DOI: 10.1111/j.1365-263X.2011.01129.x

INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY

Hardness and microshear bond strength to enamel and dentin of permanent teeth with hypocalcified amelogenesis imperfecta

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International Journal of Paediatric Dentistry 2011; 21: 314–320

Background. Adhesive procedures are often required to restore teeth affected by hypocalcified amelogenesis imperfecta (HAI).

Aim. To evaluate the hardness of enamel/dentin of teeth affected by HAI and the bond strength to these substrates, as well the influence of 5% NaOCl on bond strength.

Design. Permanent molars presenting HAI and sound third molars were used. Enamel surfaces were wet-flattened and Knoop hardness was assessed. The two-step, etch-and-rinse adhesive Single Bond 2 (3M ESPE) was applied and resin cylinders bonded to the surfaces and submitted to microshear testing. The subjacent medium dentin was then exposed by wet-grinding. Hardness readings and microshear testing were carried out again. The relationship between hardness and bond strength was assessed by nonlinear regression analysis.

Results. Hardness of normal enamel was higher than hardness of enamel affected by HAI, whereas dentin hardness did not differ from normal to HAI-affected teeth. Enamel and dentin hardness were similar for teeth affected by HAI. Higher bond strengths were obtained to the normal tooth tissues. Dentin bond strength was higher than enamel bond strength. NaOCl exposure did not influence bond strengths. A positive linear relationship between enamel hardness and bond strength was observed.

Conclusion. HAI imposes challenges to bonding to enamel and dentin.

Introduction

Amelogenesis imperfecta (AI) is a heterogeneous group of hereditary disorders that may affect the enamel[1] of some or all teeth in the primary and/or permanent dentition. AI has been reported as an isolated finding with an autosomal dominant, autosomal recessive, or X-linked mode of inheritance.[2] The enamel defects are highly variable, ranging from deficiency in the structure to defects in the mineral and protein contents. Clinically, AI can be categorized into two main groups: hypocalcified and hypoplastic.[3] The former is mainly related to lower mineral content, whereas the latter is characterized by the presence of enamel defects, a thin enamel layer, or even absence of the enamel layer. Some authors also add hypomaturation as another type of AI,[3,4] this condition being an abnormality in the maturation stage, characterized by opaque enamel.[3] Occasionally, AI occurs in association with other features as part of a syndrome, such as amelo-onycho-hypohidrotic syndrome, Morquio syndrome, Kohlschutter syndrome, and trico-dento-osseous syndrome.[5,6]

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A significant clinical complication of the AI is the aesthetic issue related to the enamel appearance. The poor aesthetics results from surface roughness, staining, and abnormal crown shapes because of enamel loss.[7,8] Several treatments have been proposed for patients with AI such as the extraction of the compromised teeth and placement of a removable or an implant-supported fixed prosthesis.[9,10] This approach, however, is sometimes too invasive and presents high incidence of complications. A more conservative approach is the use of bonded composite restorations.[11]

The fundamental principle of bonding to dental hard tissues is based on micromechanical interlocking of the adhesive resin with the enamel and dentin.[12] While bonding to enamel depends on the micromechanical retention to the etched substrate,[13] bonding to dentin relies on hybridization with the exposed collagen mesh.[14] In etch-and-rinse adhesives, a conditioner (usually 30–40% phosphoric acid) dissolves the hydroxyapatite crystals and creates spaces for infiltration.[15] This has been a successful approach to bond to healthy enamel as it presents high mineral content. High failure rates have, however, been described for bonding to enamel affected by AI.[16] As the structure of the substrate has a key role on the performance of adhesive agents,[17] the lower mineral content for the enamel affected by hypocalcified AI might be deleterious to the bonding procedure.[18]

While bonding to normal teeth is extensively studied, however, the adhesive performance of teeth affected by hypocalcified AI has been seldom evaluated. Furthermore, little information is available on the structure of the dentin subjacent to enamel affected by hypocalcified AI. Thus, the aim of this study was to evaluate the hardness of enamel and dentin of permanent teeth affected by hypocalcified AI and the bond strength of an etch-and-rinse adhesive to these substrates. In addition, as it has been demonstrated that protein removal by exposure to sodium hypochlorite solution may improve the bond strengths to enamel with hypocalcified AI,[18] the effect of NaOCl application on the bond strength was also investigated.

