Altered levels of inorganic elements and carbon in both dentin and enamel in primary teeth from patients with osteogenesis imperfecta

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Running title: inorganic elements in osteogenesis imperfecta primary teeth

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Abstract
Osteogenesis imperfecta (OI) is an inherited disorder of collagen which might manifest in dentin. OI is due to a collagen mutation where glycine is substituted by larger amino acids disturbing collagen fibril coiling. The defect collagen might impair dentin mineralization. As OI is a collagen disturbance, a majority of publications has focused on the organic components of tissues. The inorganic elemental deviation pattern in OI type I is not very well understood, especially not in dental tissues. The aim of this study was to analyze the elemental content pattern in dentin and dental enamel for 12C, 23Na, 24Mg, 31P, 39K, 77CaCl and 88Sr, by means of Secondary Ion Mass Spectrometry (SIMS) in addition to X-Ray Micro
Analysis (XRMA) of Ca, P, O and C. The relative importance of critical elements for the outcome OI was analyzed using rule induction analysis. The conclusion from this study is striking differences in element composition of dental tissues from OI patients. Alterations in hydroxyapatite constituents Ca, P and O, together with carbon, were found in dentin as well as in enamel. Findings included elevated levels of phosphorus, calcium and magnesium in DI dentin. Oxygen levels in DI dentin were lowered, and fluorine levels in the central parts of enamel were also lowered. In enamel, carbon and chlorine were elevated. Rule induction analysis revealed elevated tissue levels of carbon to be obligate for the outcome OI in both dentin and enamel. These changes probably reflect aberrations in matrix mineralization during early odontogenesis. Changes observed in enamel are probably due to indirect effects of imperfect mesenchymal - ectodermal interactions during early tooth formation.

Key words: osteogenesis imperfecta, primary teeth, dentin, enamel, inorganic, carbon, element, secondary ion mass spectrometry, X-ray microanalysis
Introduction

The heritable bone disease osteogenesis imperfecta (OI) is in more than 90% of all cases caused by mutations in the collagen type I pro-α1(I) (COL1A1) and pro-α2(I) (COL1A2) chain genes where the mutation causes glycine to be substituted by larger amino acids [1-3]. Before the molecular basis of OI was understood, the disease was classified into four types based on clinic, inheritance and radiographic findings [4]. Today, this classification has expanded to at least seven types due to, i.a., molecular genetic analyses [5]. The phenotype varies from mild to lethal. OI can be associated with or without dentinogenesis imperfecta (DI). In OI the expression of DI is present in a continuum from subclinical to severe dentin pathology [6]. Clinic and histology of DI is not always clearly correlated to specific types of OI, even though caused by the same collagen mutation [7].

By volume, dentin is the dominating dental tissue. Approximately 70% of the dentin is mineralized. The unmineralized organic part accounts for 20%, and water 10%. As in bone, the primary organic component of dentin is collagen type I, the remaining part constituting different non-collagenous proteins where dentin phosphoprotein and dentin sialophosphoprotein (DSPP) predominate [8, 9].

Just as OI, DI is inherited in an autosomal dominant mode, affecting dentinogenesis. DI is subclassified into three types [10]. The least severe is type I, being associated with the collagen defect in OI. Type II is attributed to mutations of the DSPP gene [11-13]. The most severe form, type III, also being caused by a DSPP mutation, was first identified in the Brandywine triracial isolate population in Maryland, USA [9, 10, 14-17]. The incidence of DI is 1:6000 to 1:8000 [10, 16, 17]. Varying grades of structural dentin defects with small pulp chambers and radiographic findings are found in either, or both, the primary and permanent dentitions [10, 16, 17]. The dental aberrations, often more prominent in the primary dentition, comprise atypical dentin with irregular tubules, remnants of capillary inclusions, obliterated pulps and irregular mineralization, in addition to deviations in association with the dentin-enamel junction [16-19]. Apart from clinical and histological aberrations some biochemical features have been reported in OI. In OI type I a higher mean (7%) mineralization density has been revealed [20]. Contents of magnesium, fluoride and sodium in bone in an OI-mouse model were found to vary significantly, in addition to a significant difference in the magnesium content in teeth [21]. The Ca/P-ratio in OI-bone has been reported to decrease. It has been suggested that this decrease is due to a mineral composition deviation of the carbonapatite normally found in bone [21-23]. Reports on biochemical features of DI-dentin are very scarce. In contrast to OI-bone, DI-dentin has been reported to have a loss in both Ca, P and Mg, and having a higher Ca/P-ratio [24].

