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ATOMIC FORCE MICROSCOPY STUDY OF HUMAN TOOTH ENAMEL SURFACES

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Abstract

Human enamel features from individual crystals up to prisms were observed by atomic force microscopy (AFM). Low magnification images of vestibular tooth surfaces show the existence of enamel prisms appearing as deep holes. Individual, parallel enamel crystals show lateral faces elongated and formed by the (100) planes of hydroxyapatite (HA). Height differences between (001) faces create the roughness of enamel surface. Individual (001) crystal faces can be observed clearly at higher magnification and show the characteristic hexagonal shape with 60° angles between (100) faces. This study confirms the applicability of AFM for studying biological hydroxyapatite crystals.

Key Words: Atomic force microscopy, human enamel, tooth enamel, enamel crystal, hydroxyapatite, adsorption.

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Introduction

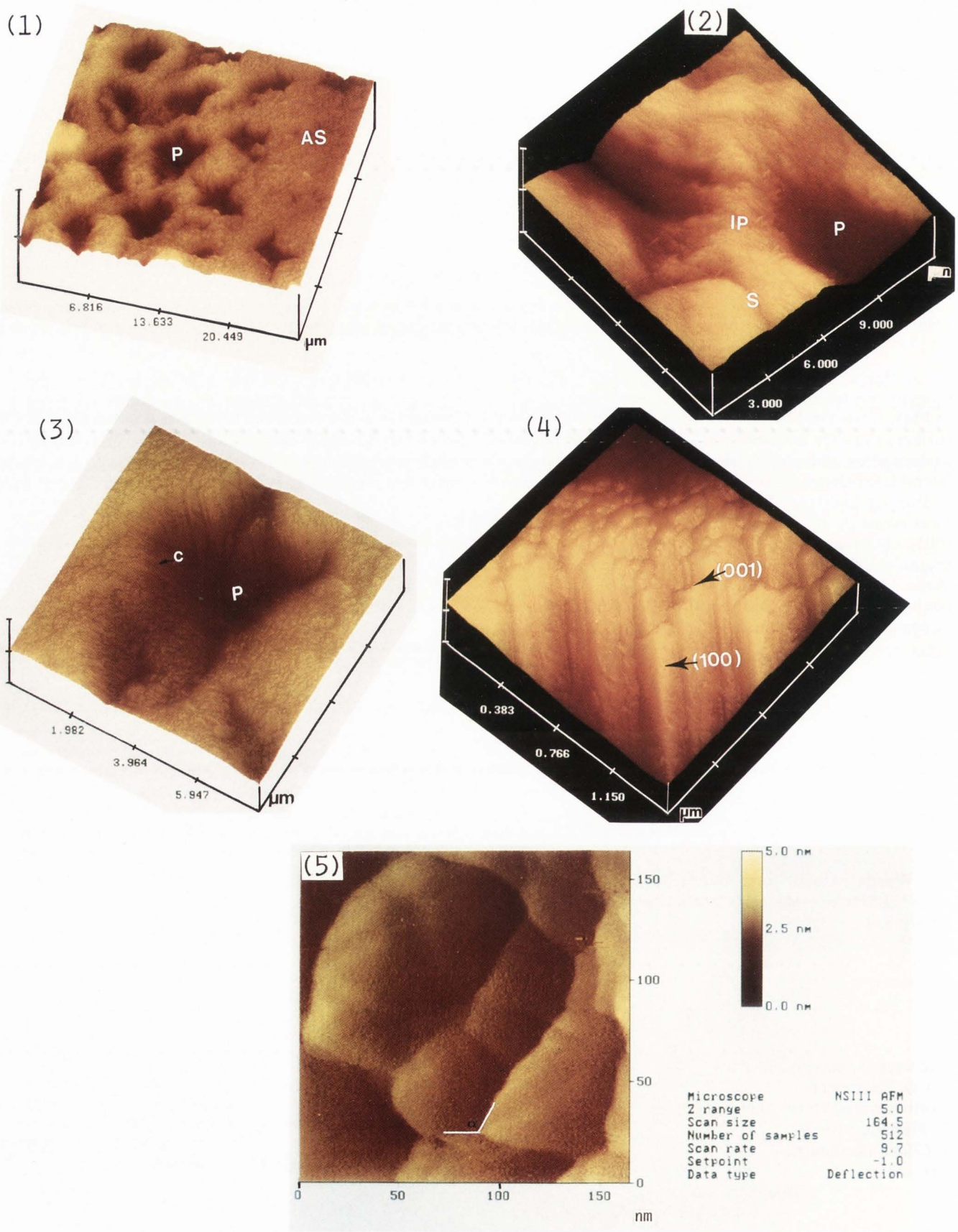
Surface features of unerupted and erupted enamel were previously studied by scanning electron microscopy (SEM; see for example, Haikel and Frank, 1982; Boyde *et al.*, 1988) and by high resolution scanning electron microscopy (HRSEM; see Apkarian *et al.*, 1990). These investigations were carried out on fixed, dehydrated, and metal-coated specimens. Such preparation stages can induce specimen alterations and result in a misunderstanding of surface structures. SEM secondary electron images give only pseudo 3-dimensional (3-D) information and stereo microscopy is needed for 3-D information (Joy, 1984).

