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Linking the clinical presentation of molar-incisor hypomineralisation to its mineral density

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Background. The *in vitro* methods used for the assessment of the severity of molar-incisor hypomineralisation (MIH) are not available for clinicians faced with questions regarding the severity in clinical cases, and the best management approach.

Aim. To assess whether there is a relation between the severity of the defects in MIH enamel (represented by reduction in the mineral density) and the clinical presentation (represented by the colour of the defect and its laser fluorescence).

Design. The colour of enamel was recorded (normal, white, yellow or brown) in specific areas for ten extracted first permanent molars with MIH defects and ten extracted sound teeth. Laser fluorescence (LF) and mineral density (MD) were measured for the same areas. A mixed model, using sample/tooth as a random effect, was used to estimate the relationship between the MD and the colour-coding, and between the MD and LF readings.

Results. The between-samples correlation coefficient for the colour coding and the MD was 0.99 ($P < 0.001$), and 0.83 ($P < 0.001$) for the LF and MD.

Conclusions. The degree of staining of MIH enamel, as assessed visually or by LF, may be used clinically to reflect the severity of the defect.

Introduction

Among other factors, the severity of hypomineralisation or enamel loss in molar-incisor hypomineralisation (MIH) is a major determinant for the choice of treatment. In young individuals, minimally to moderately affected molars may be restored with composite resin restorations or metal crowns, while for severely affected molars in otherwise a healthy mouth, extraction may be an option at the appropriate developmental stage of occlusion development[1,2].

The *in vitro* methods available for assessment of the severity of MIH (e.g., measuring the mineral density (MD) of enamel with X-ray microtomography (XMT)[3,4], or measuring the mechanical properties of enamel with nanoindentation testing (Mahoney *et al.*, 2004a,b; Xie *et al.*, 2007) are accurately quantitative. Nanoindentation testing employs a small diamond indenter, and measures the hardness and elastic modulus of the specimen under study. XMT is a miniaturised version of the conventional computerised tomography (also known as CT scanning). It is used to visualize hidden details of structures and to perform quantitative analyses such as measuring the mineral density of mineralised tissues[5]. Unfortunately, these are laboratory-based methods and are not available for clinicians in everyday practice who are faced with questions regarding the severity in clinical cases, and the best management approach.

The main severity assessment tool available for the dentist is direct visual inspection. Dentists may determine the severity of MIH enamel defects according to their colour, with dark/brown enamel usually labelled as worse than yellow enamel, which is in turn worse than white/chalky enamel. For example, Leppaniemi *et al.*[6] classified MIH molars into three categories: molars with atypical restorations in the worst category, molars with rough and broken enamel in the second category, and

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molars with only colour change (opaque, yellow or brown) into the least severe category. Chawla *et al.*[7] modified Leppaniemi *et al.*'s index, and classified MIH molars with white-cream opacities as mild hypomineralisation, and teeth with yellow-brown discolouration and/or post-eruptive breakdown as moderate to severe. This colour-based classification is applied to other enamel defects, such as those caused by fluorosis. For example, the Tooth Surface Index of Fluorosis (TSIF)[8] classifies stained teeth in a worse category than white opaque lesions, even if the opaque lesion was occupying a wider area of the tooth.

None of these indices have been tested for validity and sensitivity, and they are merely based on 'experienced' observations. It is perhaps obvious that posteruptive breakdown of enamel is the most severe form of MIH defect. But how accurate is it to report that MIH molars with brown defects are more severely affected than MIH molars with opaque defects?

Another method available for dentists that may prove useful in assessing the severity of MIH is the laser fluorescence (LF) of enamel as measured by a chair-side device such as DIAGNOdent (KaVo, Biberach, Germany). DIAGNOdent is traditionally used as an adjunct method in diagnosing dental caries. However, in a recent study, the severity of MIH (as assessed by the reduction in the mechanical properties of enamel) showed good correlation with the DIAGNOdent readings, suggesting a possible role for LF devices in labelling the severity of MIH[9].

The aim of the present *in vitro* study was to assess whether there is a relation between the severity of the defects in MIH enamel (represented by reduction in the MD) and the clinical presentation (represented by the colour of the defect and its LF).

