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Original Article

Nature's answer to sealing the skin barrier: characterization of dental enamel and root cementum

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Abstract: Objective: The human body has evolved to maintain homeostasis through the covering of skin and mucous membranes, which separate the internal environment from the harsh and variable external milieu. Few structures naturally penetrate these coverings, and teeth are the only exception in human beings. Dental enamel and root cementum at the cervical region is of interest since that area represents the interface between a tooth and gingival tissue. A better understanding of these features might give valuable insights for designing percutaneous implants. The aim of this study was to characterize dental enamel and root cementum at the cervical region of healthy human teeth by using atomic force microscopy (AFM). Field-emission scanning electron microscopy (FESEM) was used for comparison. Methods: Twenty five freshly extracted sound human maxillary first premolars extracted for orthodontic treatment reasons were included in the study. Five premolars were used for FESEM and twenty for AFM. The enamel surface on the buccal side of the tooth was analyzed in a narrow area, 1 mm coronal to the zenith of cemento-enamel junction (CEJ). The cementum surface on the buccal side of the tooth was analyzed in a narrow area, 1 mm apical to the zenith of CEJ. The value of Ra and Rq was measured. Results: The gross appearance of the cervical enamel was characterized by overlapping wave-like layers. The border of each enamel layer was highly undulated and formed in most areas a distinct rounded step towards the underlying layer. The exposed surface of each enamel layer was relatively smooth with very shallow depressions of Tomes' processes pits. The cementum was characterized by the position of the Sharpey fibers, generally present in one or both of two distinct ways; either they appear as projections above the general plane of the mineralizing front or as a depression in this front. The mean Ra and standard deviation in μm were: enamel, 0.46 ± 0.20; cementum, 0.65 ± 0.28. The mean Rq and standard deviation in μm were: enamel, 0.58 ± 0.22; cementum, 0.79 ± 0.32. There were statistically significant differences ($P < 0.05$) among Ra and Rq between enamel and root cementum. Conclusions: Dental enamel surface and root cementum surface at the cervical region showed a clear difference in topography. Enamel surfaces were slightly smoother than root cementum surfaces. The findings from the study of the morphology of teeth gave us valuable insights for designing percutaneous implants.

Keywords: AFM, cementum, cemento-enamel junction, enamel, morphology, SEM

Introduction

Metal implants which temporarily or permanently breach the natural barrier of skin to infection have a number of clinical applications. Examples include dental and auricular implants anchored to bone, external fixators and Ilizarov frames, and intraosseous transcutaneous amputation prostheses. The longevity of percutaneous devices is often impeded by complications like exit-site infection, marsupialization or extrusion [1, 2]. Numerous approaches have been made in designing implants that overcome the problems encountered with percutaneous devices.

Biomaterials always show amazing properties, such as the structural color of butterfly wings [3], dry adhesion of gecko's foot [4], water collection of Namib Desert beetle [5], self-cleaning of lotus leaf [6] and so on, that are envied by researchers. These properties of biomaterials

are closely related with the structure optimized through million years of evolution. The human body has evolved to maintain homeostasis through the covering of skin and mucous membranes, which separate the internal environment from the harsh and variable external milieu. Few structures naturally penetrate these coverings, and teeth are the only exception in human beings. Dental enamel and root cementum at the cervical region is of interest since that area represents the interface between a tooth and gingival tissue. However, knowledge about normal variation of surface topography of natural teeth is limited. A better understanding of these features might give valuable insights for designing percutaneous implants.

Studies on the cemento-enamel junction (CEJ) using replica technique and scanning electron microscopy (SEM) have been reported previously [7]. However, SEM has its limitation as a method for topographical evaluation on tooth surfaces. This method requires special specimen preparations and examination conditions. Specimens for SEM must be dehydrated and coated with a conductive material, e.g. gold-palladium in the majority of the cases. These procedures could change natural conditions and part of surface structures. As a common method to observe surface morphology, SEM provides a good visual description of surface morphology, but it is a purely qualitative method and, prone to subjective interpretation. In addition, three-dimensional measurements can not be made directly using SEM. Therefore, in order to quantitatively characterize the topography of a tooth surface for biomimetic engineering, there is a need to adopt new methods that can provide both quantitative and three-dimensional topographic data.

