Analysis of Tooth Surface Elements by Ion Beam Analysis

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Abstract: To examine the applicability of elastic recoil detection analysis (ERDA) in studying element constituents of dental hard tissues.
The concentration of all elements using high-energy heavy ions was detected in extracted teeth of both dentitions. The main elements present in enamel (calcium, phosphorus, oxygen, hydrogen), along with nitrogen, carbon, fluorine, sodium, magnesium and zinc, were measured. Concentrations and depth profiles were calculated and compared with simulation data generated using two programs, KONZERD and SIMNRA.
Enamel calcium, phosphorus, oxygen, hydrogen, nitrogen, carbon, fluorine, sodium, magnesium and, occasionally, zinc were detected. One-third of samples showed a constant concentration of the constituents over the analyzed depth, whereas the remaining samples had pronounced surface contaminations of carbon and nitrogen. Although calculation of concentrations with KONZERD gave expected values, simulation with SIMNRA was not possible since no agreement could be obtained between simulated and measured results for the elements.

Key words: Dental enamel, Elastic recoil detection analysis, ERDA, Ion beam analysis

Introduction

The anatomic crown of every tooth is covered by enamel, the most highly mineralized tissue found in the human body. Enamel consists of about 96% inorganic materials, mainly hydroxyapatite crystallites, 4% organic material, and water.[1,2]

The acquired enamel pellicle is an organic film covering the surfaces of teeth which is formed by selective binding of glycoproteins from saliva.[3–5] Proteins, enzymes, immunoglobulins, glucose, and mucins have all been shown to be components of the pellicle. It forms a layer about 0.7-1.0 µm thick and protects enamel by reducing demineralization upon acid challenge. However, it has also been shown to be the means by which microorganisms attach to the tooth leading to the formation of dental plaque and caries.[3, 5–7] The earliest sign of a carious lesion is the appearance of a chalky white spot on the surface of the tooth, indicating an area of demineralization of enamel.[8] As the lesion continues to demineralize, it can turn brown and will eventually turn into a cavitation.[9–13] In the early lesions the surface of the enamel (5-10 µm) may appear to be intact, despite demineralization processes occurring beneath the surface. Although, these processes are reversible at this stage by means of fluoride application or by increasing uptake of calcium and phosphorus, it is important to gain an insight into the effects of demineralization on tissue elements.[14–18]

The model of an intact surface pellicle covering an area of demineralization has been confirmed by a number of microscopic and radiographic techniques.[19–22] However, none of them assessed whether any changes were occurring within the pellicle itself and the area beneath an intact pellicle. Although a number of nuclear analytical methodologies, such as proton induced x-ray emission (PIXE), particle induced gamma-ray emission, (PIGE), nuclear reaction analysis (NRA), hydrogen NRA (H-NRA), nuclear activation analysis (NAA), or Rutherford backscattering spectroscopy (RBS) are available to investigate these changes, elastic recoil detection analysis (ERDA) is the only technology capable of providing depth profile analyses of all chemical elements found in the outer surface of dental tooth substances

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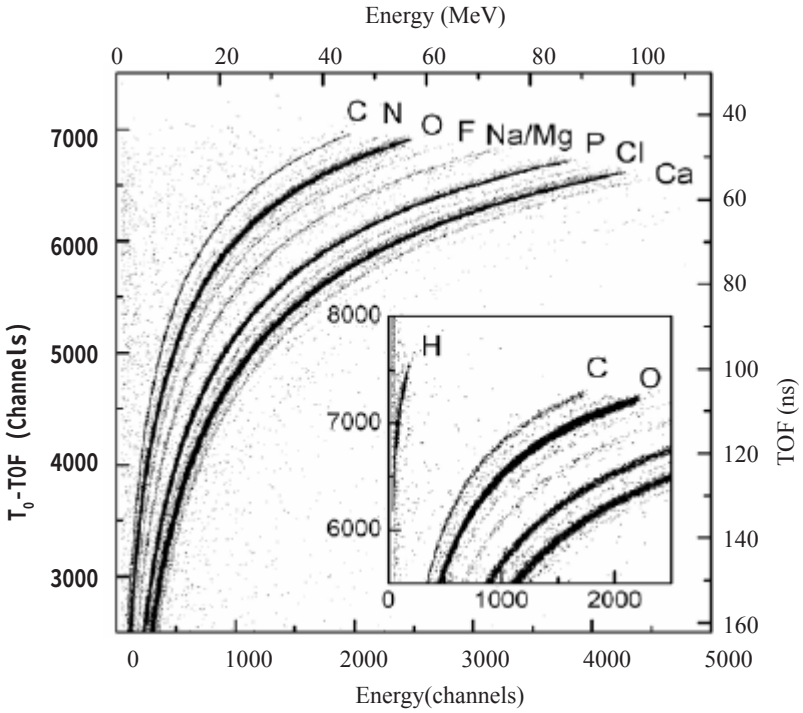

