Pulpal Status of Hypomineralized Permanent Molars
Helen D. Rodd, BDS, PhD 1 • Fiona M. Boissonade, BDS, PhD 2 • Peter F. Day BDS, M Paed Dent 3

Abstract: Purpose: Young patients with hypomineralized teeth frequently complain of symptoms suggestive of dentin hypersensitivity. It has been proposed that these symptoms may be exacerbated by an underlying pulpal inflammation. The purpose of the study was to determine the pulpal status of hypomineralized teeth. Methods: The experimental material comprised 25 sound and 19 hypomineralized permanent first molars obtained from children requiring dental extractions under general anesthesia. Pulp sections were processed for indirect immunofluorescence using combinations of: (1) protein gene product 9.5; (2) leukocyte common antigen; and (3) Ulex europaeus I lectin. Image analysis was then used to determine the percentage area of staining of each label. Results: Innervation density was significantly greater in the pulp horn and subodontoblastic region of hypomineralized teeth than in sound teeth. Immune cells were most abundant within pulps of hypomineralized teeth exhibiting enamel loss. Vascularity was found to be similar for both hypomineralized and sound teeth, but was significantly greater in hypersensitive hypomineralized samples. Conclusion: This study provides biological evidence that inflammatory changes may be present within the pulpal tissue of these teeth. (Pediatr Dent 2007;29:514-20) Received September 21, 2006 / Revision Accepted February 25, 2007.

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Molar incisor hypomineralization (MIH) is a clinical diagnosis used to describe “hypomineralization of systemic origin of 1 to 4 permanent first molars, frequently associated with affected incisors.” 1 Affected molars present with well-demarcated white/yellow or brown/yellow enamel opacities. In severe cases, the defective enamel is lost shortly after tooth eruption, exposing underlying dentin. Epidemiological data would suggest that MIH is a common problem and may affect between 3% and 25% of all children. 2,3 The etiology remains speculative, but may include: (1) a variety of environmental factors; (2) childhood illnesses; and (3) medical complications. 4-6 The extent and severity of the enamel defects are dependent on the phase of amelogenesis occurring at the time of the insult. 7

Histological examination of the hard tissues in MIH has found that the affected enamel is very porous. 7 Using secondary ion mass spectrometry, Jälevik and colleagues reported that the MIH enamel has a lower calcium and phosphate content than normal teeth. 8 Thus, the rapid posteruptive enamel loss seen in some MIH patients is likely to relate to its inherent weakness.

The clinical management of young MIH patients is directed towards early occlusal coverage to: (1) protect from future tooth tissue loss; (2) protect against caries; and (3) alleviate reported sensitivity. 9 MIH children frequently complain of extreme sensitivity to cold or sweet stimuli and even tooth-brushing. 10,11 Several clinicians have also observed that the sensitive nature of these teeth may complicate restorative management. 11,12 It appears that some hypomineralized teeth remain sensitive to instrumentation, despite the use of local anesthesia. Furthermore, it has been suggested that pain experience during dental treatment has led some MIH children to be significantly less compliant and more dentally anxious than their peers. 10

Interestingly, despite clinical observations that hypomineralized molars are sometimes difficult to anesthetize, no studies have attempted to identify the pathophysiological mechanisms underlying this problem. Jälevik and Klinberg, however, hypothesize that a subclinical pulpal inflammation in MIH teeth could lead to hypersensitivity. 10 Certainly the presence of porous enamel and exposed dentin may favour ingress of bacterial contaminants, thereby inciting pulpal inflammation.
inflammation. Tissue inflammation may, in turn, lead to a number of morphological and cytochemical changes within sensory neurons, resulting in sensitization of these nerve fibers.13,14

This study’s aim, therefore, was to carry out a quantitative assessment of pulpal innervation and inflammation in hypomineralized molars in order to gain some understanding of the pain-related symptoms associated with this condition.