Recently, atomic force microscopy (AFM) has been used to study the structural topography of enamel crystals in healthy and developmentally affected enamel [8-12]. Variations in morphology and roughness have also been reported in healthy enamel or dentin treated with different conditioning agents using AFM [13-19]. AFM technology is considered a significant advance in high-resolution imaging of biological materials. The main advantage of AFM is its ability to provide quantitative data at the nanometer level in all three dimensions with their respective images, and no sample preparation is needed [20-23]. Soft or hard tissue samples can be imaged under fluids or stains in embedded or coated conditions. Since tissues are examined in a much closer to natural condition using AFM than SEM, the surface morphology obtained in an AFM image is more likely to represent the natural conditions.

The aim of this study was to quantitatively characterize and compare the topography of dental enamel and root cementum at the cervical region of healthy teeth by AFM. Field-emission scanning electron microscopy (FESEM) was used for complement. We will use quantitative and qualitative findings from the study of the morphology of teeth to develop a device that mimics their structure, which will create a tight seal between the implant and the host tissues.

Materials and methods

Subjects and sample preparation

Subjects aged from 12 to 16 years old undergoing extraction of maxillary first premolars for orthodontic treatment reasons were recruited and agreed to donate their extracted teeth. The study design was approved by the Ethic Committee of Research of the Capital Medical University of Beijing, China. The informed consent was obtained. A total of 35 healthy erupted maxillary first premolars were collected from the Department of Stomatology, Beijing Anzhen Hospital, Capital Medical University, Beijing. The extraction of the teeth was performed following ordinary routines, i.e. teeth were gently removed with forceps and a luxator. Extreme care was taken not to touch the cervical portion of the teeth during extraction. Immediately after extraction, all teeth were cleaned and disinfected in an ultrasonic bath. Soft tissues on the teeth were removed by 30 minutes of sonication in 5% sodium hypochlorite solution. Following that procedure, the teeth were gently and mechanically cleaned with a new soft toothbrush for 30 seconds, while being rinsed in water. All procedures were performed by the same operator. All teeth were screened under a microscope (Nikon smz1000, Tokyo, Japan) at 10x magnification. Out of 35, 10 premolars with dental restorations, dental caries, cracks, structural defects or any damage resulting from forceps during extraction were excluded. Twenty five premolars with healthy enamel and

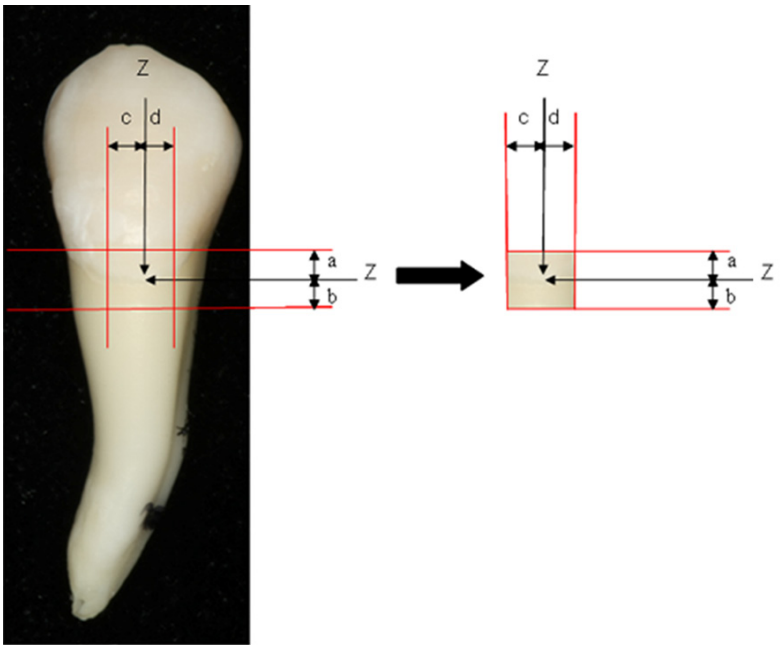

Figure 1. Schematic diagram of sample preparation. The surface on the buccal side of the tooth was analyzed in a square area. The teeth were prepared for analysis by removing the occlusal crown, 1 mm coronal to the zenith of cemento-enamel junction (CEJ), and the root, 1 mm apical to the zenith of CEJ, and toward the mesial surface, 1 mm mesio to the zenith of CEJ, and toward the distal surface, 1 mm disto to the zenith of CEJ, using a high-speed diamond rotary instrument with water-air spray. The samples for analysis were obtained by making a section about 2 mm thick and parallel to the buccal side of teeth. The surface was used for FESEM and AFM analyses. Z represents the zenith of CEJ, (a = b = c = d = 1 mm).

cementum were included in the study. Five premolars were used for FESEM and twenty for AFM. The selected teeth were placed in normal saline and stored in a refrigerator at 4°C till sectioning. The teeth used for the study were within 2 months of storage.