Figure 1. Typical scatterplot of all detected recoils from a measurement of an intact natural tooth surface (21, permanent anterior tooth) with the long TOF spectrometer.
The different elements are clearly separated. Insert shows the scatterplot from the measurement of the same spot with the short TOF spectrometer for the determination of hydrogen content.

with good sensitivity.[23–31] This study aims to use ERDA to analyze whether surfaces can be intact after out-diffusion of ions from the subsurface lesion and whether there are consequences of these processes detectable in the early carious lesion.

Materials and Methods

Seventeen teeth from fourteen dental patients, extracted for orthodontic and other reasons, were used in this study and stored in formalin before use. After rinsing in water, the tooth samples were sawed from buccal to oral, vacuum dried and mounted in a target holder proximal surface uppermost ensuring minimal curvature of the sample.

Elastic recoil detection analysis (ERDA) measurements (Ion-Beam Laboratory ISL of the Hahn-Meitner Institute, Berlin) were performed on healthy and white spot lesions of untreated enamel. ERDA allows the analysis of layer structures used in solid state physics and is based on the Rutherford scattering theory of atomic nuclei.[31] For the measurements, the samples were irradiated with high energy heavy ions in a vacuum of 10^{-7} mbar between 10 minutes and 1 hour. 230 MeV Xe ion beams with ion currents of approximately 2nA and beam spot sizes of 0.4 mm^2 were used. The measurements were performed at a scattering angle of 60° with a short TOF spectrometer and at an angle of 40° with a long TOF spectrometer.[32] As a result of elastic scattering of the ions,

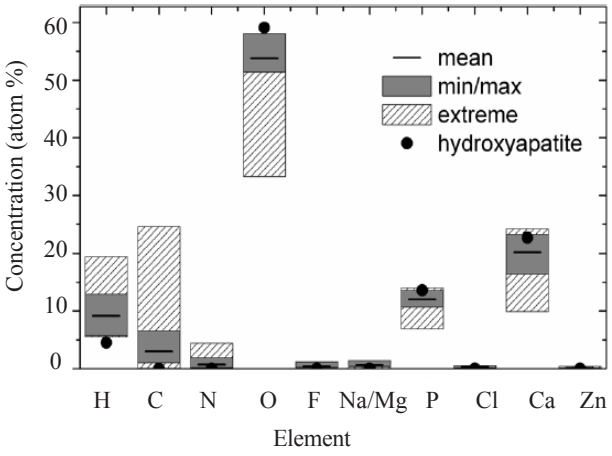

Figure 2. Values for the element concentration for all investigated teeth in atom % calculated with KONZERD.

recoiled sample atoms leave the sample and hit the detector system where the energy and Time-of-Flight (*TOF*) for a fixed distance *s* were measured.