As a consequence of defect connective tissue proteins in bone, dentin, and indirectly enamel, this might
influence the mineralization process [11, 12, 25]. As OI is a connective tissue disorder, most publications have focused on the organic components of mineralized tissues, in addition to morphology and chemo-topographical patterns [16-19]. The pattern and composition of inorganic components, however, is not very well understood. Whether the mineralization processes in bone and dentin with defects in their organic matrices are similar, is not known. The aim of this study was to reveal elemental composition, relative abundance, and interrelationships for some critical inorganic elements in primary dentin, and enamel, in primary teeth from patients with OI, by means of elemental analysis and rule induction analysis.

Materials and methods
Eight primary teeth with no clinical signs of DI, from 7 patients with OI types I, III and IV were used for analysis. As controls, 36 primary teeth from healthy patients were used. After storage in 70% ethanol the teeth were embedded in epoxy-resin (Epofix®, Electron Microscopy Sciences, Fort Washington, USA) un-demineralized sagittal sections with a thickness of 110 µm from the tooth crown midline were cut in a Leitz Low Speed Saw Microtome (Leitz®, Wetzlar, Germany). For orientation all sections were analyzed in a polarization microscope in order to identify the areas for elemental analysis. Sections obtained from the tooth crown midline were chosen for analysis.

Secondary Ion Mass Spectrometry (SIMS) - In the SIMS analysis F, Na, Mg, K, Cl, Sr, as well as 44Ca (Table 1) were measured at 10 locations along a line through dentin and enamel with gradual steps of 50 µm (Fig. 1). The measurements were performed in a Cameca IMS 3F ion probe [25]. The specimen surfaces were bombarded with O- to emit secondary ions, which were separated according to mass/charge ratio. The diameter of the analyzed area was 10 µm. Both positive and negative secondary ions were recorded. All data for each element mass number were normalized to the mass number of the calcium isotope ^{44}\text{Ca} in order to compensate for intensity fluctuations due to mineralization irregularities on the sample surface. For each measurement the total ion yield was 100%, i.e. values for separate elements are semi-quantitative.

X-Ray Microanalysis (XRMA) and scanning electron microscopy (SEM) - Sections for SEM were etched for 45 seconds with 30% phosphoric acid, carefully rinsed with de-ionized water, and coated with gold by vapor deposition. Sections for XRMA-analysis were coated with carbon. The SEM and XRMA analyses were performed in a Philips SEM 515 at 12 kV; EDAX DX4, ECON-detector (Philips, Eindhoven, The Netherlands) [26, 27]. In the XRMA measurements C, Ca, P, and O (Table 1) were analyzed in 10
measurement points in enamel and dentin along a line (Fig. 1). For all measurements, emitted X-rays were detected while continuously fast scanning a small window of 6.1 µm x 4.3 µm at a magnification of 650 x. The relative amounts of C, Ca, P, and O are considered semi-quantitative.

**Rule induction analysis** – In order to reveal patterns in the inorganic composition of dental tissues a rule inductive analysis was performed. The XRMA data for C, O, P and Ca were imported to an inductive analysis program spread sheet (XpertRule Analyser®, Attar Software Ltd., Lancashire, UK) [28-30]. Numerical data from each element (attributes) were set in separate columns, each row representing measurements from separate patients. In a separate column the discrete diagnosis variables OI or Control (outcome) were entered. The results are presented in hierarchic diagrams (knowledge trees) in which the importance of every attribute is specified by its position and level in the knowledge tree. The higher in the tree, the more important for the outcome. In the rule induction process a knowledge tree is generated by repeatedly splitting the given data set according to different attributes until terminal points (leaves) are reached. The accuracy of the knowledge tree may be validated against a test data set. The program randomly selects 50% of the data for induction of rules (training) while the remaining 50% is used in the verifying process (test). In the Verify option in Analyser a table is displayed showing the accuracy of each leaf by comparing the probabilities of the leaf outcome in the training and testing data, which is expressed as correctly classified percentage. The inductive analysis was carried out in all five locations in the enamel and in all five in the dentin, respectively. In the hierarchical trees the shortest way to the outcome OI with highest level of probability, most often being 100%, was identified. These ways through the trees are compiled for all measurements in. Breakpoint values are stated for every element. Analyzed attribute groups, the elements, are specified in the left column of Table 2 and Table 3. The last attribute group (Na Mg Cl K Sr) is similar to the last but one group, except for F, which is excluded. The levels in the trees are stated for measurement locations 1 to 5 within enamel and dentin, respectively.