The teeth were sectioned as shown in **Figure 1**. The sectioning was accomplished using a high-speed diamond rotary instrument using water-air spray. The enamel surface on the buccal side of the tooth was analyzed in a narrow area, 1 mm coronal to the zenith of CEJ. The cementum surface on the buccal side of the tooth was analyzed in a narrow area, 1 mm apical to the zenith of CEJ.

Field-emission scanning electron microscopy

Five specimens were dehydrated in progressive acetone solutions (20%, 50%, 70%, and 90% acetone in distilled water and acetone at 100%), by successive 15-minute passes at room temperature. Before proceeding to the final dehydration, the specimens were desiccated by the critical point method using CO_2, and amyl acetate as a method of transference. Finally they were fixed with epoxy resin to provide rigid support. The surface morphology of dental enamel and root cementum was studied under Field-emission SEM (FESEM; FEI-QUANTA 200F, Eindhoven, The Netherlands) at an accelerating voltage of 8 KV in low vacuum mode.

Atomic force microscopy

Twenty samples were evaluated at the same scan size (50 × 50 μm^2). After cleaning and careful drying with a soft paper napkin, the sample was mounted on the AFM and fixed to the sample holder by double-sided adhesive. The surfaces of each sample were analyzed immediately at 3 randomly selected positions on enamel and root cementum, respectively, with a Bio-AFM (5500 Agilent Corp, CA, USA) in a contact mode. A Picoscan controller and a contact Mode control-box were used to control the scanner, acquire and convert analog signals to digital signals, which were then transferred to a computer via USB port. Picoscan 5.0 software was used to give command to the controller and to display data. The AFM was operated using standard silicon nitride cantilevers (Digital Instruments, CA, USA) with force constant-typical of 0.4 N/m on all samples. The nominal curvature radius of a tip is 20-40 nm. All data were collected as 256 × 256 data arrays in the trace direction. Raw AFM images were subjected to first order flattening prior to further analysis.

Recorded topographic parameters included: Ra (μm): The arithmetic mean deviation from a mean plane within the sampling area. Rq (μm): The root mean square deviation from a mean plane within the sampling area.