Scanning tunnelling microscopy (STM; Binnig and Rohrer, 1982) and atomic force microscopy (AFM; Binnig *et al.*, 1986) furnish quantitative 3-D information with the z information as precise as the x-y information. AFM investigations can be performed on uncoated non-conductive specimens, including biological dry specimens, without preparation. Since enamel is composed of hydroxyapatite (HA) consolidated into a ceramic, AFM is well adapted to the study of enamel. In the case of ceramic materials, tip-sample interactions in AFM are controlled by capillary forces. The presence of liquid reduces the capillary force problems and improves AFM images of ceramics (Drake *et al.*, 1989). We performed these studies on human enamel surfaces by an AFM equipped with a liquid stage filled with ethanol.

In this paper, we report one of the first observations of human tooth enamel surfaces at high resolution by AFM (see also Kasas *et al.*, 1993). Enamel prisms and individual crystals were observed, thus demonstrating the ability of AFM to furnish useful information on biological crystals. Future goals are the imageing of biological calcified tissue surfaces at atomic resolution in order to follow dynamical surface processes on biological crystals, such as crystal nucleation, crystal growth, crystal dissolution and protein adsorption.

Material and Methods

Two unerupted third molars extracted for orthodontic reasons were collected from an 18 year old


(1)
P
AS
6.816
13.633
20.449
µm
(2)
IP
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S
9.000
6.000
3.000
(3)
c
P
1.982
3.964
5.947
µm
(4)
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0.383
0.766
1.150
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150
100
50
0
0
50
100
150
nm
5.0 nm
2.5 nm
0.0 nm
Microscope NSIII AFM
Z range 5.0
Scan size 164.5
Number of samples 512
Scan rate 9.7
Setpoint -1.0
Data type Deflection

Figure captions (for Figures on the facing page, color plate)

AS: aprismatic surface; **P**: enamel prisms; **IP**: interprismatic enamel; **S**: prism sheath; **C**: enamel crystals.

Figure 1. Human enamel surface; scan size: 27 mm; z range 5 mm.

Figure 2. Human enamel surface; scan size: 12 mm; z range 3.5 mm.

Figure 3. Human enamel surface; scan size: 7 mm; z range 3 mm.

Figure 4. Human enamel surface; scan size: 2.1 mm; z range 1 mm; (001): crystal faces formed by (001) lattice plane; (100): crystal faces formed by (100) lattice planes.

Figure 5. (001) enamel crystal faces; scan size: 164 nm; z range: 5 nm.

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patient. Specimens were rinsed in 0.1 M cacodylate buffer at pH 7.4 and tangential sections to the vestibular cuspid were prepared using a Bronwill TSR 77 thin sectioning machine. Enamel sections were immersed in 10% sodium hypochlorite for 30 minutes in order to remove organic material adsorbed on the surface and to extract the organic material located between crystals. After four successive washes in distilled water, specimens were dried at room temperature.

Observations were performed without any further preparation in an AFM (Nanoscope III, Digital Instrument, Santa Barbara, CA, USA). This AFM was equipped with an optical microscope (Nikon, Tokyo, Japan) and a TV camera which were used to select areas of enamel surfaces for observation. We used commercially available Si_3N_4 tips (NanoProbes, Digital Instruments, Santa Barbara, CA, USA). This triangular tip, with a length of 115 mm, has a spring constant of 0.58 N/m. Strong interactions related to capillary forces between tips and ceramics, in our case HA, perturb tip movements. To overcome this problem, all work was carried out at room temperature with a liquid stage filled with pure ethanol and with Si_3N_4 tips. In order to obtain high resolution images, the AFM was put under a glass bell to isolate the microscope from ambient noise vibrations. The enamel sections were glued to steel disks and placed on a magnetic disk on the top of the piezoelectric translator. The maximum range scanned was 130 x 130 mm^2 and images of 512 x 512 or 256 x 256 pixels were recorded. After appropriate 3-D visualization and colorization, the images were printed with a colour printer (Mitsubishi, Tokyo, Japan).

Results

Low magnification images of vestibular tooth surfaces showed the existence of aprismatic and prismatic areas (Fig. 1). Prismatic areas probably corresponded to perikymatia grooves. Enamel prisms in prismatic areas showed the characteristic arcade organisation. Prisms appeared as deep holes separated by interprismatic enamel (Figs. 1-3). Hole bottoms appeared to have irregular triangular shapes. The mean diameter of the 12 measured holes was 2.8 $\pm$ 0.95 mm. The measured diameter corresponded to the diameter of the circle which contained the triangular structure. These holes corresponded to highly porous Tomes' process pits. Enamel surface appeared flat and smooth in aprismatic areas, while the transition between prismatic and aprismatic enamel was irregular.