Using the formula $E = \frac{M}{2}\left(\frac{s}{TOF}\right)^2$

where E = energy and M = mass of the recoiled atoms, an

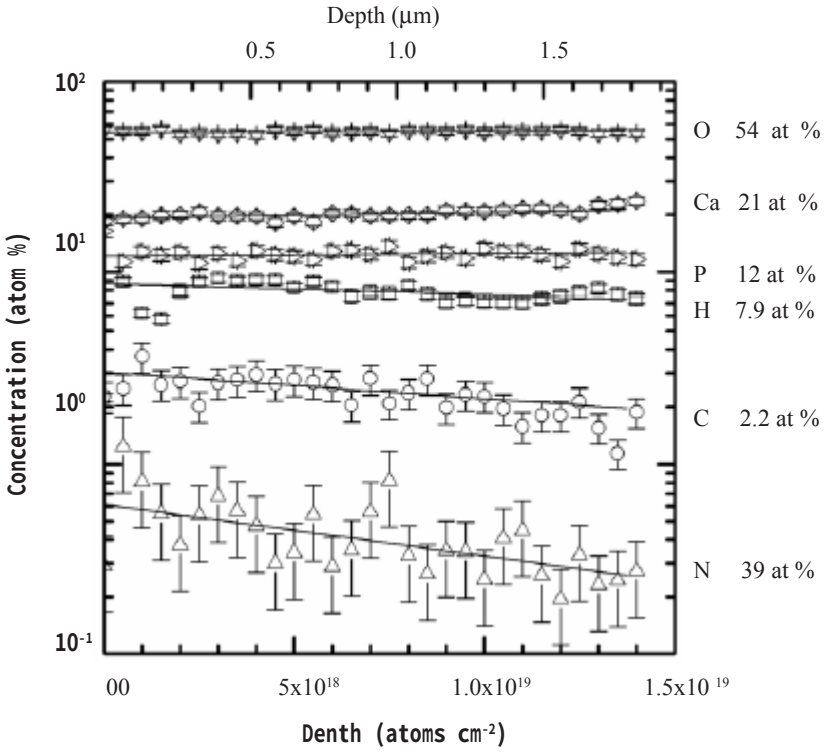

Figure 3. Depth profiles of the main elements for a (18, upper wisdom tooth) tooth calculated with KONZERD. The concentrations of the heavier elements were constant over the analyzed depth. The concentrations of hydrogen, carbon, and nitrogen decrease with increasing depth. The conversion of the calculated area density given in atoms per cm^2 into a geometrical thickness was performed using a density of 2.92 g/cm^3 for hydroxyapatite and the averaged concentrations are shown.

identification and separation of the various atomic elements in a 2-dimentional plot of time vs. energy could be recorded. This, in turn, allowed the energy spectra for each element of the sample to be obtained. Sample composition, element concentration and depth profiles were calculated from the energy spectra directly and by means of simulation using the computer programs KONZERD[33] and SIMNRA.[34]

To investigate the effect of tooth surface roughness on the measurements taken from the untreated enamel samples, further studies were performed to allow comparisons with that of polished tooth samples. Three additional teeth were embedded in resin (brand of resin-Buehler GmbH, Düsseldorf), sawed with a low-revving saw and polished with diamond-studded paper with decreasing grain size and, finally, silicon carbide foil with a grain size of 0.3 µm. Quality was confirmed using light and scanning electron microscopy (SEM, CamScan MaXim2040S, CamScan Electron Optics Ltd, USA).

Results

Enamel calcium, phosphorus, oxygen, hydrogen, nitrogen, carbon, fluorine, sodium, magnesium, and occasionally, zinc were detected. *Figure 1* depicts a typical graph of the detected atoms of a healthy tooth surface. The recoils with the maximum energy of a particular mass originate from the surface.The energy reduces with increasing depth. The density of the points reflects the corresponding element concentration in the tooth.

A third of the samples showed a constant concentration of the constituents over the analyzed depth, whereas the remaining samples had pronounced surface contaminations of carbon and nitrogen. The minimum and maximum element concentrations were calculated with the program KONZERD and averaged over a depth range from $2 \cdot 10^{18}$ atoms cm^{-2} to $12 \cdot 10^{18}$ atoms cm^{-2}, allowing possible contributions from surface contaminations to be avoided (*Figure 2*). Extreme values from samples with very high carbon contamination were excluded from averaging but are included for comparison.

From *Figure 2*, it can be seen that the teeth consisted of about 90 atom % (at %) hydroxyapatite. The residual 10 at % was composed mainly of hydrogen, carbon and other elements with fractions of less than 1 at % each.A higher than expected hydrogen excess of about 5 at % compared to pure hydroxyapatite was detected. The mean values for the concentration of the main components, except hydrogen, were in reasonable agreement with the theoretical values assuming pure hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (*Figure 3*). Despite the best possible selection of samples the slopes of the simulated spectra did not match the measured ones. However, this affected all elements simultaneously, allowing determination of the concentrations with