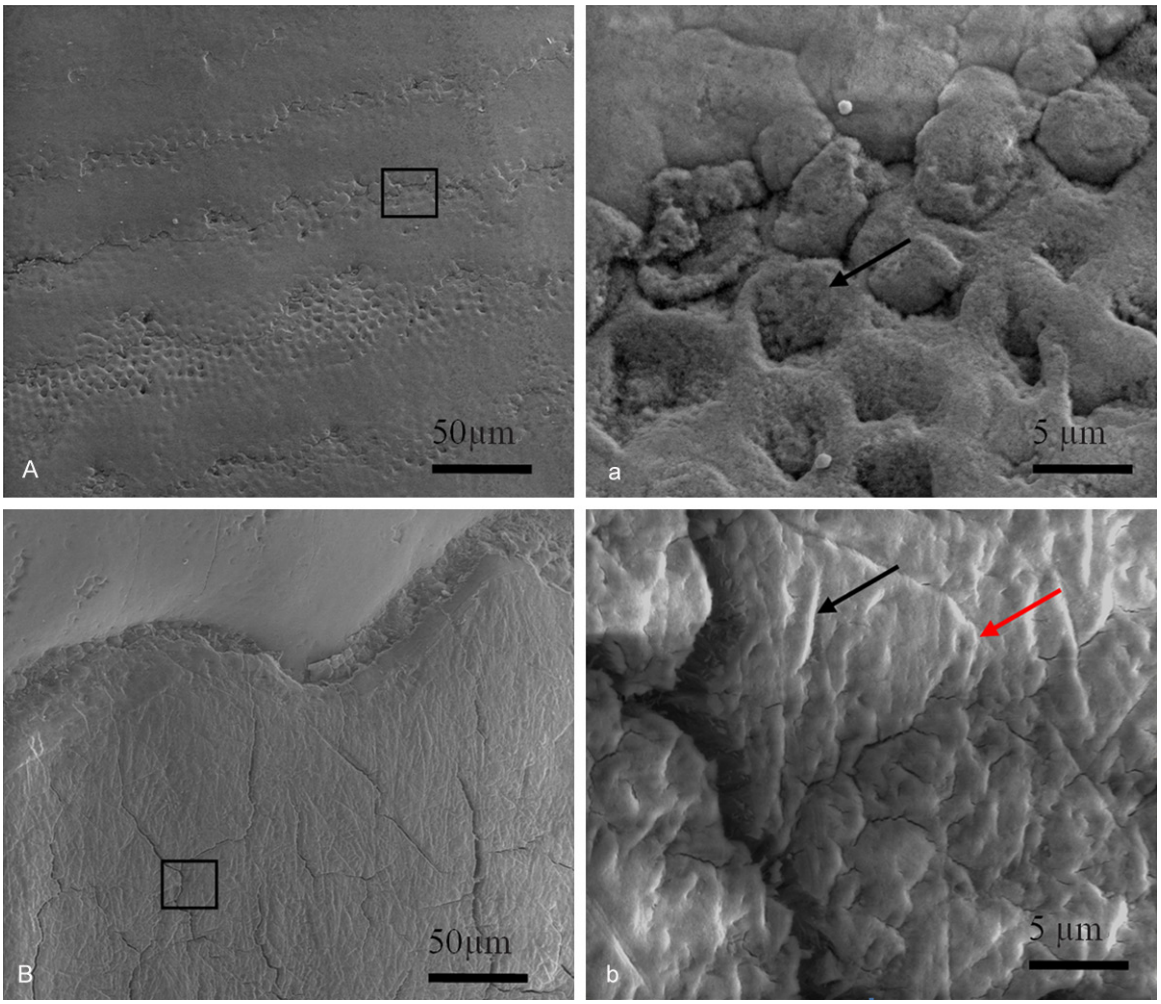

Figure 2. The SEM images of dental enamel and root cementum. A (magnification: 500×) is the dental enamel surface, bar represents 50 μm, a (magnification: 5000×) is the zoom of the square in A. bar represents 5 μm. Arrow indicates a Tomes' process pit. B (magnification: 500×) is root cementum surface, bar represents 50 μm, b (magnification: 5000×) is the zoom of the square in B, bar represents 5 μm. Black arrow indicates hypermineralization, red arrow indicates hypomineralization. The surface cracking is an artifact of dehydration.

Statistical analysis

All data were expressed as mean ± standard deviation and range. The One-Sample Kolmogorov-Smirnov Test and Homogeneity of Variance Test were used to test the distribution of variables. Statistical analysis between means was performed using nonparametric Mann-Whitney U test. The level of significance was determined at 0.05.

Results

Figure 2A (low magnification) and **Figure 2a** (high magnification) show the surface morphology of dental enamel observed by FESEM. The gross appearance of the cervical enamel was characterized by overlapping wave-like layers (**Figure 2A**). The border of each enamel layer was highly undulated and formed in most areas a distinct rounded step towards the underlying layer. Each layer was apparently separated from the underlying one by a slight gap corresponding to the opening of a stria of Retzius. At higher magnification, the very border of each enamel layer consisted of numerous irregular rounded peninsulas each of which fitted into a Tomes' process pit of the underlying layer (**Figure 2a**). The exposed surface of each enamel layer was relatively smooth with very shallow depressions of Tomes' processes pits. The diameter of the Tomes' process pit was 4 to 6 microns. Small round defects and crater-like

