Introduction

Scanning Probe Microscopy (SPM) is a set of experimental methods used in imaging of surface structures with subatomic resolution (8). Beside physics and chemistry of surfaces the method is useful also in biological sciences.

One of the clones of SPM is Atomic Force Microscopy (AFM). AFM is based on mapping of an atomic-force field on a surface of an examined sample. The method is useful not only in physics and chemistry; it can be also applied in biological fields. Special construction of AFM scanner enables to follow biological samples in liquid environments. Artifacts caused by dehydration of samples are removed this way. Dentin of human teeth is a vital hydrated tissue. It is strongly sensitive to dehydration and drying that are commonly used in preparation of samples in examinations by Scanning Electron Microscopy (SEM). We describe our experience in examination of dentin surfaces of extracted human third molars using contact method of AFM under moist conditions.
standard procedure for preparation of dentin samples in SEM (Scanning Electron Microscopy) a sample is dehydrated in graded acetone series, dried and coated in sputtering device after its fixation. The occurrence of artifacts caused by shrinking (15) cannot be eliminated in practice because dentin is strongly sensitive to dehydration.

We have investigated the possibility to examine dentin surface of human teeth under moist conditions using AFM. This method that is not based on aggressive chemicals can help in excluding artifacts.

**Method**

Human extracted third molars without decay stored in solution of 0.5 % chloramine for the period of less than one month after their extraction at the temperature of 4 degrees of Celsius (according to the rules ISO TR 11 405) have been used. After removing soft tissue and debris anatomical crown and apical part of a root have been separated using a diamond disc. 3-mm high dentin disc has remained after this procedure. A thin layer of cementum on the surface is removed with a low-speed handpiece under water cooling. Then the outer surface is polished by paper discs Sof-Lex (3M ESPE). Every dentin slice is divided into two halves and placed into distilled water in an ultrasonic purified apparatus for 30 minutes. The samples have then been examined by AFM in Laboratory of Atomic Force Microscopy.

AFM Explorer manufactured by ThermoMicroscopes (USA) has been applied using contact mode with tips from silicon nitride (type 1520–00, ThermoMicroscopes). The imaged surface area has gone from 5 to 100 µm and resolution of 300 points per row has been used. Maximum measurable changes of the surface profile have been 10 µm. Dentin samples have been stored in distilled water and processed under moist conditions.

**Results**

Figures 1–4 show dentin surfaces and they also demonstrate problems that have been faced using AFM as a method for imaging. A flat dentin surface with well-shown dentinal tubules without smear layer are depicted in Fig. 1. Dentinal tubules as well as obvious drafts caused when preparing a sample are shown in Fig. 2. Dentinal tubules surrounded by a wall (caused probably by mechanical preparation of the sample) can be seen in Fig. 3. Morphology of a dentinal tubulus is visualized in 3D graph in Fig. 4.

Samples were small and that is why they had to be fixed to a bedding in order to allow for a convenient manipulation.

Natural curvature of a tooth root in two planes and a small roughness occurring on dentin surface after removing cementum complicated imaging and could also lead to a damage of the used tip. This problem has been removed polishing the surface by fine paper discs Sof-Lex under water cooling.

We have had to solve the problem of contamination of sample surfaces. If a surface is not clean enough a lot of strips occur in the image; they are caused by small particles that are moved on a surface by the tip. Removal of smear layer that arises on the surface after its instrumentation and prevents dentinal tubules from imaging is necessary. Removal of smear layer as well as plugs in dentinal tubules can be safely reached polishing the samples and subsequently putting them into an ultrasonic bath for 30 minutes.

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**Fig. 1:** Picture of a flat dentin surface with opened dentinal tubules without smear layer obtained by AFM (scan area 100x100 µm).

**Fig. 2:** Visible drafts on a surface of a dentin sample caused by mechanical preparation and depicted by AFM (scan area 100x100 µm).
of smear layer applying 37 % solution of phosphoric acid or combining phosphoric acid with natrium hypochloride lead to demineralization, event. deproteination of dentin.

Disadvantage of AFM approach is the fact that taking an image requires approx. five minutes. We obtain information about the sample surface just bellow the tip that covers a small area. Information about the rest of a sample surface is missing owing to a small scanned area and this complicates statistical analysis of the surface. Samples have to be moved mechanically bellow the tip which also causes troubles in a real experiment. To compare with Scanning Electron Microscopy (SEM), this method enables to visualize a large area of the surface and a subsequent enlargement of resolution is possible. We show pictures of surface – dentin morphology obtained by SEM for comparison. Samples have been prepared using a standard method for Scanning Electron Microscopy (dehydration in a grated acetone series, drying in CPD-030, coating by a 5-nm layer of gold and palladium). Observation has been done using microscope SEM Tesla BS 340. Surface of a dentin sample with well shown tubules is shown in Fig. 5; also evidence about mechanical instrumentation is present. Apertures of tubules are without a smear layer. Surface of dentin shown under a higher resolution is depicted in Fig. 6. Apertures of tubules are mostly covered by smear layer, presence of surface distortions caused by instrumentation is visible.

**Discussion and Conclusions**

It is evident from literature that AFM is a frequently used method for imaging of dentin. It is mainly useful in studies of a collagen network of dentin and its changes caused by different chemical agents to dentin (3,15). Changes in intertubular and peritubular dentin caused by interaction with phosphoric acid, self-etching primers, conditioners

![Fig. 3: Dentinal tubules are surrounded by a wall in this image taken by AFM (scan area 25x25 µm).](image)

![Fig. 4: 3D-image of dentin surface taken by AFM provides full information (scan area 5x5 µm).](image)

![Fig. 5: Dentin surface with open dentinal tubules as seen by SEM; visible drafts remind mechanical preparation; (original magnification x1000, bar represents 20 µm).](image)

![Fig. 6: Dentin surface with concealed apertures of dentinal tubules (original magnification x5000, bar represents 5 µm).](image)
and other agents used in bonding of restorative materials (2,11,14,17,19) can be investigated. Also interaction of dentin adhesives with tooth hard tissues can be observed (1,10,12).

AFM has been applied in investigations of ultramorphology of superficial and deep dentin and its mechanical properties (6,15). Roughness and elasticity of hydrated peritubular and intertubular dentin has been followed (7). Atomic Force Microscopy enables to observe micromorphology noncarious cervical lesions (13) as well as functional width of the dentino-enamel junction (4). This method also provides information about dentin roughness (16) and enables to register changes in nanomechanical properties of dentin during its storage (5).

We would like to note at this point that AFM has also some limitations. An inevitable use of a cantilever results in many difficulties and restrictions in measurements and sample preparation (20). Furthermore, the high cost of AFM systems prevents their widespread industrial and clinical use, and they are difficult to produce in laboratories that do not specialize in AFM technology (20). When a tip scans across a sample it induces a dynamic interaction force between the tip and the surface (18). The dynamic behavior is complicated and a precise analysis is difficult, but it can influence resolution of the surface image (18).

According to our experience AFM method is not suitable for the estimation of properties of larger areas on a surface. It is time-consuming and requires a flat surface that is accessible to a tip of an AFM microscope. This means a rather strong restriction to flatness of a sample as a whole as well as that concerning flatness of its detailed parts. It is without doubts that AFM brings new possibilities in imaging of dentin surfaces. For this reason, further spreading of this nondestructive method is expected in near future. We can see its main advantage in the fact that this method enables to study wet and chemically non-modified surfaces and so it does not faces troubles connected with artifacts caused by dehydration as the other methods do.

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