable of this augmentation if exposed to favourable conditions. The spectrum of surface anatomy observed under SEM would also indicate it is possible, even after significant breakdown, to “seal off” the surface with putative corresponding decreases in patient discomfort and caries risk.

Despite similarities it must be remembered, both in study design and clinical care, that caries and MIH are not truly analogous conditions. SEM imaging reveals surface characteristics which putatively contribute to bacterial adhesion and, even on apparently “intact” surfaces, pore sizes sufficient to allow bacterial ingress. These features are present on what would normally be low caries-risk areas of sound teeth and help account for the atypical caries and restoration patterns commonly observed in MIH patients. They suggest a benefit from sealing or “re”-mineralising parts of the crown not otherwise considered at risk. Similarly the higher carbonate content means any caries risk assessment or intervention should recognise a deficiency exists not only in the quantity but in the quality of mineral present. Higher carbonate content also has implications for the aetiology of MIH. Since carbonate levels sometimes exceeded those reported for developing enamel it seems unlikely that they are simply an expression of enamel maturation failure. An alternative explanation could be the exposure of ameloblasts to a metabolic uncoupler resulting in a carbon dioxide rich, but mineral poor, environment during maturation. The metabolic poisoning of the ameloblast during mineralisation would also be consistent with the persistence of ameloblastin, a secretory phase protein, in MIH enamel.¹⁴

In summary, the present study has described the highly variable nature of MIH-affected enamel in terms of reduced mineral content, reduced hardness, increased porosity, increased carbonate content and defective ultrastructure including significant surface defects on apparently intact lesions. The reported sparing of cervical areas has been confirmed but

the categorisation of severity according to clinical appearance has only been supported to a limited extent and requires further validation.

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References

1. Weerheijm KL, Jalevik B, Alaluusua S. Molar-incisor hypomineralisation. *Caries Research* 2001;**35**:390-1.
2. Jalevik B, Klingberg GA. Dental treatment, dental fear and behaviour management problems in children with severe enamel hypomineralization of their permanent first molars. *International Journal of Paediatric Dentistry* 2002;**12**:24-32.
3. Mejare I, Bergman E, Grindefjord M. Hypomineralized molars and incisors of unknown origin: treatment outcome at age 18 years. *International Journal of Paediatric Dentistry* 2005;**15**:20-8.
4. Crombie FA, Manton DJ, Weerheijm KL, Kilpatrick NM. Molar incisor hypomineralization: a survey of members of the Australian and New Zealand Society of Paediatric Dentistry. *Australian Dental Journal* 2008;**53**:160-6.
5. Alaluusua S. Aetiology of Molar-Incisor Hypomineralisation: A systematic review. *European Archives of Paediatric Dentistry* 2010;**11**:53-8.
6. Crombie F, Manton D, Kilpatrick N. Aetiology of molar-incisor hypomineralization: a critical review. *International Journal of Paediatric Dentistry* 2009;**19**:73-83.
7. Heijs SCB, Dietz W, Noren JG, Blanksma NG, Jalevik B. Morphology and chemical composition of dentin in permanent first molars with the diagnose MIH. *Swedish Dental Journal* 2007;**31**:155-64.
8. Fagrell TG, Dietz W, Jalevik B, Noren JG. Chemical, mechanical and morphological properties of hypomineralized enamel of permanent first molars. *Acta Odontologica Scandinavica* 2010;**68**:215-22.

9. Mahoney E, Ismail FS, Kilpatrick N, Swain M. Mechanical properties across hypomineralized/hypoplastic enamel of first permanent molar teeth. *European Journal of Oral Sciences* 2004;**112**:497-502.
10. Mahoney EK, Rohanizadeh R, Ismail FS, Kilpatrick NM, Swain MV. Mechanical properties and microstructure of hypomineralised enamel of permanent teeth. *Biomaterials* 2004;**25**:5091-100.
11. Jalevik B, Noren JG. Enamel hypomineralization of permanent first molars: a morphological study and survey of possible aetiological factors. *International Journal of Paediatric Dentistry* 2000;**10**:278-89.
12. Farah RA, Swain MV, Drummond BK, Cook R, Atieh M. Mineral density of hypomineralised enamel. *Journal of Dentistry* 2010;**38**:50-58.
13. Fearne J, Anderson P, Davis GR. 3D X-ray microscopic study of the extent of variations in enamel density in first permanent molars with idiopathic enamel hypomineralisation. *British Dental Journal* 2004;**196**:634-8; discussion 25.
14. Farah RA, Monk BC, Swain MV, Drummond BK. Protein content of molar-incisor hypomineralisation enamel. *Journal of Dentistry* 2010;**38**:591-6.
15. Mangum JE, Crombie FA, Kilpatrick N, Manton DJ, Hubbard MJ. Surface Integrity Governs the Proteome of Hypomineralized Enamel. *Journal of Dental Research* 2010;**89**:1160-65.
16. Jalevik B, Odelius H, Dietz W, Noren J. Secondary ion mass spectrometry and X-ray microanalysis of hypomineralized enamel in human permanent first molars. *Archives of Oral Biology* 2001;**46**:239-47.
17. Jalevik B, Dietz W, Noren JG. Scanning electron micrograph analysis of hypomineralized enamel in permanent first molars. *International Journal of Paediatric Dentistry* 2005;**15**:233-40.