Methods
The experimental material comprised both hypomineralized and sound (noncarious and unaffected by any enamel defect) permanent first molars obtained from children requiring routine dental extractions under general anaesthesia. Sound permanent first molars were occasionally extracted, together with 1 or more permanent first molars of poor prognosis, as part of an orthodontic treatment plan in which compensating extractions were indicated in certain clinical situations.15

Prior to tooth removal, children were interviewed to ascertain a simple pain history. A positive pain history was recorded for children who reported that they had regularly experienced sensitivity to hot, cold, sweet, or mechanical stimuli. A history was elicited for both sound and hypomineralized teeth. The South Sheffield Research Ethics Committee, a local branch of the UK National Research Ethics Service, granted ethical approval for the study and informed consent was obtained from all parents/guardians.

Immediately following simple forceps extraction, a groove was cut on the buccal aspect of each crown and the teeth were split longitudinally using an osteotome and surgical mallet. The teeth were then placed in fixative (4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer; pH 7.4) for 24 hours at 4°C. The coronal pulp was carefully removed and placed in phosphate-buffered saline (PBS) for 24 hours at 4°C before being placed in 4ºC before being placed in PBS containing 30% sucrose solution for cryoprotection (5 hours at 4°C). The pulp tissue was then embedded in Tissue-Tek OCT compound (Bayer Diagnostics, Basingstoke, UK), and 10-µm longitudinal sections were cut from each tooth pulp and collected on poly-D-lysine-coated glass slides.

Immunocytochemistry and lectin histochemistry. Immunostaining was performed using an indirect immunofluorescence method.17 Slides were first washed in PBS containing 0.2% Triton X-100 (Bayer Diagnostics, Basingstoke, UK), (PBST; 2 x 10 minutes) and then incubated in phosphate-buffered saline and triton (PBST) containing 10% normal goat serum (Vector Laboratories, Peterborough, UK) for 30 minutes at room temperature. Following this, sections were triple labelled using a mixture of:

1. a monoclonal antibody to protein gene product 9.5 (PGP 9.5)—a general neuronal marker (rabbit anti–human PGP 9.5, dilution 1:1000, Ultraclone, Isle of White, UK);
2. a monoclonal antibody to leukocyte common antigen (LCA)—a universal marker for leukocytes (mouse anti-human LCA, dilution 1:100, Dako, Bucks, UK); and
3. biotinylated Ulex Europaeus agglutinin I lectin (UEIL)—a marker of human vascular endothelium (dilution 20µg/ml, Vector, Vector Laboratories, Peterborough, UK).

The antisera and UEIL were diluted in PBST containing 5% normal goat serum, and sections were incubated for 24 hours at 4°C.

Before incubating, slides were then washed again in PBS (2 x 10 minutes) for a further 90 minutes at room temperature using a mixture of fluorescent secondary antibodies:

1. goat antirabbit IgG conjugated to fluorescein isothiocyanate (dilution 1:20, Vector);
2. horse antimouse IgG conjugated to Texas red (dilution 1:100; Vector); and
3. 7-amino-4-methyl-3-acetic acid–conjugated streptavidin (dilution 1:25, Vector).

The fluorescent labels were diluted in PBST containing 2% normal goat serum. Slides were finally washed again in PBS (2 x 10 minutes) before mounting with Vectashield (Vector).

Immunohistochemical controls for PGP 9.5 and LCA were performed by incubating sections with the antibody diluent alone. The specificity of the lectin reaction was tested by inhibiting lectin binding with the use of 0.2 M L-fucose (Vector) dissolved in PBS containing 0.2% PBST. No positive labelling was seen in any of the controls.

Analysis of labelling. Sections were viewed using a Zeiss axioplan fluorescent microscope, and all analysis was performed by one investigator (HDR). Slides were prepared and precoded such that the investigator was blinded as to the experimental subgroup. Three different fields were subject to quantitative analysis:

1. the mesiobuccal pulp horn;
2. the occlusal subodontoblastic region between the 2 pulp horns; and
3. the midcoronal pulp region (Figure 1).