Materials and methods

The research project was approved by the Ethical Research Committee of State University of Montes Claros, Brazil (protocol 1304/08). Five unerupted permanent molars were extracted from three patients presenting hypocalcified AI. The patients were members of the same family, including two twin sisters, having an autosomal recessive syndrome characterized by the presence of gingival fibromatosis, intrapulpal calcifications, and several unerupted teeth. Figures 1 and 2 show the clinical and radiographic phenotypes of one member of the family. The parents signed a free and informed consent form stating their willingness and freedom of choice to participate in the study.

Five sound third molars extracted from other patients, stored in 0.05% thymol saline solution for no more than 3 months, were used as controls. All molars were sectioned to their

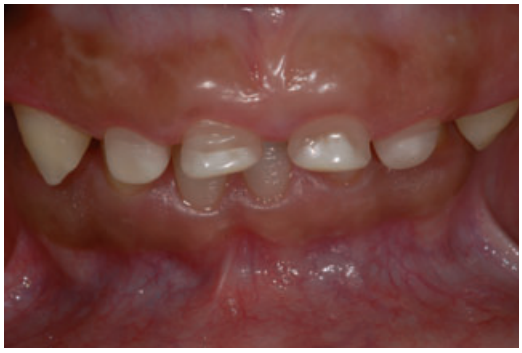

Fig. 1. Clinical phenotype of a patient diagnosed with hypocalcified amelogenesis imperfecta. Note the thin enamel layer in the upper incisors and defects in the cervical area.

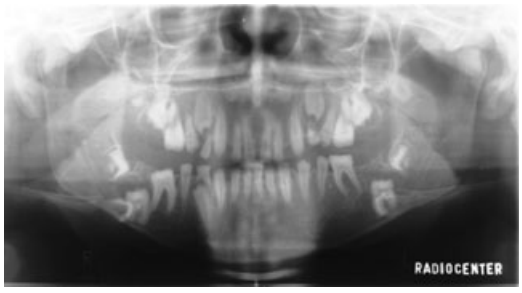

Fig. 2. Panoramic radiograph phenotype of the patient shown in Figure 1. Note the presence of several unerupted molars. Some of these were extracted and used in the present study.

mesio-distal axes, parallel to the long axis of teeth, using a slow-speed diamond saw under water cooling. Each half was embedded in acrylic resin with the buccal/lingual face exposed. The surface was slightly wet-ground with 1200-grit SiC abrasive paper to obtain a flat area in enamel. Three Knoop hardness indentations were made on the ground surface under a load of 50 g for a 10-s dwell time (HMV-2; Shimadzu, Tokyo, Japan). The Knoop hardness number (KHN, kgf/mm^2) for each specimen was recorded as the average of the three readings.

Half the number of hemi-sections was assigned to receive conventional bonding procedures, whereas the correspondent hemi-section of the same tooth was soaked in 5% NaOCl solution for 1 min before the adhesive procedure. The specimens treated with NaOCl were rinsed with water and air-dried. The two-step, etch-and-rinse adhesive system Single Bond 2 (3M ESPE, St Paul, MN, USA) was applied to all samples according to the manufacturer's instructions. The solvent was gently evaporated for 5 s using compressed air and the adhesive photoactivated for 10 s (XL3000; 3M ESPE, 600 mW/cm^2). Two polyvinyl tubes with a cylinder-shaped orifice (1 mm inner diameter × 2 mm height) were placed onto the surfaces, filled with resin composite (Filtek Z350; 3M ESPE), and photoactivated for 20 s. After 24 h, a shear bond test was conducted on a mechanical testing machine (DL2000; EMIC, São José dos Pinhais, PR, Brazil). A thin steel wire (0.2 mm diameter) was looped around each cylinder and the shear load applied to the base of the cylinder at a crosshead speed of 0.5 mm/min until failure. The average value of the two bonded cylinders for each tooth was recorded as the microshear bond strength (MPa) for that specimen.