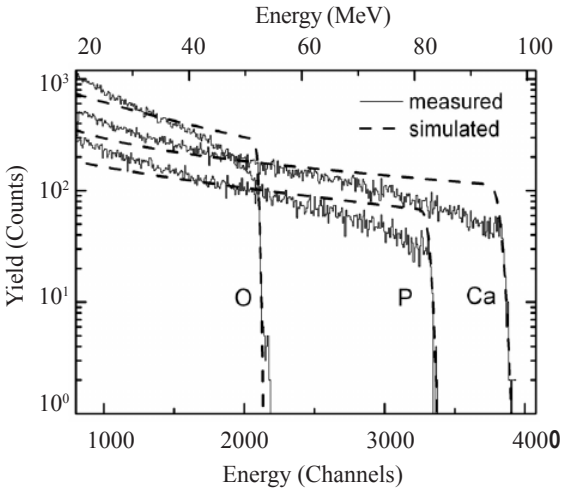

Figure 4. Measured (open symbols) and simulated (line) energy spectra of the main elements for a measurement of a (55, second upper primary molar) tooth.
The high energetic edges correspond to the surface and lower energy to increased depth. The calcium atoms with 20 MeV arise from a depth of about 1.5 µm. The different slopes of the measured and simulated spectra are evident.

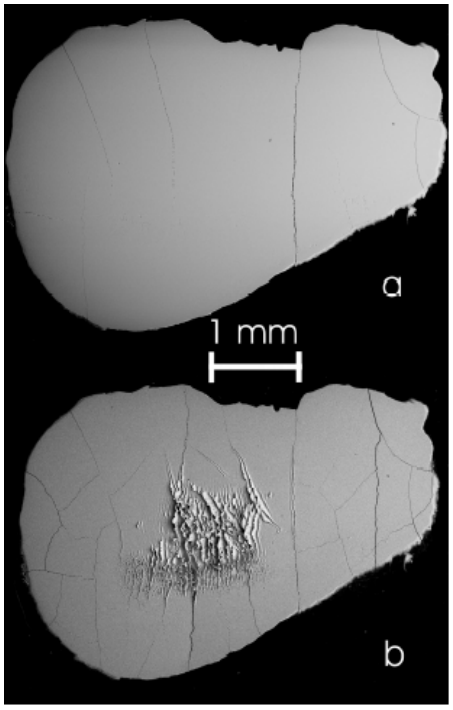

Figure 6. Scanning electron micrograph (SEM) pictures of a polished tooth 65 cut (a) before and (b) after two ERDA measurements with 230 MeV Xe ions.
The total fluence amounts to about $3.6 \cdot 10^{14}$ ions/cm^2. Besides the increased number of micro cracks, the formation of waves perpendicular to the beam direction is visible. The overlapping of the two beam spots is reflected in the different heights of the wave structures.

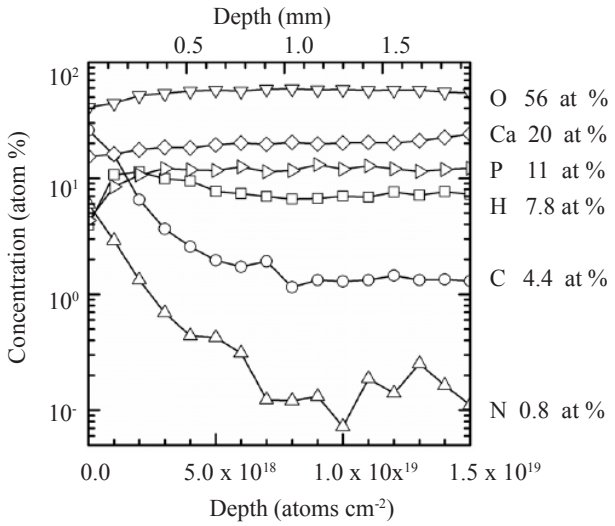

Figure 5. Depth profiles of the main elements for a (55, second upper primary molar) tooth calculated with KONZERD.
The concentrations of carbon and nitrogen decrease steeply from the surface to the bulk, indicating the existence of a film on top of the tooth surface. The averaged concentrations are given for comparison.

KONZERD. Although the calculation of the concentrations with KONZERD gave expected values (*Figure 3*), simulation with SIMNRA was not possible since no agreement could be obtained between simulated and measured results for the elements (*Figure 4*). Simulation of oxygen, phosphorus, and calcium spectra in a second deciduous molar (55: deciduous tooth) measured with the long TOF spectrometer and assuming a constant concentration for all elements, gave a steeper slope than for the measured values. The assumption of a depth dependent concentration for the elements did not solve the problem, since the effect occurred for all element spectra.