18. Fagrell TG, Lingstrom P, Olsson S, Steiniger F, Noren JG. Bacterial invasion of dentinal tubules beneath apparently intact but hypomineralized enamel in molar teeth with molar incisor hypomineralization. *International Journal of Paediatric Dentistry* 2008;**18**:333-40.
19. Rodd HD, Boissonade FM, Day PF, Rodd HD, Boissonade FM, Day PF. Pulpal status of hypomineralized permanent molars. *Pediatric Dentistry* 2007;**29**:514-20.
20. Rodd HD, Morgan CR, Day PF, Boissanade FM. Pulpal expression of TRPV1 in molar incisor hypomineralisation. *European Archives of Paediatric Dentistry* 2007;**8**:184-8.
21. Chawla N, Messer LB, Silva M. Clinical studies on molar-incisor-hypomineralisation part 2: development of a severity index. *Eur Arch Paediatr Dent* 2008;**9**:191-9.
22. Sobel AE. Interrelationship of tooth composition, body fluids, diet, and caries susceptibility. *Annals of the New York Academy of Sciences* 1960;**85**:96-109.
23. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *Journal of Dental Research* 2001;**80**:2066-70.
24. Angmar B, Carlstrom D, Glas JE. Studies on the ultrastructure of dental enamel. IV. The mineralization of normal human enamel. *Journal of Ultrastructure Research* 1963;**8**:12-23.
25. Buchalla W, Imfeld T, Attin T, Swain MV, Schmidlin PR. Relationship between nanohardness and mineral content of artificial carious enamel lesions. *Caries Research* 2008;**42**:157-63.
26. Theuns HM, van Dijk JW, Jongebloed WL, Groeneveld A. The mineral content of human enamel studied by polarizing microscopy, microradiography and scanning electron microscopy. *Archives of Oral Biology* 1983;**28**:797-803.

Figure Legends

Figure 1. a) clinical appearance of the lesion with black lines indicating position of section relating to subsequent images. Images demonstrating enamel lesion appearance, and good agreement between techniques, using: b) light microscopy, including visual designation of affected (1) and apparently unaffected (2) enamel used for sampling purposes (original magnification x1); c) transverse microradiograph demonstrating hypomineralisation (original magnification x2) and; d) polarised light microscopy image in air (RI 1.00) demonstrating areas of increased porosity (black) and normal enamel (blue) (composite image, original magnification x5).

Figure 2. Lesion appearance under polarised light (composite images) corresponding to the intact (top row) and broken down (bottom row) lesions in Figures 3 and 4 respectively. From left to right full lesions are shown in: a) air, enamel $\leq 1\%$ porosity in blue; b) water, enamel $\leq 5\%$ porosity in blue; c) Thoulet's solution RI 1.41, enamel $\leq 10\%$ porosity in blue and; d) Thoulet's solution RI 1.47, enamel $\leq 25\%$ porosity in blue. The central inset showing an expanded view of the images in water demonstrates the presence of a surface layer (blue, porosity $\leq 5\%$) overlying each lesion.

Figure 3. Transverse microradiograph (TMR) of an intact MIH lesion shown in cross section (corresponding to Figure 2, top row). White lines with coloured numbers marked on the TMR image indicate line scans corresponding to the adjacent graphs showing the mineral content profile of each line scan and demonstrate the aberrant pattern in MIH enamel.

Figure 4. Transverse microradiograph (TMR) of an MIH lesion with post-eruptive breakdown shown in cross section (corresponding to Figure 2, bottom row). White lines with coloured numbers marked on the TMR image indicate line scans corresponding to the adjacent graphs showing the mineral content profile of each line scan: line 1 demonstrates the normal mineral content gradient in a relatively unaffected area, while lines 2-10 demonstrate the aberrant pattern in MIH enamel.