After testing the bond strength to enamel, the surfaces of the same specimens were wet-ground with 600 and 1200-grit SiC abrasive papers until a flat surface in medium dentin was obtained. The specimens were ultrasonically cleansed in distilled water for 20 min. Knoop hardness readings were taken on dentin, following the same procedure described for enamel. Thereafter, the surfaces were wet-polished with 600-grit SiC abrasive papers to standardize the smear layer. The bonding procedures, resin cylinders built-up, and shear testing followed the same methods described for enamel. After the bond tests, the surfaces were evaluated under optical microscopy at 40× magnification to verify the failure modes.

Hardness data were analysed by two-way ANOVA and Tukey's test. The factors evaluated were 'presence of AI' and 'substrate'. Bond strength data were submitted to three-way ANOVA and Tukey's test. The factors evaluated were 'presence of AI', 'substrate', and 'NaOCl exposure'. The relationship between hardness and bond strength was assessed by nonlinear regression analyses with bond strength as dependent variable. All analyses were conducted at a significance level of $P < 0.05$.

Results

Results for hardness are shown in Table 1. The factors 'presence of AI' and 'substrate' were both significant, as well was their interaction ($P < 0.001$). The power of performed test was 1 for all conditions. Hardness of normal enamel was significantly higher than hardness of enamel affected by AI ($P < 0.001$), whereas

Table 1. Means (SD) for hardness (KHN, kgf/mm^2).

Hypocalcified AI	Enamel	Dentin
Present	53.3 (13.7)^{A,b}	57.2 (9.5)^{A,a}
Absent	360.4 (89.5)^{A,a}	51.1 (5.2)^{B,a}

KHN, Knoop hardness number; AI, amelogenesis imperfecta.
Means followed by distinct capital letters in the same row, and distinct small letters in the same column, are significantly different at $P < 0.05$.

Table 2. Means (SD) for bond strength (MPa).

Hypocalcified AI	Enamel	Dentin
Present	14.2 (4.8)^{B,b}	24.6 (5.4)^{A,b}
Absent	24.0 (7.6)^{B,a}	30.3 (6.4)^{A,a}

AI, amelogenesis imperfecta.
Distinct capital letters in the same row indicate differences between enamel and dentin. Distinct lowercase letters in the same column indicate differences between normal and affected tissue.