The method used to quantify labelling has been described previously.16,18,19 Essentially, computer-assisted image analysis software (Image-Pro Plus v. 3.0; Media Cyber-
netics, Silver Spring, Md) was used to create a digital image from the microscopic image. The percentage area of staining (PAS) for PGP 9.5-, LCA-, and UEIL-labelled tissue was then automatically determined within each field of analysis.

Statistical analysis. One-way analysis of variance (ANOVA) was employed to test for statistically significant differences for PAS PGP 9.5, LCA, or UEIL according to the tooth status (sound, hypomineralized with intact enamel, and hypomineralized with enamel loss). This was followed by Tukey’s test for multiple pairwise comparisons of mean values. An independent t test was used to determine whether there was any significant difference in PAS PGP 9.5 (innervation density). Innervation density was significantly different according to tooth status in the pulp horn and occlusal subodontoblastic region (P =.023 and P =.028, respectively, ANOVA). Further pairwise analysis revealed that, in the pulp horn region, hypomineralized samples with intact enamel had a significantly greater innervation density than sound teeth (P <.047, Tukey’s test). In the subodontoblastic region, innervation density was significantly greater in the pulps of hypomineralized teeth with enamel loss than was the case for sound teeth (P <.038, Tukey’s test). There were, however, no significant differences in pulpal innervation between subgroups within the midcoronal region.

Immune cells. Leukocyte common antigen-immunoreactive (LCA–ir) cells were sparse or absent within the coronal pulp of sound samples (Figure 2e). There was, however, frequently a marked increase in LCA–ir cells within the pulps of hypomineralized teeth exhibiting enamel loss (Figure 2f). ANOVA revealed that there were significant differences in mean PAS for LCA in the pulp horn and midcoronal regions according to tooth status (P <.001 and P <.032, respectively, ANOVA). Further pairwise comparisons revealed that there were significantly more LCA–ir cells within the pulps of hypomineralized teeth with enamel loss compared to sound teeth or hypomineralized samples with intact enamel (P <.05, Tukey’s test; Figure 3b).

Pulpal vascularity. Labelling for UEIL clearly revealed the overall distribution and varying morphology of the pulp microvasculature. Analysis of pooled data for mean PAS UEIL confirmed that there were no significant differences in vascularity between sound and hypomineralized samples (Figure 3c).

Figure 1. Schematic diagram showing areas subject to quantitative analysis within the coronal pulp of sound and hypomineralized teeth.*

*1=pulp horn; 2=occlusal subodontoblastic region; 3=midcoronal region. E=enamel; D=dentin; P=pulp; BV=blood vessel; NT=nerve trunk; NF=nerve fibers.
Each area of analysis represents 0.22mm² of pulp tissue.

Figure 2. Innervation and immune cells in sound and hypomineralized teeth.

Figure 3. Vascularity of sound and hypomineralized teeth.
Findings in relation to pain history. For the 11 hypomineralized teeth from children who were considered to provide a reliable pain history, there were no significant differences in mean PAS for PGP 9.5 or LCA between reportedly sensitive (N=6) and asymptomatic (N=5) samples ($P > 0.05$, independent sample t test). There was, however, a significantly greater mean PAS for UEIL in sensitive hypomineralized samples (N=6) compared to asymptomatic hypomineralized teeth (N=5) within the:
1. pulp horn (mean PAS UEIL MIH asymptomatic= $0.82 \pm 0.66$; mean PAS UEIL MIH sensitive= $1.58 \pm 0.35$; $P = 0.013$; Figures 2g-h); and
2. midcoronal region (mean PAS UEIL MIH asymptomatic= $0.92 \pm 0.24$; mean PAS UEIL MIH sensitive= $1.86 \pm 1.04$; $P = 0.049$).