The Mann-Whitney U-test of medians was used for statistical analyses to analyze possible differences between DI-teeth and control teeth for both SIMS and XRMA.

**Results**

*Sodium and potassium*

In OI the levels of Na were lower in enamel, being significantly lower (p < 0.05) in the midpart. In OI-dentin the Na-levels were higher, with a significant difference (p < 0.05) close to the enamel-dentin
The K-levels were lower in OI-enamel. In OI-dentin the K-levels were higher, with a significant difference \((p < 0.05)\) in the midpart. (Fig Na K 1). For both Na and K the surface levels in OI-enamel were higher than in controls.

**Magnesium, calcium and strontium**

In OI the levels of Mg were lower in enamel. In OI-dentin the Mg-levels were higher, with significant differences \((p < 0.05)\) in the midpart and close to the enamel-dentin junction (Fig Mg 1). The Ca-levels were similar to controls in OI-enamel. The Ca-levels were significantly higher \((p < 0.05 - 0.01)\) in the mid part of OI dentin (Fig Ca 2). In both enamel and dentin the levels of Sr were elevated in OI, being significantly higher \((p < 0.01)\) at the enamel surface (Fig Sr 3).

**Carbon, phosphorus and oxygen**

In OI the levels of C were significantly higher \((p < 0.05)\) at the surface and in the mid parts of enamel. In OI-dentin the C-levels were higher, with a significant rise \((p < 0.05)\) close to the dentin-pulp Interface (C 1). The P-levels were similar to controls in OI-enamel. In OI-dentin the P-levels were significantly higher at the enamel-dentin junction \((p < 0.01)\) and in the mid part \((p < 0.05 - 0.01)\). (P 2). In both enamel and dentin the O-levels were lowered in OI, with significant differences at the enamel-dentin junction \((p < 0.05 - 0.01)\) and throughout the dentin \((p < 0.001)\) (O 3).

**Fluorine and chlorine**

In OI the levels of F were lower in enamel, being significantly lower \((p < 0.05)\) in the mid part. In OI-dentin the F-levels were lower, being close to similar to controls at the dentin-pulp interface (Fig F 1). The Cl-levels were increased in OI-enamel, with significant differences in the mid part \((p < 0.05 - 0.01)\) and at the enamel-dentin junction \((p < 0.05)\). In OI dentin the Cl-levels were higher with a significant rise in the dentin mid part \((p < 0.05)\) (Fig Cl 2).

**Calcium/phosphorus-ratio**

The Ca/P weight % ratio was practically identical in all locations in normal and OI-teeth for both enamel and dentin except for close to the enamel-dentin junction in dentin (Fig Ca/P).

**Rule induction analysis**

For the attribute group C,O,P, Ca, measured by means of XRMA, a uniform pattern was seen in enamel. The outcome OI could be identified with 100% probability already after three breakpoint branchings in
the trees. An increase in C, in addition to a decrease in O was seen in several locations in the top level. Increased levels of Ca were found in level two. A similar pattern was identified for the attribute group Ca/P, Ca/C, P/C. In the top level a lowered P/C ratio was found. In levels two and three elevated levels of the Ca/C- and Ca/P-ratios were seen. For the attribute group F Na Mg Cl K Sr, measured by means of SIMS, a concordant pattern was found. In levels one and two elevated levels of Cl and lowered levels of F were observed. If F was excluded as an attribute (last attribute group) elevated Cl levels were still seen, in addition to elevated levels of Na (Table induct enamel).