Materials and methods

Enamel samples

Ethical approval to collect and use the teeth was obtained from the New Zealand Multi-Region Ethics Committee (MEC/06/12/177, March 2007). Ten first permanent molars with different severities of MIH (from 10 different participants) and ten mature permanent teeth (from 10 different participants) with sound enamel were collected following planned extractions. The MIH teeth were collected from children aged between 8.5 and 11.5. MIH defects are distinguishable from carious lesions by their shape, colour and location on teeth. None of the sound teeth or the MIH teeth showed any signs of dental caries. The teeth were stored at 4 °C in distilled water with thymol crystals until the time of investigation, which was typically within a week of extraction. Besides cleaning the teeth from dental plaque and blood, no other treatment or preparation of the enamel was carried out before taking the photographs, LF measurement and the XMT.

Light photographs and colour-coding

A digital camera (Nikon D80; Nikon Inc., Melville, NY, USA) fitted with a macro-lens (Tamron SP Di AF 90 mm 1:2.8 Macro 1:1; Tamron Inc., Commack, NY, USA) and a ring flash (Sigma Ring Flash EM-140 DG; Sigma Corporation of America, Ronkonkoma, NY, USA) was used to capture macro-photographs for all the surfaces (buccal, lingual, proximal and occlusal) of each tooth used in this study. Following the criteria of the Developmental Defects of Enamel Index (DDE index)[10], each tooth was cleaned, but not thoroughly dried before the photographs were taken.

When a photograph was taken, the camera was clamped to a stand about 15 cm away from the tooth, which was fixed to a rotating stage in front of a blue background. The photographs were faithful recordings of the extracted teeth. The burn-out effect of excess lighting was eliminated by immediately examining each photograph for clarity and validity and if the photograph was not clear or if a defect was not clearly seen, the photograph was repeated until the appearance on the photograph matched the clinical view.

The photographs served as a means of assessing the severity of each MIH defect based on enamel colour. Three examiners (R.F. and two independent experienced dentists) independently viewed the photographs

of the five surfaces of the 20 teeth used in the study on a 17 inch LCD computer screen. Specific areas (132 in total with an average of 6.6 areas per tooth) on the smooth surfaces of the teeth and the cusp tips, for which MD was measured and LF readings recorded, were indicated on the photographs for the examiners to colour-code (Fig. 1). More than one area was colour-coded on each photograph. The examiners were instructed to give the colour-code based on Table 1. One month later, R.F. repeated the colour-coding. All colour coding was carried out before the MD was measured to prevent any bias.

This severity classification was based on several indices specifically developed for enamel defects[6–8,10]. Since hypoplasia has been shown not to be present in MIH[3,4], no reference to hypoplastic-type lesions was made. When more than one defect affected a tooth surface, each defect was scored independently.

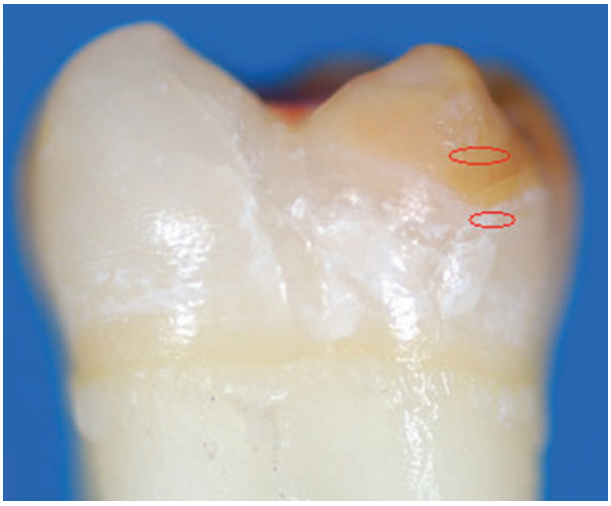

Fig. 1. A photograph of the lingual surface of a study tooth. The red circles indicate the areas for colour-coding by the examiners. The MD and LF for the same areas were also measured.

Table 1. Degree of severity of MIH defects as seen visually.

Enamel colour	Code
Normal	1
White/chalky opacity	2
Yellow opacity	3
Brown opacity	4
Posteruptive breakdown	5

Laser fluorescence measurement

This assessment was made with the DIAGNOdent pen. The flat tip (1 mm in diameter) was calibrated, before use with every tooth, using the ceramic reference provided with the pen, as recommended by the manufacturer. After drying the enamel with a 3-in-1 syringe for approximately 8 s, LF was measured on the specific areas of the teeth that had been colour-coded, and recorded on print-outs of the photographs taken for each tooth. The LF readings were recorded once only.