Figure 5. Photograph showing clinical appearance of tooth with red oval indicating area shown in subsequent SEM images demonstrating surface features (top row). SEM images demonstrating disorganised internal structure with hollow rod cores (bottom row).

Table 1.

Summary of physical and chemical findings for MIH-affected enamel including subdivision into clinical characteristic categories. “All” represents pooled data from all hypomineralised lesions irrespective of clinical appearance unless otherwise designated in the first column in which case it represents pooled data from control teeth or from apparently sound areas of affected teeth.

	All	Colour		Surface	
		White/ cream	Yellow/ brown	Intact	PEB
Physical (n=10)					
Microhardness (GPa)					
Mean $\pm$ s.d. (range)	1.8 $\pm$ 1.1 (0.1-6.7)	2.1 $\pm$ 1.0 (0.4-5.1)	1.5 $\pm$ 1.0 (0.1-6.7)	1.8 $\pm$ 1.1 (0.1-6.7)	1.6 $\pm$ 0.7 (0.3-3.8)
Apparently sound	4.4 $\pm$ 1.0				
Control	4.4 $\pm$ 1.0				
Porosity $\geq 1\%$ <5%					
% of lesion (range)	33.4 (12.3-71.0)	47.1 (22.5-71.0)	27.5 (12.3-51.0)	36.0 (15.9-71.0)	29.5 (12.3-51.0)
average % vol min (range)	74.8 (67.5-85.1)	71.1 (67.5-74.7)	75.3 (68.7-85.1)	73.4 (67.5-85.1)	74.1 (68.1-80.4)
Porosity $\geq 5\%$ <10%					
% of lesion (range)	34.4 (11.1-79.1)	29.8 (11.1-63.4)	36.4 (18.3-79.1)	37.4 (11.1-79.1)	29.9 (18.3-36.1)
average vol% min (range)	61.9 (56.2-66.7)	57.4 (56.2-58.6)	62.5 (58.2-66.2)	59.5 (56.2-63.5)	63.4 (61.0-66.2)
Porosity $\geq 10\%$ <25%					
% of lesion (range)	18.4 (0-36.2)	14.0 (7.4-20.6)	20.2 (0-36.2)	16.6 (0-34.6)	21.1 (6.5-36.2)
average vol% min (range)	50.5 (38.2-58.7)	52.0 (51.0-52.9)	50.4 (38.2-58.7)	47.1 (38.2-52.9)	53.5 (46.2-58.7)
Porosity $\geq 25\%$					
% of lesion (range)	13.8 (0-28.8)	9.0 (0-20.4)	15.9 (0-28.8)	10.1 (0-28.8)	19.5 (15.0-24.2)
average vol% min (range)	43.0 (30.0-53.2)	43.3 (39.8-46.7)	42.7 (30.0-53.2)	39.8 (30.0-46.7)	46.9 (42.7-53.2)
Mineral content, area analysis (vol% min)					
Mean (range)	58.8 (53.0-72.7)	63.7 (62.9-64.5)	57.5 (53.0-72.7)	61.2 (53.6-72.7)	55.1 (53.0-58.1)
Median (range)	58.7 (50.6-73.2)	63.8 (63.6-64.0)	57.4 (50.6-73.2)	61.4 (53.0-73.2)	54.6 (50.6-58.8)
Mode (range)	61.7 (50.6-74.5)	64.7 (64.4-65.0)	61.0 (50.6-74.5)	63.5 (50.8-74.5)	59.1 (50.6-72.1)
Minimum (range)	45.7 (31.7-58.8)	38.6 (32.1-45.0)	47.5 (31.7-58.8)	42.3 (31.7-58.8)	50.7 (47.1-52.8)
Control	Mean 81.4	Median 83.4	Mode 85.5	Min 69.7	
Chemical (n=15)					
Carbonate content (wt%)					
Mean $\pm$ s.d. (range)	6.6 $\pm$ 2.1 (4.2-10.8)	5.8 $\pm$ 1.3 (5.0-7.4)	7.3 $\pm$ 2.5 (5.0-10.8)	5.5 $\pm$ 0.6 (5.0-6.2)	6.8 $\pm$ 2.9 (4.2-10.8)
Apparently sound	4.1 $\pm$ 0.8				
Control	4.1 $\pm$ 1.1

Figure 2

Figure 3

Figure 4

Figure 5

Characterisation of developmentally hypomineralised human enamel**ACKNOWLEDGEMENTS**

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