In dentin a less distinct pattern was seen for the attribute group C,O,P, Ca. The outcome OI could be identified with 100% probability after at most four breakpoint branchings in the trees. In the top levels a decrease in O, in addition to an increase in P was seen. Increased levels of C were found higher in the tree. For the attribute group Ca/P, Ca/C, P/C, a less uniform pattern was found, with elevated levels of both P/C and Ca/C, in addition to lowered levels of Ca/P. For the attribute group F Na Mg Cl K Sr, a concordant pattern was found. In the top levels, an increase in K and decrease in F was observed. If F was excluded as an attribute (last attribute group) lowered levels of Na were seen (Table induct dentin).

Discussion
The main findings of this study were elevated levels of phosphorus, calcium and magnesium in DI dentin. The levels of oxygen in OI dentin were lowered, and fluorine in the central parts of DI enamel was also lowered. In enamel from OI patients the levels of carbon and chlorine were elevated.

Primary teeth from patients with OI, with or without DI, have previously been described from a histomorphological point of view [7, 16-19]. Most morphological aberrations have been observed in teeth from patients with OI type III and IV. This study has, by means of SIMS, XRMA and rule induction analysis, demonstrated critical differences in elemental composition in both dentin and enamel in primary OI-teeth. The differences observed in enamel are suggested to be indirect effects. During odontogenesis, secretion of predentin and enamel matrix and initial crystal formation are closely coupled, in space and time [31]. As both dentin and enamel form as a result of a series of mesenchymal – ectodermal interactions [32], deficiencies in one tissue, e.g. dentin collagen, might also affect the other, enamel, due to these interactions.

In the hydroxyapatite crystal lattice of dentin and, especially, enamel, a number of inorganic elements are found as elemental inclusions. Some of these elements might reflect events during the hard tissue
formation where huge amounts of ions are transported through the odontoblast or ameloblast cell layers. One possible role for some of the different inorganic elements, other than Ca\(^{2+}\) and PO\(^{4-3}\), might be as co-transport ions in plasma membrane transporters of the mineralizing cells [33]. Sodium, e.g., is transported in parallel to phosphate in many cell types. Other explanations for the presence of non-Ca\(^{2+}\) non-PO\(^{4-3}\) elements might be poor selectivity of cell ion channels, or as catalysts for lowering the activation energy during formation of hydroxyapatite.

Measurements of the inorganic composition of dental tissues have previously been performed utilizing several different techniques. All techniques have advantages and possible limitations. Data obtained with different methods must be interpreted and compared with care. Also the teeth chosen for analysis must be handled, with care. As an example, it has been shown that country of origin has an important influence on the elemental composition of dental tissues [34]. Secondary ion mass spectrometry (SIMS) technique has been used for more than 20 years for such analyses. By means of SIMS, consistent patterns of inorganic elements from dental enamel and dentin have been shown [18, 19, 26, 27]. Dental enamel and dentin consist of great extents of the calcium phosphate salt hydroxyapatite. A high sensitivity of X-ray micro analysis (XRMA) for the hydroxyapatite constituents Ca, P, O, in addition to C, makes this technique a suitable complement to SIMS [26, 27, 35].

Fluorine is found in all mineralized tissues. The role of fluorine during dental enamel formation is still not fully elucidated. Its presence during matrix mineralization, however, introduces significant changes in the dental tissue chemical composition [36, 37]. It is known that fluorine might induce calciumphosphate salt formation without being incorporated itself [38]. Fluorine has earlier been demonstrated to have a high degree of co-variation with potassium [28]. As K has been suggested to be involved in initial apatite formation [39], this might reflect the role of F in the same process. In OI, the only differences were found in the enamel midpart with significantly lower levels (Fig F 1). Thus, fluorine gives no clues to the origin of the altered calcium and phosphate composition in OI dentin. Together with sodium chlorine is a component of the abundant NaCl. The chlorine levels in OI-enamel increased significantly in the midpart and at the enamel-dentin junction. Chlorine has previously been reported to be an element with a high degree of co-variation with fluorine in the enamel surface [29], in addition to a high degree of co-variation with sodium [28]. In this study the levels of both F and Cl were highest in surface enamel, probably reflecting this. In OI-dentin the chlorine levels were significantly higher in the midpart (Fig Cl 2). As a comparison, elevated levels of chlorine have been reported in newly mineralized dentin, close to the predentin [40].