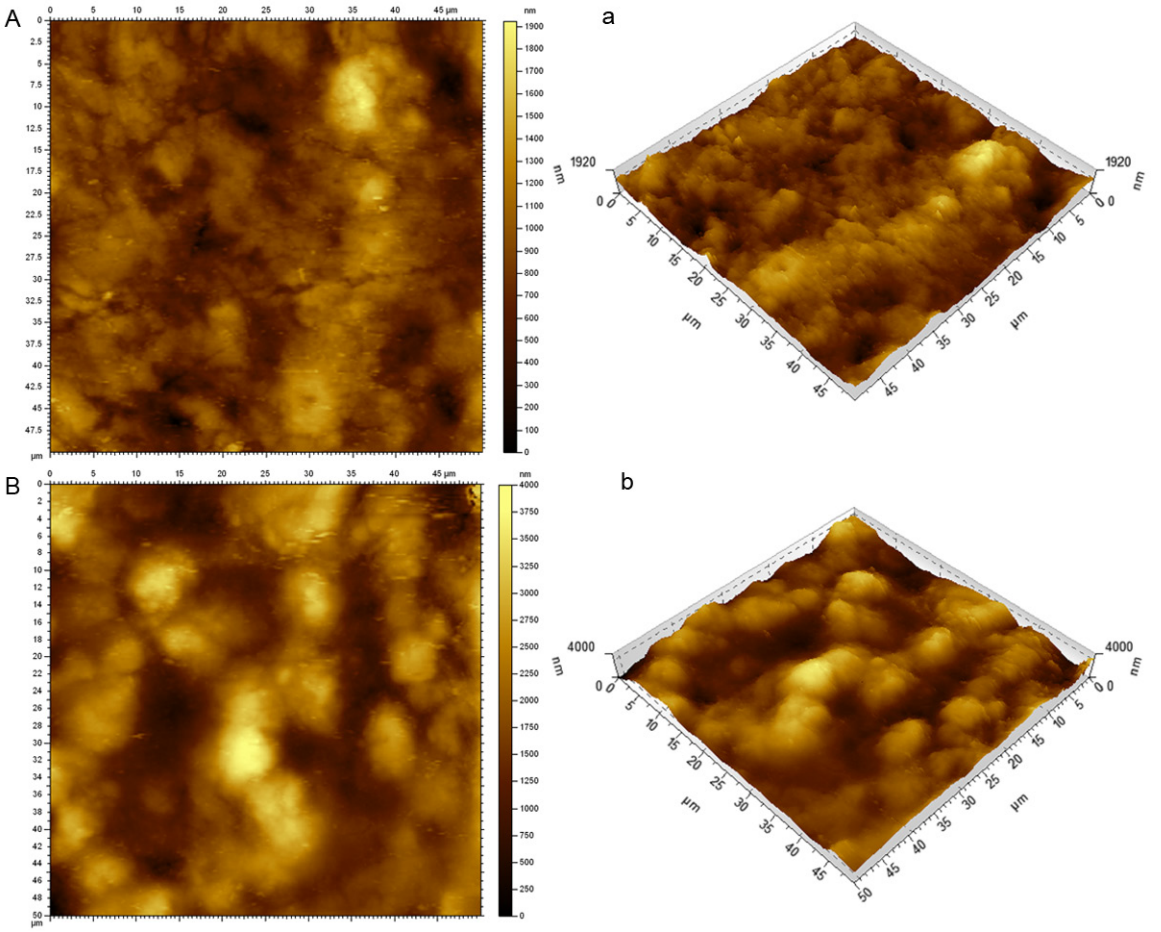

Figure 3. Representative AFM topography images and three-dimensional modified AFM images of the surface of the enamel and cementum by AFM show a clear difference in topography. The surface of the enamel appears smoother than cementum. A and a are the dental enamel surface, B and b are the root cementum surface.

focal holes were occasionally noted in the cervical enamel zone.

Figure 2B (low magnification) and **Figure 2b** (high magnification) show the surface morphology of root cementum observed by FESEM. The cementum was characterized by the position of the Sharpey fibers, generally present in one or both of two distinct ways; either they appear as projections above the general plane of the mineralizing front or as a depression in this front. The projections, low rounded mounds, represent Sharpey fibers that are mineralized to a degree beyond that of the original fibers, whereas the depressions represent the site of Sharpey fibers that are not mineralized to the same degree as the original fibers.

Figure 3A and **Figure 3a** show representative AFM topography images and three-dimensional modified AFM images of the surface of the enamel by AFM. **Figure 3B** and **Figure 3b** show representative AFM topography images and three-dimensional modified AFM images of the surface of the cementum by AFM. As shown in **Figure 3**, the surface of the enamel appears smoother than cementum.

The two roughness parameters were used to quantitatively evaluate the surface topography of the enamel and cementum. The distribution of all variables was nonparametric. The mean values, the standard deviation, and the range of Ra and Rq for each group are shown in **Table 1**. The mean Ra and standard deviation in µm were: enamel, 0.46 ± 0.20; cementum, 0.65 ± 0.28. The mean Rq and standard deviation in µm were: enamel, 0.58 ± 0.22; cementum, 0.79 ± 0.32. For Ra and Rq measured, the Mann-Whitney U test showed a significant difference between the surfaces of enamel and cementum ($P = 0.01$).

