ORIGINAL RESEARCH ARTICLES

MINERAL DENSITY OF HYPOMINERALISED AND SOUND ENAMEL

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Molar incisor hypomineralisation, X-ray microtomography, enamel mineral density, characterisation.

Introduction
The term Molar Incisor Hypomineralisation (MIH) was first proposed in 2001 by Weerheim (1). MIH is a demarcated qualitative defect of enamel of systemic origin, affecting one or more first permanent molars, often affecting the permanent incisors (2). The affected teeth show clearly demarcated enamel opacities of normal thickness with a smooth surface and white, yellow or brown in colour (2). Internationally, the current MIH prevalence ranges between 3 and 44% with an average of 15%, which, considering its aesthetic and functional consequences, make it a major public health problem (3). Currently, the aetiology of MIH is not clearly elucidated, but researchers agree that it is likely to be multifactorial in origin (4). MIH consists of an alteration in the action of the ameloblasts during the maturation phase (5). Authors determined the mineral density of hypomineralised enamel in permanent teeth by means of X-ray MicroComputed Tomography (XMCT) (6-10). Here, from our preliminary results, we aimed to distinguish hypomineralised enamel from sound enamel by mean of XMCT with a sample including eight hypomineralised teeth.

Materials and Methods
The tooth material consisted of a convenience sample of eight permanent first molars, collected from seven patients with a clinical diagnosis of MIH, extracted as part of an orthodontic treatment plan at the Teaching Hospitals of Bordeaux. Diagnosis of MIH was made by one of two experienced paediatric dentists. X-ray Micro-Computed Tomography (XMCT) was performed on the MIH teeth. The material was digitised using high resolution microtomography at the laboratory PLACAMAT (UMS 3626) in Bordeaux (Microtomograph X GE V/TOME/X S equipment). The scanning parameters were 120 kV, 147 µA for the x-ray tube, an exposure time of 500ms with four integrations per projection, 2550 projections/360° and a copper filter of 0.1 mm was placed on the source in order to reduce beam hardening artefacts. The voxel size was 7x7x7 µm3. The final volume was reconstructed in 16-bit. The microCT images were compiled using software Avizo® 7.0.1 (FEI, OR, USA). Measures were performed at the same coronal heights. This requirement is because in the permanent dentition the occlusal enamel is more mineralised than the cervical enamel (11, 12). Thus, the most apical plane of section through the cervix that shows a continuous ring of enamel was located, and the images were then recompiled according to this plane (13). Five
slides including an enamel defect were selected. The average of grey levels contained in a square (1 mm²) was calculated in hypomineralised and normal enamel for each slide. This square is placed in the centre of the defect and at the same distance to the enamel surface in normal enamel (Figure 1). This procedure was performed using Plot Profile ImageJ® 1.45 software (NIH, USA).

Non-parametric statistical tests (Wilcoxon test for paired samples) were carried out from Statistica® software Package Version 7.1 software (Statsoft® Dell, OK, USA) to compare data from these two areas (stained and normal).

The research protocol (DC-2015-2415) was registered at the “Comité de Protection des Personnes Sud-Ouest et Outre Mer III” and the “Ministère de la recherche”. Data were anonymised.

### Results

The mean of five measures per area (hypomineralised and normal) for each tooth are presented in Table 1. The relative grey scale values were markedly higher in normal enamel compared to hypomineralised enamel with an average difference of 9148 (SD=2455). There was a mineral density reduction of 19%. The Wilcoxon test for paired samples was conducted for 80 measures in hypomineralised and normal enamel. The test showed that mineral density reduction was statistically significant (p value = 3.57E-08).

![Figure 1: Image from micromotomographic analysis of a MIH first permanent molar. The grey level average was calculated for each square in hypomineralised enamel (dark area) and normal enamel (light area). A red square is placed in the centre of the hypomineralised enamel and a blue square is placed in normal enamel at the same distance from the enamel surface.](image)

<table>
<thead>
<tr>
<th>MIH area</th>
<th>Normal area</th>
<th>Difference</th>
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<tbody>
<tr>
<td></td>
<td>Mean (GS)</td>
<td>SD (GS)</td>
</tr>
<tr>
<td>MIH_1</td>
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</tr>
<tr>
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<tr>
<td>MIH_8</td>
<td>26546</td>
<td>626</td>
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**a)** Mean and standard deviation (SD) of five measures performed per area (normal and hypomineralised) for each MIH tooth. **b)** Results from the Wilcoxon test for paired samples performed (p value) between the 80 measures calculated in the hypomineralised and normal enamel. (GS : Grey Scale; * : statistically significant).

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<tr>
<td><strong>Mean</strong></td>
<td>9148</td>
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<td><strong>SD</strong></td>
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<td></td>
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<tr>
<td><strong>p value</strong></td>
<td>3.57E-08*</td>
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density was significantly lower in hypomineralised enamel than it was for normal enamel (p<0.0001; Table 1).

Discussion
The present microtomographic analyses of hypomineralised enamel showed a reduction (mean 19%) in mineral density. To our knowledge, the mineral density of MIH enamel has been the subject of five studies using back-scattered electron or x-ray microcomputed tomography (6, 7, 9, 10, 14-16). The samples of reviewed studies included from 2 to 15 teeth affected by MIH. In all studies, a decrease in mineral density was shown in hypomineralised enamel versus unaffected enamel (6-10, 14, 16). From these five studies, on average, a reduction in mineral density of 18% was calculated. Studies by Farah et al. included the largest sample set analysed by micro-computed tomography (10 MIH teeth) (9, 15). Parameters vary depending on studies, for example, previous studies used a voxel size of 15µm³ whereas in our study a higher resolution was obtained (7µm³). Currently, authors perform analyses in 2D, however 3D analyses should be used. 2D data analysis leads to a loss of dimensional information compared to 3D (17). A standardisation of the methodology with clearly defined parameters is necessary to perform meta-analyses.

Conclusions
The present study of hypomineralised teeth has shown a significantly decrease in mineral density in hypomineralised enamel compared to normal enamel.

Acknowledgements
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Bibliography


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