Sodium, together with potassium, takes part in the maintenance of the cellular plasma membrane potential. Sodium is the most abundant ion in the body. In every cell, including dentin- and enamel
forming cells, sodium is continuously extruded in parallel to a cellular uptake of potassium. In bulk enamel Na has previously been reported to have a high degree of co-variation with phosphate [29]. When odontoblasts and ameloblasts handle large quantities of calcium and phosphate for matrix mineralization, they also handle considerable amounts of sodium and potassium. Initial apatite formation happens close to sodium and potassium ion fluxes. High levels of K have previously been reported in predentin, close to the mineralization front [40]. Initial dentin formation has been suggested to be “triggered” by, i.e., potassium. It is speculated that local increases in concentration of monovalent potassium may act as an activator of predentin matrix mineralization. At the mineralizing front, micro-areas with a strong co-enrichment of phosphate and potassium are found. During the beginning of the calcium enrichment and the subsequent apatite mineral formation, the content of potassium decreases significantly [39]. In OI-dentin the K-levels were higher, with a significant difference in the midpart, (Fig Na K 2) perhaps reflecting insufficient transformation of phosphate into hydroxyapatite. A similar mechanism for sodium in dentin is suggested. In OI-dentin the Na-levels were higher with a significant difference close to the enamel-dentin junction (Fig Na K 1). A similar role for enamel mineralization has not been demonstrated.

Magnesium and strontium are elements closely related to calcium. These neighbors in the periodical system are divalent cations. The elements have earlier been shown to have a high degree of co-variation in enamel [28], possibly indicating similar fates and roles. One can speculate whether mineralizing cells occasionally substitute Ca for either Mg or Sr during the cellular transport of ions to the site of mineralization. The small amounts of Mg and Sr found in dental tissues might, possibly, represent "mistakes" during hard tissue formation. Or, they do take part in the mineralization process. In OI-dentin the Mg-levels were higher, with significant differences in the midpart and close to the dentin –pulp junction (Fig Mg 1). Similarly, in newly formed rat incisor dentin the levels of Mg have previously been reported to be elevated [41]. This might indicate a role for Mg in early hydroxyapatite formation. As a comparison, the levels of Mg in normal deciduous postnatal enamel have been reported to be lower than in prenatal enamel [42]. This was not confirmed in this study concerning normal enamel, but could be seen in OI-enamel. In a previous study Mg was reported to have a high degree of co-variation with the organic marker element carbon in enamel [29]. This was confirmed in this study, with a high resemblance between the Mg and C graphs. This might indicate a role for Mg in early matrix – hydroxyapatite interactions. In enamel the levels of Sr were significantly higher at the enamel surface in OI (Fig Sr 3). In normal teeth Sr has been reported to exhibit stable values within the dental tissues [42] being the case for both OI and controls in both dentin and enamel.