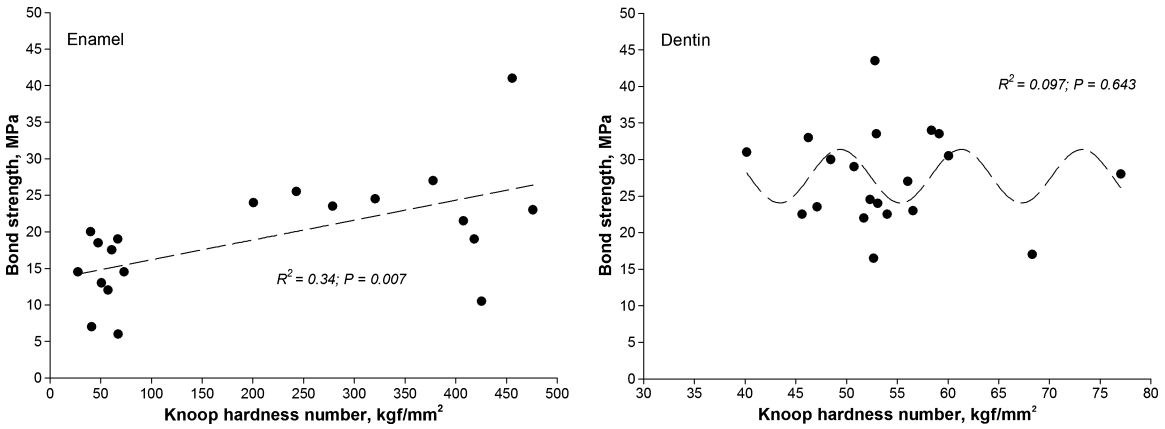

Fig. 3. Non-linear regression plots with bond strength as dependent variable. The relationship between hardness and bond strength followed a significant positive linear behavior for enamel, while the model for dentin was not significant, showing a waveform behavior.

dentin hardness did not differ from normal to AI-affected teeth ($P = 0.308$). Comparing enamel and dentin, both substrates showed similar hardness for teeth affected by AI ($P = 0.348$), whereas enamel hardness was significantly higher than dentin hardness for normal teeth ($P < 0.001$).

Results for bond strength are shown in Table 2. The factors 'presence of AI' and 'substrate' were both significant ($P < 0.001$), whereas the factor 'NaOCl exposure' ($P = 0.992$) and any interaction between the three factors ($P \geq 0.118$) were not significant. Therefore, bond strength data in Table 2 are presented disregarding the factor 'NaOCl exposure' in order to compare only the factors that were statistically significant. Comparing the bonding to normal *versus* AI-affected teeth, significantly higher bond strengths were obtained to the normal tooth tissues ($P < 0.001$). When comparing the substrates, the dentin bond strength was significantly higher than the enamel bond strength for both the normal and affected teeth ($P < 0.001$). Mixed modes of failure were detected for all conditions.

Results for the nonlinear regression analysis are shown in Fig. 3. The relationship between hardness and bond strength followed a significant positive linear behaviour for enamel ($R^2 = 0.34$; $P = 0.007$). In contrast, the model for dentin was not significant, following a waveform behaviour ($R^2 = 0.097$; $P = 0.643$).

Discussion

The present results showed that the enamel affected by hypocalcified AI presented similar hardness values to the underlying dentin and significantly lower values as compared with normal enamel. These findings are in line with the results of a previous study[19] and might be explained by the lower mineral content of enamel affected by hypocalcified AI. The enamel of normal teeth is a highly mineralized tissue containing large crystals organized in a prismatic structure. The formation of this structure is believed to occur under rigorous control by ameloblasts through interaction with a number of organic matrix molecules.[1] The initial stage of enamel development is characterized by the secretion of a protein-rich, partially mineralized matrix. During maturation, this matrix is removed by proteases with associated growth of hydroxyapatite crystals until the enamel reaches its final hardened state.[1,20] Genetic mutations may result in the hypocalcification of enamel; the altered tissue shows incomplete biomineralization[1,4,5,20] and thus lower hardness. El-Sayed *et al.*[20] reported approximately 40% of mineral reduction on enamel affected by hypocalcified AI.

No significant differences in hardness were observed for normal dentin and dentin underlying hypocalcified enamel. A recent study[20] has described that the underlying dentin of primary teeth with AI preserves its

normal architecture, which would explain the similar hardness. In contrast, Sánchez-Quevedo *et al.*[21] reported that the subjacent dentin may present higher calcium levels in response to the enamel disorder, with the altered dentin showing a morphological pattern similar to sclerotic dentin. As the hypocalcified enamel is more porous, the continuous irritation may permit the stimulation of odontoblasts, resulting in dentin sclerosis. This alteration could increase the mineral content on the surface and lead to the occlusion of dentin.[22] In their study, Sánchez-Quevedo *et al.*[21] used erupted molars, contrasting with the unrupted molars used in the present investigation. This may explain the differences in findings between the studies.

