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Deviations of inorganic and organic carbon content in hypomineralised enamel

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ABSTRACT

Objectives: The purpose of this study was to discriminate hypomineralised enamel of permanent first molars from normal enamel by means of spectroscopic methods.
Methods: The present study was conducted using Multi spot Raman Fourier Transform Spectroscopy, Diffuse Reflectance Infrared Fourier Transform Spectroscopy (FTIR) and X-ray diffraction (XRD).
Results: Raman-spectroscopy indicated significantly more B-type carbonate and hydrocarbons in hypomineralised enamel diagnosed as MIH (Molar Incisor Hypomineralisation). From XRD analysis, no changes in crystallinity of the enamel apatite could be found.
Conclusions: Using multi spot Raman-spectroscopy, a significant molecular discrimination Q3 between normal and hypomineralised enamel could be made.

1. Introduction

The condition Molar Incisor Hypomineralisation (MIH) is a developmental disturbance of the enamel in permanent first molars and incisors seen as a Hypomineralisation of the enamel. It has a reported prevalence between 2.8% and 37.5%.[1–3] MIH is associated with a number of subjective and objective problems and complications, such as dental fear and anxiety, severe loss of enamel, hypersensitivity, increased treatment need and problems in performing proper filling therapy.[4–6] Clinically, it is difficult to estimate the degree of Hypomineralisation and the risk for loss of enamel. However, it has been shown that there is a relation between hardness values and the colour of the hypomineralised enamel, with yellow lesions being softer, i.e. with a higher degree of porosity, than white lesions.[7] Thus, while variation in porosity between normal enamel specimens is small, porosity in hypomineralised enamel specimens can vary from a few percent to more than 5%. However, neither hardness nor porosity is very specific for diagnosing MIH.

The microstructure of the hypomineralised enamel (HME) in permanent teeth diagnosed MIH has been described in several microscopy and spectroscopy based studies as having less distinct prism sheaths and disorganised enamel with lack of organisation of the enamel crystals. The mechanical

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properties, i.e. the hardness and modulus of elasticity, generally have lower values compared with normal enamel.[8–12]

Elemental analyses of enamel diagnosed MIH shows a profound reduction in the mineral content, together with an increase in organic constituents, i.e. the organic matrix.[13,14] The organic matrix, which in fully developed normal human enamel constitutes around 4% by volume of the enamel, is composed of at least two major types of enamel-specific matrix proteins, i.e. amelogenins and enamelins. Amelogenins are proline-rich, hydrophobic proteins and constitute approximately 90% of the matrix proteins prior to maturation. Amelogenins have been suggested to regulate the mineralisation of enamel and experimental inhibition of amelogenin translation has been found to result in interference of crystal growth and orientation. More specifically, amelogenin has been proposed to undergo self-assembly into nanospheres,[15] possibly with the purpose to guide the crystallisation process into long thin crystallites.[16]

The present study draws on a preceding microscopic and spectrometric study in which significantly higher concentrations of magnesium and lower concentrations of chloride ions was found in hypomineralised enamel compared to normal enamel.[17] Bicarbonate ions are known to exchange across the ameloblast plasma membrane for chloride ions, and a situation with low concentration of chloride ions in the matrix could therefore indicate higher concentration of bicarbonate ions in the matrix. Furthermore, earlier studies on the organic carbon content in normal versus hypomineralised enamel are inconclusive, i.e. supporting higher as well as lower median concentrations in hypomineralised enamel.

We hypothesise that the content of inorganic carbon (bicarbonate) and organic carbon, i.e. matrix proteins such as amelogenin is higher in hypomineralised enamel compared to normal enamel. The aim of the present study is to confirm the hypothesis by means of Raman and infrared spectroscopy.

2. Material and methods

2.1. Tooth material

Five permanent first molars with the diagnosis MIH, extracted from five patients in Gothenburg, Sweden were used for Raman and infrared analyses. After 24 h in 70% ethanol, the teeth were embedded in an epoxy-resin (Epofix®, Electron Microscopy Sciences, Fort Washington, PA, USA) and cut sagittally bucco-lingually in two halves in a Leica SP1600 Saw Microtome (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). From one of the halves, sections with a thickness of approximately ≈120 μm, were prepared in the saw microtome. The central sections through the one of cusps were used for histological examination.

2.2. Preparation of enamel powder

For complementary infrared and XRD analyses, milligrams of enamel powder of the outermost layers of hypomineralised and normal enamel were collected from a fifth extracted permanent first molar using a diamond bur. In order to distinguish hypomineralised enamel from normal, the preparation was performed under a stereo microscope (Leica M80 with 8:1 zoom, 0.75x–6x, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany).

2.3. Light and polarised light microscopy

All sagittal and radial sections were examined in a Leica M80 stereo microscope in incident light with a matt black background. Digital images were taken of all sections using a Leica digital camera (Leica DFC420C, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) equipped with Leica Application Suite LAS V3.7.0 (Leica Microsystems AG, Heerbrugg, Switzerland). All sections were examined in an Olympus polarised light microscope (Olympus, Tokyo, Japan), according to previously described routines.[18]

2.4. Morphological and crystalline identification

X-ray diffraction (XRD) was used for identification of the crystalline mineral phases in dental enamel powder. The XRD investigations were performed using a Bruker d8 Advance instrument in θ–θ mode, with an optical configuration involving primary and secondary Göbel mirrors. The sample foils were mounted on a rotating low-background Si-single-crystal sample holder. Continuous scans at a rate of 1 degree/min were applied. By adding repeated scans, the total data-collection time for each sample amounted to at least 15 h. A Fourier smoothing was applied to the scans and the background was removed. Analyses of the diffraction patterns were done by Bruker software together with the PDF2 databank.

2.4.1. Scanning electron microscopy (SEM)

One of the samples was etched with phosphoric acid for 45 s, carefully rinsed with de-ionised water and cleaned in an ultrasonic bath. The surface was coated with gold by vapour deposition and SEM images of normal and hypomineralised enamel were taken in a Hitachi VP-SEM S-3400 N.

2.5. Spectroscopic studies of enamel surfaces

In a molecule, the relative positions of the atoms are not fixed but fluctuate continuously as a consequence of different types of vibrations. A molecule can absorb infrared radiation if the radiation has the same frequency as one of these fundamental vibrational modes of the molecule. Raman spectroscopy relies on inelastic scattering, or Raman scattering, of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the vibrational modes in the system. The two methods provide complementary information.

In this study, Raman spectroscopic studies were not invasive, whereas infrared spectroscopic studies required pulverisation of the enamel sample.

2.5.1. FT-infrared spectroscopy

The spectrometer used was a Nicolet Magna-IR 560 equipped with a KBr beam splitter, a deuterated triglycerine sulfate

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