In elemental analysis of biological tissues carbon is considered a marker of the organic tissue component. Carbon is the key element in all organic compounds. Previously, carbon, in the form of carbonate, was detected in all stages of dental enamel maturation [43]. It has been shown that the content of carbon, in
the form of carbonate, decreases in parallel to enamel matrix maturation. Organic carbon containing matrix is substituted by hydroxyapatite. In case of imperfections in the matrix in can be speculated that the organic, C-containing, matrix is not fully replaced by hydroxyapatite. Carbon containing matrix components remain within the tissues. In OI the levels of carbon were significantly higher at the surface and in the midparts of enamel (fig C emalj). In OI-dentin the C-levels were higher with a significant rise close to the dentin-pulp Interface (C 1). Elevated levels of carbon in hard tissues might reflect remaining organic matrix due to imperfect hydroxyapatite induction mechanisms. These findings were in accordance with earlier studies utilizing SIMS imaging on human primary dental enamel [17]. Alterations in the elemental composition of the hydroxyapatite constituents calcium, phosphorus and oxygen were found in both dentin and enamel. The calcium levels were significantly higher in the midpart of OI dentin (Fig Ca 2). The lowest Ca-levels were found in dentin close to the pulp, being in contrast to previously reported higher contents in predentin [40]. It has been reported that reduced expression of the key protein dentin sialophosphoprotein, causing the defect in DI type II, is correlated to increased levels of Ca in mice [11]. In OI-dentin the phosphorus levels were significantly higher at the enamel-dentin junction and in the midpart (P 2). The lowest levels for both OI and controls were found in dentin close to the pulp, being in agreement with previously reported low levels in predentin [40]. In both enamel and dentin the oxygen levels were lowered in OI, with significant differences at the enamel-dentin junction and throughout the dentin (O 3). In most sites the interrelationship between calcium and phosphorus, the Ca/P-ratio, did not differ significantly between test and controls. It is concluded that the mineral in OI dentin and enamel is hydroxyapatite. The weight % ratio of Ca/P was practically identical in all locations in DI-teeth for both enamel and dentin except for close to the enamel-dentin junction in dentin, probably reflecting a very early mesenchymal – ectodermal interaction disturbance (Fig. Ca/P).

All above mentioned data were subject to a machine learning method, a computerized rule induction analysis. Rule induction analysis is a non-statistical technique to find patterns and rules in complex data, e.g. an extensive collection of elemental analysis measurement data [28, 29]. In the rule induction analysis, outcomes from experience are identified, being the basis for construction of hierarchical “trees”, in which the relative importance of individual data is stated. For any chosen outcome, e.g. the diagnosis OI, all relevant data, and their mutual order, are shown in trees. The higher in the tree, the higher the importance for the outcome. In the top level of the enamel trees an increase in C was seen, in addition to a decrease in O. In tree level two increased levels of Ca were found. A lowered P/C ratio was found, indicating a higher C level and a lowered P level. Elevated levels of the Ca/C ratio indicate a higher Ca content. These findings are in accordance with the increased levels of the Ca/P-ratios, indicating higher Ca and lower P contents of the enamel. In addition, elevated levels of Cl and lowered levels of F were observed. The rule induction analysis pattern was not as evident for dentin as for enamel. In dentin,
increased levels of C and decreased levels of O were found, just as for enamel. In dentin, an increase in P was seen, in contrast to enamel. Elevated levels of the Ca/C and P/C ratios might indicate elevated levels of both Ca and P. In addition, elevated levels of K and lowered levels of F were observed. Surprisingly, the rule induction analysis pattern was more evident for enamel than for dentin, despite OI being a mesodermal, connective tissue, disease which should primarily affect dentin in teeth. The divergences in dental enamel are most probably induced indirectly during mesenchymal - epithelial interactions during early tooth formation. To conclude, the rule induction analysis demonstrated an enamel pattern with elevated levels of C and Ca, and lowered levels of P and O. A different pattern was found in dentin, with increased levels of C, in addition to both Ca and P. The increased C levels might reflect a higher content of organic components in both enamel and dentin, the enamel observations most probably being indirect, secondary, effects of an impaired dentin formation.

The overall conclusion from this study, including individual data from SIMS and XRMA measurements, in addition to the rule induction analysis, is the striking difference in element composition of dental tissues from OI teeth, both dentin and, above all, enamel. Even though the analyzed teeth were not diagnosed DI they exhibited apparent deviations in elemental composition. It seems that DI is a continuous spectrum disease, ranging from subclinical findings to severe tissue injuries. Main findings were elevated levels of phosphorus, calcium and magnesium in OI dentin. The elevated levels of Ca and P were confirmed by the rule induction analysis. The levels of oxygen in OI dentin were lowered, and fluorine in the central parts of OI enamel was also lowered. In enamel from OI patients the levels of carbon and chlorine were elevated, the elevated levels of C being confirmed by rule induction analysis. These changes most probably reflect aberrations in the matrix to be mineralized during early dentinogenesis. The changes observed in enamel are probably due to indirect effects of impaired dentin collagen during mesenchymal-ectodermal interactions at early tooth formation.
References


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Legends to figures

Figure 1
Photo of primary tooth demonstrating the locations of SIMS- and XRMA-measurements in enamel and dentin. In enamel 1 corresponds to the enamel surface, 2, 3 and 4 correspond to the enamel bulk, and 5 enamel close to the enamel-dentin junction. In dentin 1 corresponds to dentin close to the enamel-dentin junction, 2, 3 and 4 correspond to the dentin bulk, and 5 dentin close to predentin.