X-ray microtomography

The XMT system used in the study was SkyScan 1172 (SkyScan N.V., Aartselaar, Belgium). For measuring the MD of enamel, four hydroxyapatite internal standards/phantoms of known densities (1.74–3.06 g/m^3) were used. The four phantoms were stacked in a small plastic tube and the study tooth was placed on top of them before placing the group into the XMT machine. For quantification of MD, the reconstructed cross-sectional images were imported to special software for quantitative measurement, CT Analyser (Version 1.5.0.0, SkyScan N.V., Aartselaar, Belgium). Full description of the parameters used in recording the X-ray projections, reconstructing the images, and the method used to quantify MD was presented in a previous paper[4]. The specific areas for which colour-coding was performed and LF recoded were identified on the reconstructed images and MD was measured. The area where the colour-coding was performed can be accurately identified on the CT scans by measuring the distance between the area and the cusp tip as well as between the area and the cementum–enamel junction. This insured that MD measurements accurately reflected the LF measurements and the colour codings.

Statistical analysis

To assess inter-examiner and intra-examiner reliabilities for colour-coding, intraclass correlations between the different examiners and for the same examiner, respectively, were

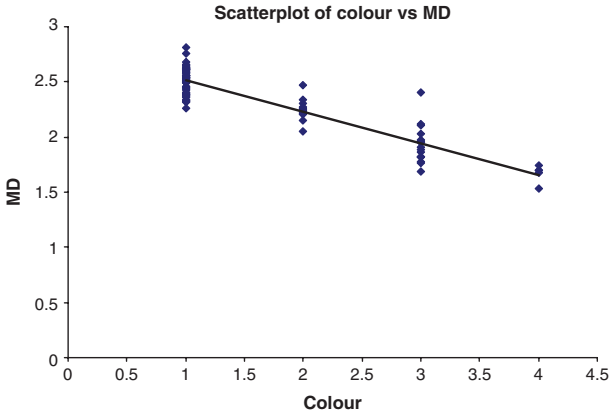

Fig. 2. Scatter plot for colour-coding and MD (g/m^3) from all the samples (132 areas in total).

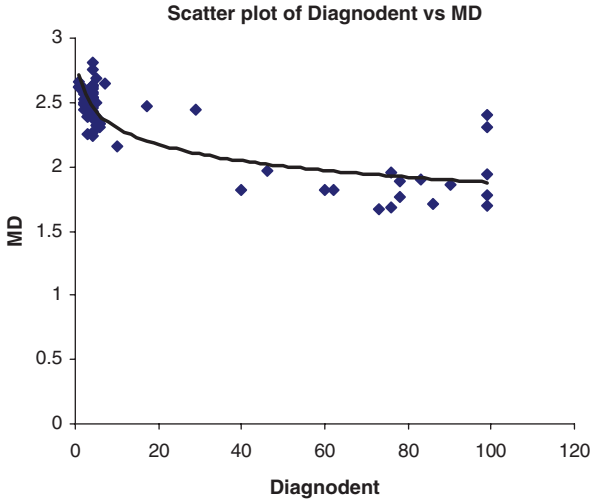

Fig. 3. Scatter plot for LF and MD (g/m^3) from all the samples before log transformation (132 areas in total). Note the curvilinear nature of the plot.

calculated. A mixed model, using sample/tooth as a random effect, was used to estimate the relationship between the MD and the colour-coding, and between log transformed MD measurements and log transformed LF readings [due to the curvilinear nature of the produced scatter plot (Fig. 3)]. Using Stata™ 10.0, both between sample and within sample correlations[11,12] were calculated.

Results

Fig. 1 is a photograph of the lingual surface of one of the teeth in the study, with specific indication for the examiners of the areas where the enamel colour was to be coded.

There was substantial agreement[13] between the examiner R.F.'s repeated colour-codings (intraclass correlation coefficient = 0.76, kappa = 0.68) and between R.F. and one of the other examiners (intraclass correlation coefficient = 0.76, kappa = 0.65). There was a fair agreement[13] between R.F. and the second examiner (intraclass correlation coefficient = 0.60, kappa = 0.38). When assessing the correlation with LF and XMT readings, the colour-codings recorded by R.F. were used.