Concentration depth profiles of the main elements for a tooth (55, second upper primary molar) were calculated with KONZERD (*Figure 5*). It can be seen that the concentrations of carbon and nitrogen decreased steeply from the surface to the bulk, indicating the existence of a structure that is not part of the tooth structure probably the dental biofilm (pellicle). In addition, a correlation between the carbon and nitrogen concentrations was evident.

Light microscopy and SEM were used to investigate the effect of tooth surface roughness on the measurements (*Figure 6*). With both methods some cracks were found, which were shown to increase if the specimens were submitted to high vacuum. Furthermore, small scratches from the cutting were detected. SEM pictures taken before and after the measurement showed superficial roughness and wave forming on the beam spot area.

Discussion

The main enamel components have been reported as calcium

(38%), phosphorus (18%), magnesium (0.4%), carbon dioxide (2.5%), water (3%), and organic substances (1%).[35–38] Many studies using methodologies such as atomic force microscopy (AFM), PIXE or quantitative light-induced fluorescence (QLF) were unable to measure oxygen levels, despite it being a major component in enamel, since the analytical methodology involve carbonization of the samples.[39–43] In contrast, ERDA does not involve any manipulation of the samples and, therefore, has the capability to measure the total atomic concentration of all constituents within the sample. In addition, ERDA allows the quantification of both hydrogen and oxygen within the sample.

The excess hydrogen observed in the samples was interesting since there was no evidence of additional chemically bonded water in the samples as the oxygen values were not in excess. It is possible that the hydrogen excess was derived from organic material within the sample. This is supported by the value obtained for carbon content of approximately 3 at %, which gave a ratio of 2:1 hydrogen: carbon as observed in organic matter. It is also possible that the nitrogen originated from organic material, particularly since a correlation between the carbon and nitrogen content was observed in the samples. Previous studies have reported the fraction of carbon dioxide as 2.5 weight % which corresponds to 1.4 at % carbon.[2, 20, 44] This value agrees with the minimum value obtained in this study and would suggest that some of the carbon in the samples represented superficial contamination. The fluorine concentration in the samples was consistent with other studies, though factors such as the fluoride content of drinking water, dentition, and methodology used may affect the actual values obtained in individual studies.[20, 45, 46] Concentrations of trace elements such as chlorine, sodium, magnesium and zinc were generally consistent with other studies.[47, 48]

Although the applicability of ERDA for the chemical characterization of tooth surfaces could be demonstrated, a comparative analysis of sound and demineralized enamel was hindered by a number of factors which could be attributable to the properties of the samples. Since ERDA is a highly sensitive technique, any surface contamination of the sample may result in varying element concentrations. Some of the variations seen between samples in this study reflect contamination by the dental biofilm or formalin attached at the dental biofilm. Similarly, changes in sample composition such as cracking, release of water and gaseous reaction products during the irradiation, ion beam analysis or vacuum in the scattering chamber may account for some of the sampling variation. Another important factor was the influence of surface geometry on the measurements. A plane and smooth sampling surface was assumed for the calculations which is not the case for natural tooth surfaces. Although rigorous attempts were made to ensure optimal samples, the slopes of the simulated spectra did not match the measured ones suggesting that surface roughness of the teeth was affecting the measured values. However, this affected all elements simultaneously and allowed the determination of the concentrations with KONZERD, though concentrations could not be determined by SIMNRA. Attempts to investigate the effect of surface roughness using SEM showed the formation of waves in the analysis area. This has been demonstrated using ion irradiation where amorphous solids showed the formation of waves following bombardment with phosphates being particularly sensitive to ion irradiation.[49, 50] The induced roughness was dependent on the number of particles, which in this study led to a measurement time dependent difference between simulated and measured spectra. Since the ion beam affected the samples, the only way to improve the analysis was to reduce the number of impinging primary ions for the measurement which, in turn, led to an increase in the concentration errors and inaccuracies in the concentration depth profiles. The individual effects of each of these factors were difficult to predict and made assumptions for the simulation analysis practically impossible.

Conclusions

EDRA allowed detection of all elements in expected concentrations, with the exception of natural teeth covered by the dental biofilm, where high carbon and nitrogen values were obtained. Due to factors that significantly influence the measurements, ERDA is unsuitable as a standard investigative tool for analyzing the main elements of dental enamel.

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