Figure Na K
Weight % graphs of Na and K in enamel and dentin from DI (●) and controls (■) measured at 5 locations within enamel and dentin respectively. In DI the levels of Na (1) and K (2) were lower in enamel. In DI-dentin the Na- and K-levels were higher.

Figure Mg, Ca, Sr
Weight % graphs of Mg, Ca and Sr in enamel and dentin from DI (●) and controls (■). In DI the levels of Mg were lower in enamel. In DI-dentin the Mg-levels were higher in the midpart and close to the enamel-dentin junction (1). The Ca-levels were similar to controls in DI-enamel. The Ca-levels were higher in the midpart of DI dentin (2). In both enamel and dentin the levels of Sr were elevated in DI (3).

Figure C, P, O
In DI the levels of C were higher at the surface and in the midparts of enamel. In DI-dentin the C-levels were higher close to the dentin-pulp Interface (1). The P-levels were similar to controls in DI-enamel. In DI-dentin the P-levels were higher at the enamel-dentin junction and in the midpart. (2). In both enamel and dentin the O-levels were lowered in DI with significant differences at the enamel-dentin junction and through out the dentin (3).

Figure F Cl
In DI the levels of F were lower in enamel. In DI-dentin the F-levels were lower, being close to similar to controls at the dentin-pulp interface (1). The Cl-levels were increased in both DI-enamel and dentin (2).

Figure Ca/P
The Ca/P weight % ratio was practically identical in all locations in normal and DI-teeth for both enamel and dentin except for close to the enamel-dentin junction in dentin.
### Tables

**Table 1.** Ion species analyzed by means of SIMS and XRMA. SIMS-values were normalized to those of $^{44}\text{Ca}$.

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<th>$^{23}\text{Na}$</th>
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<th>$^{47}\text{CaCl}$</th>
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<td><strong>XRMA</strong></td>
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<td>$^{16}\text{O}$</td>
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Table 2. Rule induction analysis data for five locations (top row) in dental enamel (A), and dentin (B), respectively. Four element groups are specified in the attributes column. The last attribute group (Na Mg Cl K Sr) is similar to the last but one group, except for F, which is excluded. In the tree level column the relative importance for the outcome OI is specified. The higher the level, the higher the relative importance for the outcome OI. For every element, the breakpoint levels in the induction trees are stated. The probability for induction analysis to identify the outcome OI is stated in the last level for every location.

<table>
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<td>Cl &gt; 0.002</td>
<td>Cl &gt; 0.001</td>
<td>F &lt; 0.00006</td>
<td>Cl &gt; 0.0006</td>
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A (enamel): For the attribute group C O P Ca an increase in C and Ca and a decrease in O was seen. For the attribute group Ca/P, Ca/C, P/C, a lowered P/C ratio and elevated levels of the Ca/C- and Ca/P-ratios were seen. For attribute group F Na Mg Cl K Sr elevated levels of Cl and lowered levels of F were observed. If F was excluded (last attribute group) elevated Cl levels were still seen, in addition to elevated levels of Na.
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<th>location 3</th>
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<td>OI 100%</td>
<td>OI 100%</td>
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<td>K &gt; 0.04</td>
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B (dentin): For the attribute group C O P Ca a decrease in O and increase in P and C were seen. For the attribute group Ca/P, Ca/C, P/C elevated levels for both P/C and Ca/C and lowered levels of Ca/P were seen. For attribute group F Na Mg Cl K Sr an increase in K and decrease in F were observed. If F was excluded (last attribute group), lowered levels of Na were seen.
Figures

Figure 1
Figure Na K

Figure Mg, Ca, Sr

Figure C, P, O

Figure F Cl

Figure Ca/P