Table 2 shows the means and ranges of MD and LF for each colour-coding category. The scatter plot for the overall compiled results from all the enamel samples for MD measurements vs. colour-codings showed a linear relation (Fig. 2), while the scatter plot for MD *versus* LF readings showed a curvilinear relation (Fig. 3). The correlation coefficients for within samples, and between samples were calculated as recommended by Bland and Altman[11,12] and are shown in Table 3.

The regression coefficients indicate that for every change in the colour-code (e.g., from yellow to brown) there is a 0.28 g/m^3 reduction in MD (95% CI: −0.31, −0.25) and for every unit increase in DIAGNOdent readings there is an 8% reduction in MD (95% CI: −0.1, −0.07).

Discussion

Reporting the intra- and inter-examiner reliabilities is critical for establishing the validity of a certain classifying scheme. Many studies investigating the severity of developmental enamel defects fail to report intra- and inter-examiner reliabilities. In a comprehensive review of the literature, Rozier[14] found that about 60% of the studies using fluorosis indices failed to report inter- and intra-examiner reliabilities. The intra-examiner reliability found in the present study was substantial according to Dunn[13], and the inter-examiner reliability was fair to substantial. These results are comparable to other studies in which photographs or direct clinical inspection were used to colour-code enamel defects and stains[8,14–16]. The inherit subjectivity in determining the colour of enamel accounts for the

Table 2. Means and ranges of MD and LF for each colour-code. No MD or LF could be recorded for the 'Posteruptive breakdown' category since, as the name indicates, no enamel exists in this category.

Enamel colour (code)	Number of samples	Mean (and range) LF	Mean (and range) MD (g/m^3)
Normal (1)	40	4.17 (1–29)	2.51 (2.26–2.81)
White/Chalky opacity (2)	22	26.6 (3–99)	2.24 (2.04–2.46)
Yellow opacity (3)	42	75.84 (40–99)	1.93 (1.68–2.4)
Brown opacity (4)	28	86 (73–99)	1.67 (1.52–1.74)
Posteruptive breakdown (5)	Not applicable	Not applicable	Not applicable

Table 3. Results analysed for within-samples and between-samples correlations.

Independent Variable	*r* with MD (within samples)	*r* with MD (between samples)	Regression coefficient (S.E)	Significance
Colour	0.88	0.99	−0.28 (0.014)	$P < 0.001$
DIAGNOdent	0.85	0.83	−0.08 (0.006)	$P < 0.001$

disagreements between the examiners. If training had been provided for the examiners, the agreement would have improved. However, the aim of the study was to reflect the real clinical situation were no training is provided to the clinician.

Conventionally, enamel defects indices have been implicitly or explicitly[8,17] used to label the severity of defects in terms of aesthetics, and therefore most of the indices have concentrated on anterior teeth. In contrast, the present study focuses on molars, and assesses the severity in terms of function rather than aesthetics. The lower the MD of enamel, the higher the chances are for breakdown under normal occlusal loading[18].

In dental fluorosis, it is argued that staining can be considered to be a basis of severity when aesthetics is of concern but not when considering the pathological changes in fluorosis[14]. This is because staining may be a posteruptive feature in fluorosis, with porous enamel absorbing external stains. However, in doing so, we will have ignored that the extent of stains that enamel tends to absorb may depend on its porosity[19] particularly in the more severe forms of fluorosis. Moreover, what may be posteruptive staining in fluorosis, is not necessarily posteruptive in MIH defects.

The intention of this study was not to recommend the use of photographs as a means of assessing enamel defects, nor to present a new index for labelling the severity of MIH defects. A severity index should critically identify the specific areas of the tooth affected by the defects, the number of teeth affected, and possibly include the symptoms enamel hypomineralisation may cause[7,14]. The present study considers MIH at the defect level rather than at the surface, tooth or individual level. Also, for an index to prove to be valid and reliable, it must sustain further research and scrutiny. The aim of the present study was merely to investigate whether the general convection among dentists that the shade of enamel discoloration reflects the severity of the defect was valid or not. The strong correlation demonstrated between the severity of the defect in MIH enamel as measured by its MD, and the clinical presentation represented by the colour of the enamel and its laser fluorescence confirms this (Table 3). It also demonstrates the validity of several classification indices[7,10] for the severity of MIH defects according to the degree of staining.