The enamel affected by AI presents the loss of normal architecture, the prisms being incompletely formed, sometimes with the presence of an abnormal amorphous material obscuring the rods.[20] On the other hand, the dentin may be more highly mineralized, potentially presenting occluded tubules. These characteristics might explain the significantly lower bond strengths obtained to enamel and dentin of teeth affected by AI. The deproteinization bonding technique was also evaluated in an endeavour to improve the bond strengths. This technique was initially proposed to remove the exposed collagen mesh in the etched dentin, reducing the technique sensitivity and improving the stability of the dentin bonds.[23] As a discrepancy between the depths of dentin demineralization and resin infiltration has been associated with the use of etch-and-rinse adhesives, the unprotected collagen at the base of the hybrid layer may act as an area of stress concentration and increase the susceptibility to water degradation.[24,25] Exposure to NaOCl, however, did not have a significant effect on the bond strength to enamel or dentin, for either normal or affected teeth. This suggests that the organic content of the enamel was not high enough to interfere with the bonding ability. Although Saroğlu *et al.*[18] have reported that NaOCl was able to enhance the bonding to hypocalcified enamel, the authors used primary molars in their study, whereas permanent unrupted molars were used in the present investigation.

As the mineral content of dental tissues is both related to their hardness and potential micromechanical interlocking with bonding agents, one could expect a positive relationship between hardness and bond strength. This relationship has been evaluated before for normal or caries-affected substrates, with conflicting results reported in the literature. Adebayo *et al.*[26] detected that the bond strength of self-etch adhesives was not influenced by enamel hardness. In contrast, Wei *et al.*[27] reported a linear relationship between these two variables for both etch-and-rinse and self-etch systems. To the authors' knowledge, this is the first time the relationship between surface hardness and bond strength is reported for teeth affected by AI. For enamel, a significant linear relationship was detected, whereas the same behaviour was not observed for dentin. This finding is probably related to the fact that characteristics other than hardness have a significant impact on the dentin bond strengths, such as the dentin depth and area occupied by dentinal tubules *versus* intertubular dentin.[12]

The higher mineral content of enamel is expected to generate a better mechanical interlocking with the adhesive resin as compared with the dentin substrate, enhancing the enamel bond strengths.[12] In the present study, however, the bond strength to normal dentin was significantly higher than to normal enamel. Although this result has probably no critical clinical implications, as high shear bond strengths were obtained to both normal substrates, it might be related to the grounding procedures used to flat the surfaces for the bond test, as it has been shown that the enamel prism orientation might interfere with the bond strength results,[28] or even to the evaporation protocol for solvent removal. This result also indicates that current adhesive formulations may equally bond to enamel and dentin[29,30] provided that the bonding procedures are correctly applied.

Under clinical conditions, the structural alterations of AI-affected teeth may impose challenges to the bond of adhesive restoratives. As a linear relationship between enamel hardness and bond strength was detected, and the bond strength to dentin was higher than enamel bond strengths, it

seems that the maintenance of the disordered enamel is not crucial. Removal of the hypocalcified enamel exposing the underlying dentin may be an interesting approach to improve the performance of bonded restorations. Notwithstanding, further clinical results are necessary to investigate the best approach to bond to teeth affected by AI.

What this paper adds

- Hardness of enamel affected by hypocalcified amelogenesis imperfecta is lower compared with normal enamel, whereas the subjacent dentin shows no alteration in hardness;
- The bond strength to permanent teeth affected by hypocalcified amelogenesis imperfecta is lower than that to sound teeth;
- There is a linear relationship between hardness and bond strength to enamel;
- Exposure to 5% NaOCl solution before the adhesive procedure does not improve bond strength.

Why this paper is important to paediatric dentists

- Hypocalcified amelogenesis imperfecta imposes challenges to the bond of adhesive restorations;
- A continuing study on the alterations of teeth affected by amelogenesis imperfecta may help in developing longer-lasting adhesive restorations.

Acknowledgements

The authors thank the family for their participation. This work was funded by FAPEMIG (grant APQ-00234-09) and CNPq.

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