Table 1. Ra and Rq of enamel and cementum. n = 20 samples per group

	Ra (μm)		Rq (μm)	
Group	Mean ± SD	Range	Mean ± SD	Range
Enamel	0.46 ± 0.20[a]	0.113-1.120	0.58 ± 0.22[b]	0.154-1.230
Cementum	0.65 ± 0.28[a]	0.140-1.361	0.79 ± 0.32[b]	0.175-1.550

Values are expressed as mean and standard deviation. The smallest and largest observation values for the sample are expressed in ranges. [a]indicates significant difference between the surfaces of enamel and cementum in Ra ($p < 0.05$). [b]indicates significant difference between the surfaces of enamel and cementumin Rq ($p < 0.05$).

Discussion

Nature always gives us inspirations to fabricate functional materials by mimicking the structure design of biomaterials. Most of the knowledge of natural tissues has been obtained by structural studies, with a great contribution through microscopy techniques. Scanning Probe Micrroscopy (SPM) is a set of experimental methods used in imaging of surface structures at subatomic resolution. One of the clones of SPM is AFM. AFM is based on mapping of an atomic-force field on a surface of an examined sample. Imaging using AFM is non-destructive and samples can be visualized several times. Physical or chemical fixations as well as coating of surfaces by sputtering for a better contrast and conductivity are not necessary. Both conductive and nonconductive samples can be studied in this way. Artifacts caused by dehydration of samples are eliminated. According to the type of the contact between the tip of the cantilever and the sample, AFM can operate in three modes: contact mode, non-contact mode and intermittent contact (tapping) mode. In this study, AFM was used in the contact mode because it is the most suitable mode for the measurement of the surface roughness of hard tissues (enamel and cementum) and it has greater scanning speeds than non-contact and intermittent modes [16]. The details of the tooth surfaces analyzed and obtained with AFM in this study are complementary to the common SEM images and could be also correlated with data obtained by transmission electron microscopy (TEM).

The most recent high-resolution microscopy tool, AFM, in theory, allows imaging up to atomic level. The roughness is scale-dependent [24]. Jandtet al reported that surface roughness values obtained with AFM from different biomaterials can only be compared if the area of the obtained value was of similar size [25]. In this study, AFM measurements were taken for a 50 μm × 50 μm area of the surface. The result of the present study recorded less surface roughness on the enamel surfaces than root cementum. The human maxillary first premolar caries-free teeth displayed a mean surface roughness value (Ra) of 0.46 ± 0.20 μm on cervical enamel, which is a lower value than earlier reported by Zhang et al. [26] and Hosoya et al. [27] but similar to the observations made by Whitehead et al. [28], Eliades et al. [29] and Edblad et al. [30]. In the present study, the root cementum was determined to be rougher than enamel. The differences in roughness are in accordance to Kocher et al. [31] and Edblad et al. [30]. This difference is likely caused by the differences in chemical composition and crystalline texture between the enamel and the root.

The AFM images of the enamel surfaces did not show enamel prism terminations. This result can be explained by the fact that enamel prisms do not end directly on the outer enamel surface, but about 5-10 μm below it [32]. Hence the superficial layer of enamel has no prisms. This prismless enamel forms at the end of amelogenesis. This layer of enamel is found on the surface of both deciduous and permanent teeth [33]. It is known that prism-free enamel is gradually worn off during mastication, but it is retained in protected zones, cervically or interproximally.

Nature has already overcome the many problems that we subsequently encounter in the development of medical devices that are required to penetrate the skin. The results of this study may also be used as a possible reference for the surface engineering of tooth restorative materials, dental implant surfaces and percutaneous devices. Surface roughness is generally considered to be of great importance for tissue integration and bacterial adhesion [34]. The former is of advantage in the design of percutaneous devices; the latter is related to the risk of infection at the skin-

implant interface. Future studies in which various implantable materials with a characteristic enamel or cementum surface topography at cervical region of tooth may give an answer to the question whether or not these biomimetic topographic features can enhance soft tissue integration and prevent peri-implant mucositis.

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Disclosure of conflict of interest

None.

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