It is important to consider within-sample and between sample correlations[11,12]. Within-sample correlation analyses whether an increase in clinical severity labelling within the individual sample (e.g., a hypomineralised tooth) is associated with a reduction in MD. Differences between samples are removed and only changes within a specific tooth are

considered. The correlations were found to be strong; i.e., in any tooth, a change in colour-coding (e.g., from yellow to brown) or an increase in its LF reading should warn the dentist that he/she is dealing with an area of defective enamel with a marked decrease in its MD. For every change in colour-code there is a 0.28 g/m^3 reduction in MD, and for every unit increase in DIAGNOdent reading there is an 8% reduction in MD. This is not to indicate that a slight increase in DIAGNOdent reading for the same area over time reflects a deterioration in the condition of the enamel in that spot, as DIAGNOdent readings tend to change over time even if the condition of the enamel remains unchanged. However, as the DIAGNOdent pen is moved from one area to another on the same tooth, an increase in the readings most likely does indicate a reduction in the MD.

The between-samples correlation analysis indicates whether, in general, samples (dental enamel) with dark staining and high LF readings also tend to have low MD. The correlations were also strong (Table 3).

In order for enamel to withstand occlusal forces placed on it directly or indirectly (i.e., beneath a restoration), sufficient mechanical properties are required. The main determinant of the strength of the mechanical properties of a mineralised tissue is its MD. A strong correlation exists between the MD of bone and its mechanical properties[20] and between the MD of dental hard tissues and their mechanical properties[21–23].

Mejare *et al.*[24] found that almost half the restorations placed in MIH molars fail, and suggested considering extracting severely affected teeth as one of the alternative treatments. An in vitro investigation[25] showed that the majority of failures in restored MIH enamel are due to breakdown in the enamel underneath the restorations. This suggests that when the reduction in the mechanical properties (and MD) of MIH enamel is below a certain level, enamel can no longer withstand the occlusal forces to sustain bonded materials.

It would be helpful for the dentist to have a guide to the severity of the defect before deciding on the best management approach. Since MD measurement is not feasible in the clinical situation, colour of enamel may be a good alternative. In addition to the somewhat subjective colour labelling of enamel, LF appears to provide a similarly strong and more objective method for indicating the severity of the defect.

The fluorophores causing enhanced LF in carious teeth are claimed to be porphyrins produced by the caries bacteria[26,27]. However, bacterial porphyrins may not be the only fluorophores causing increased LF as detected by DIAGNOdent. It has been shown that calculus, plaque, composite resin restorations, remnants of polishing paste, and external stains may increase the fluorescence signal[28–30]. The fluorophore(s) responsible for the increased LF in hypomineralised enamel has yet to be identified. But regardless of its nature, since LF correlates strongly with both the MD and the mechanical properties[9] of MIH enamel, LF as measured by DIAGNOdent may be used in the clinical situation as an extra indication of the severity of the MIH defects.

Despite the correlations found in this study, it is premature to choose a specific management approach based on the degree of staining, DIAGNOdent readings or even the MD of enamel. Once a link is made between the enamel MD (or its mechanical properties) and enamel breakdown under direct or indirect occlusal loads, it may be possible to confirm more reliable restorative and clinical treatment strategies based on the clinical presentation of MIH.

In conclusion, the degree of staining of MIH enamel, as assessed visually or by LF, reflects the severity of the disease. However, due to the wide range of readings that DIAGNOdent presents for each colour, it should not be relied on as the sole indicator of severity. Just as it is used now as an adjunct in diagnosing dental caries, LF readings should be combined with other measures, such as colour and patient's symptoms, in labelling the severity.

Clinicians and patients would like to reach to an evidence-based management strategy for MIH. The management plan should take into account the severity of the individual MIH case. While the severity was measured

in terms of MD, no link has been made so far to the actual mechanical properties, which are the main determinants of the severity. It may be logical to suggest that the lower the MD, the more reduced the mechanical properties of enamel would be, but this suggestion remains to be substantiated for hypomineralised enamel.

What this paper adds

- The use of X-ray microtomography has been repeatedly proven to be a valid way for the measurement of the mineral density of sound and hypomineralised enamel.
- A strong correlation exists between the mineral density of hypomineralised enamel and its degree of staining and laser fluorescence, with darker colours and higher fluorescence demonstrating lower mineral density.

Why this paper is important to paediatric dentists

- The long-held belief that the darker the colour of hypomineralised enamel staining, the weaker it is was proven to be valid in this study.
- Just as laser fluorescence is used in the clinical situation as an adjunct in diagnosing dental caries, it may be combined with other measures, such as colour and patient's symptoms, in labelling the severity.

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