Discussion
This immunocytochemical study has shown, for the first time, that some noncarious hypomineralized molars have an underlying pulpal inflammation, as demonstrated by an increase in pulpal innervation density and immune cell accumulation. This histological presentation is similar to that described for carious permanent first molars in children of a similar age group.16,20 Children with hypomineralized molars frequently report an exquisite sensitivity to a variety of normally innocuous thermal, mechanical, and osmochemical stimuli, probably due to dentin hypersensitivity. This condition has been well described for adults, but not for children.21 Dentin hypersensitivity may arise from both an increased excitability of intradental nerves and from a more effective transmission of the stimuli to nerves.

The presence of exposed dentin (due to pre- and/or posteruptive enamel loss) and porous hard tissues in MIH leaves the dentin very vulnerable to oral stimuli. The hydrodynamic theory of dentinal fluid movement offers the most likely explanation for the resultant short sharp pain experienced.21 This pain results from the activation of high threshold mechanoreceptors (A fibres). Symptoms are likely to be exacerbated in young patients where the dentin is immature: Wide and patent dentinal tubules facilitate fluid movement and, hence, A-fiber stimulation.

Dentin sensitivity may be exacerbated by an underlying pulpal inflammation.22,23 Patients with hypersensitive dentin may perceive pain of an exaggerated intensity or longer duration than would

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Figure 2. Digital photomicrographs showing the differences in innervation density, immune cell accumulation, and vascularity in the pulps of sound and hypomineralized teeth.*

*(A) Protein gene-product 9.5 (PGP 9.5) labelling within the pulp horn region of a sound molar showing normal innervation density. (B) PGP 9.5-labelling within the occlusal subodontoblastic region of a sound molar showing normal innervation density. (C) PGP 9.5-labelling within the pulp horn region of a hypomineralized molar showing increased innervation density. (D) PGP 9.5-labelling within the occlusal subodontoblastic region of a hypomineralized molar (with no enamel loss) showing increased innervation density and neuronal thickening. (E) Triple-exposure photomicrograph of subodontoblastic region of a sound molar, with staining for PGP 9.5, leukocyte common antigen (LCA) and Ulex europaeus agglutinin I lectin (UEIL), showing an absence of immune cells. (F) Triple-exposure photomicrograph of the subodontoblastic region of a hypomineralized molar (with enamel loss), with staining for PGP 9.5, LCA, and UEIL, showing an accumulation of immune cells and increased innervation density. (G) Double-exposure photomicrograph of the pulp horn region of an asymptomatic hypomineralized molar with staining for PGP 9.5 and UEIL. (H) Double-exposure photomicrograph of the pulp horn region of a reportedly sensitive hypomineralized molar, with staining for PGP 9.5 and UEIL, showing enlarged pulpal blood vessels.
be expected to originate from a hydrodynamic stimulus alone. In MIH teeth, porous hard tissues and exposed dentin may predispose to pulpal ingress of bacteria and other oral irritants. Following tissue inflammation, a variety of morphological and cytochemical neuronal changes may occur.\textsuperscript{22-24} Changes include neuronal branching and altered expression of neuropeptides and ion channels. These features are all indicative of peripheral sensitization, whereby the threshold of neuronal activation is reduced and, on occasions, spontaneous neuronal discharge can occur.\textsuperscript{25} It has also been shown that persistent tissue inflammation and ongoing nociceptive input may be associated with central changes (central sensitization) within pain pathways.\textsuperscript{22,26} This may also, in part, explain the dentin hypersensitivity experienced by some patients.

Following administration of a dental local anesthetic, some MIH patients continue to experience pain on instrumentation or application of cold water or air. Failure to achieve adequate levels of pulpal analgesia may also be attributable to peripheral sensitization. The authors’ recent work in carious permanent first molars has shown significant changes in the expression of a number of different neuropeptides (notably substance P) as well as the thermal vanilloid receptor, TRPV\textsubscript{1}.\textsuperscript{18,19,27} In other models of tissue inflammation, significant changes in neuronal expression of the ionic sodium channels have been shown. Interestingly, an increased expression of tetrodotoxin-resistant sodium channels has been linked to hyperalgesia and altered sensitivity to local anesthesia.\textsuperscript{28,29} Further investigations into the expression of substance P, vanilloid receptors, and sodium channels would, thus, seem to be indicated in hypomineralized teeth.