The depth and diameter of the holes decreased until they disappeared as they approached the border between the prismatic and aprismatic areas. Hole borders were well defined and walls exhibited individual enamel crystals. Enlargements of these surface areas showed individual crystals running from the depth to the surface (Figs. 2 and 3). Prism centers were deeper than 3.5 nm (Fig. 3) and corresponded to the maximum measurable depth, which is limited by the angle between the cantilever and the scanning plane.

Prism sheaths were not systematically observed (Fig. 2) and appeared as fissures partially surrounding the prisms. Fissures could have been formed by the extraction of organic material from the enamel surface.

AFM allowed the study of the same specimen at different magnifications. The area on the upper left border of the prism shown in Figure 3 has been enlarged in Figure 4. Individual parallel crystals running perpendicularly to the surface were observed. It was not possible to measure their length because bottom extremities could not be detected. Lateral faces of these crystals were elongated, i.e., their length was greater than their width. The lateral faces are formed by the (100) lattice planes of HA, as observed in transmission electron microscopy (TEM; Brès *et al.*, 1991) and the (001) surface of the crystals were perpendicular to (100) faces. Height differences between (001) faces create the roughness of enamel surface. Individual (001) faces were clearly observed at higher magnification (Fig. 5). The (001) faces were at 3° to the scanning plane. This slight disorientation gave a contrast that allowed individual crystals to be clearly distinguished. The angle between two (100) faces was 120° (Fig. 5). Most of the angles measured between (100) faces were equal to 60° or 120°. The (001) faces of enamel crystals observed by AFM were hexagonal.

Discussion

Images of enamel surfaces observed at various magnifications showed structures from prisms to individual crystals. In the areas observed, we were unable to detect focal holes and enamel caps as previously described for unerupted mature enamel surfaces (Fejerskov

et al., 1984). The morphology of crystal faces and the manner in which matrix proteins interact are of great importance for cariology and crystal growth mechanisms. The (001) faces observed appeared flat without central lesions or defects. Enamel crystals observed along the c-axis (<001> faces) have an hexagonal pattern as shown by conventional TEM (CTEM; Brès *et al.*, 1991). Rhombohedral crystals as proposed by Warshawsky *et al.* (1987) were not detected.

The AFM easily imaged crystals from human tooth enamel at a resolution and a magnification which have only been attained by high resolution TEM (HRTEM; Brès *et al.*, 1991) and HRSEM (Apkarian *et al.*, 1990). A recent study of tooth structure using AFM was performed at relatively low magnification (Kasas *et al.*, 1993). CTEM and HRTEM imaging of enamel have relied upon fixed, epoxy embedded, thin sectioned specimens. SEM and HRSEM require metal coating. HRSEM imaging on chromium coated enamel is particularly sensitive to metal decoration, the thinnest metal films being 1-2 nm. The SEM resolution (65 nm) and its pseudo 3-D topographic contrast limits the range of investigation to large surface structures like enamel prisms. Information from HRSEM overcomes some of these limitations, and structural information on individual enamel crystals and surface structures of these crystals can be obtained (Apkarian *et al.*, 1990). The resolution which can be attained with type I secondary electron emission is around 10 nm, but metal decoration is always present and the 3-D information is non-quantitative. TEM and HRTEM furnish high resolution images (0.15 nm for best HRTEM) with structural information at the unit cell level (Brès *et al.*, 1991) but surface analysis is possible only with two-dimensional (2-D) profile images of surfaces. AFM is as yet the only microscopic technique which permits collection of high resolution information from surfaces with quantitative measurements of surface roughness and depth. The obtained data are not disturbed by coating, dehydration, or any other preparation technique.

AFM reduces sample preparation artifacts but introduces its own artifacts. Artifacts from tip-sample interactions are the most frequent in AFM (Blackford *et al.*, 1991). Images depend on the tip quality and often the image is a convolution of the 3-D shapes of the sample and the tip. An increase of resolution in the case of biological specimens depends on the realization of sharp tips. The studied specimen must be well stabilized within the AFM in order to avoid displacement generated by the tip. In the present case, the crystalline structure of enamel avoids artifacts created by deformations or displacements of the specimen generated by the tip scan.

In this study, we demonstrated the ability of AFM to give useful high resolution information on biological crystals and we developed another preparation technique which allowed us to obtain better images than previously described (Kasas *et al.*, 1993). This imaging technique allows new approaches to the study of dynamic mechanisms at the biological crystal surface. Further studies on the ionic mechanisms of tooth caries could now be initiated. The atomic surface structure of apatite crystals in acidic liquid media and the dynamic surface modifications induced by decreases in pH could thus be followed and imaged by AFM in real time. In pathologies, such as dental caries or osteoporosis, in which biological crystals are destroyed, AFM may furnish key information for a better understanding of such destruction. The future goals would also be to follow dynamic surface processes occurring on biological crystals including crystal nucleation, crystal growth, and protein adsorption. The understanding of adsorption processes is of prime importance in the study of biological crystal growth and for the development of new treatments.

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no **Discussion with Reviewers**.