This study found no correlation between overall innervation density and reported pain experience, which agrees with findings from a previous study on carious teeth.\textsuperscript{16} Interestingly, symptomatic MIH samples did show an increased pulpal vascularity. Dentinal stimulation may cause increased pulpal blood flow. This is likely to be due to an axon reflex, whereby stimulated intradental nerves release substance P and calcitonin gene-related peptide, which effect arteriolar dilation and increased capillary permeability.

One may hypothesize that the presence of these enlarged vessels indicates: (1) increased tissue fluid pressure; (2) a greater outward flow of dentinal fluid; and, thus (3) increased pain on dentinal stimulation.\textsuperscript{30} The present study’s findings relating to pain, however, should be viewed with

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\caption{Bar charts showing mean (±SEM) percentage area of (A) protein gene-product 9.5 (PGP 9.5) labelled tissue, (B) leukocyte common antigen (LCA) labelled tissue, and (C) Ulex Europaeus I Lectin (UEIL) labelled tissue within the pulp of sound and hypomineralized teeth.

* Significantly different from sound samples (\(P<.05\), Tukey’s test).
† Significantly different from hypomineralized teeth with intact enamel (\(P<.05\), Tukey’s test).
‡ Significant difference according to subgroup (\(P<.05\), analysis of variance).
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some caution at this stage due to the small sample. A further acknowledged limitation of the study relates to the reliability of the dental pain history obtained from the young subjects. This could be considered subjective and entirely reliant on individual recall. Consequently, further investigation is indicated with a larger sample and the use of objective sensory testing of the experimental teeth prior to extraction.

From a clinical perspective, this study’s findings would support early interventions to prevent the development of pulpal inflammation and associated hypersensitivity. One approach may be to promote the occlusion of dentinal tubules, using topical fluoride preparations or dentin bonding agents. Further clinical and laboratory investigations would seem to be warranted, however, to better understand and treat this common clinical condition.

Conclusions
Based on the immunocytochemical findings from this study, it appears that hypomineralized permanent first molars demonstrate changes in pulpal innervation, vascularity, and immune cell accumulation that are indicative of an inflammatory response.

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References
Space closure after extraction of hypomineralized molars

The purpose of this study was to evaluate the development of the permanent dentition and need for orthodontic treatment after extraction of permanent first molars due to severe molar-incisor hypomineralization (MIH). Thirty-three children who had 1 to 4 permanent first molars extracted due to severe MIH with a poor prognosis were eligible for this follow-up evaluation. The median age at the time of extraction for 27 children was 8.2 years (range 5.6–12.7). The median follow-up time was 5.7 years (range 3.8–8.3). An average of 2.6 molars per child were extracted. Fifteen children were judged to have a favorable spontaneous development of their permanent dentition without any orthodontic intervention. Seven children received or should have received orthodontic treatment for other reasons registered prior to the extraction. Five children were judged to require treatment as a result of the extractions, but three of them abstained because of no perceived treatment need. The authors concluded that spontaneous space reduction and favorable development of the permanent dentition can be expected when extracting a severely hypomineralized permanent first molar prior to the eruption of the second permanent molar.

**Comments:** Extraction of permanent first molars is a treatment option not only for hypomineralized teeth but also for the population that cannot afford extensive and expensive dental treatment. FMS

Address correspondence to Birgitta Jälevik, Specialist Clinic of Pedodontics, Sahlgrenska University Hospital Mölndal, SE-431 80 Mölndal, Sweden; e-mail: birgitta.jalevik@vgregion.se.


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