AFM Comparative Study of Root Surface Morphology After Three Methods of Scaling

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This study aim to compare the root surface morphology after scaling with Gracey's curette, ultrasonic tip, Periotor insert, using Atomic Force Microscopy (AFM). For every tooth have been analysed three different zones on the surface and for every zone we investigated concentric foursquare areas with the size of 0.5 / 1 / 2 / 5 / 10 / 20 mm. For every area, bi-dimensional-2D and three-dimensional-3D images, phase contrast and statistical parameters have been registered. Both ways to analyse data: directly, by the images comparing and the statistics revealed a more appropriate surface morphology obtained after scaling with Periotor inserts, followed by Gracey's curette and ultrasonic tip, which were approximately simillar in features.
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Periodontal disease affects a significant percentage of the population [1]. Literature in recent years describe the association of periodontal disease with systemic diseases with high risk and reciprocal influence [2, 3]. Treatment of periodontal disease and arresting its evolution thus gain great importance. The standard approach in treating periodontal disease is represented by non-surgical treatment consisting of the etiological stage followed by monitoring the patient throughout their lifetime. The standard method consists of scaling and root planing (SRP) that can be performed by a number of techniques [4]. SRP represents the gold-standard of the periodontal therapy. In the normal circumstance, the cell of oral epithelium almost always is the first to repopulate the area, thus the common result after the conservative treatment of periodontal therapy would be the establishment of long junctional epithelium [5, 6]. The ability of the fibroblast to adhere to the root surface (which is essential for the periodontal regeneration) depends on the existence of a clean, non-toxic surface, free from bacterial plaque and calculus. Therefore, the purpose of the SRP consists in obtaining a smooth and clean surface, biologically acceptable. Its efficacy is well documented in systematic [7, 8] and narrative reviews [9, 10] by the demonstration of gains in clinical attachment levels, reductions in probing pocket depths and bleeding on probing scores.

One of our previous studies investigated comparatively level of calculus and dental tissue loss, and the quality of the root surface after the instrumentation using three scaling techniques for deep periodontal pockets (>5 mm): manual with Gracey curette (G), ultrasonic with special tip for pockets up to 10 mm depth (US) and Profin oscillating handpiece with Periotor tips (PP) [11]. The study offered data related to the aggressiveness of the scaling techniques, but no informations were obtained regarding the surface roughness after instrumentation. The aim of this study was to compare the cementum surface morphology after scaling with one of the three scaling techniques using Atomic Force Microscopy (AFM).

Experimental part

Samples preparation

The study was conducted on 30 human extracted teeth by periodontal pathological reasons. The protocol for obtaining and the preservation of teeth until the evaluation was described in one of our previous study [11]. Teeth were randomly assigned to one of the three groups. Each group was instrumented with a single scaling technique. Group 1 (G) was hand instrumented using a Gracey's curette 5/6 (Hu-Friedy Mfg.Co., Inc.) applying 20 overlapping working strokes in vertical direction under a 60-70° working angle and applying an appropriate amount of pressure during the strokes. Group 2 (US) received ultrasonic scaling using a periodontal tip mounted on an ultrasonic hand-piece (Satelec P5, Acteon Group, Ltd.) working at 25 kHz for 15 s (20 strokes) in a vertical direction under abundant water irrigation. Group 3 (PP) was instrumented using a new concept of instruments, a reciprocating contra angle handpiece Profin with Periotor tips (Dentatus Ltd., Sweden), specially designed to adapt to the complex root morphology. The tips are mechanically driven with reciprocating strokes of 1.4mm length.

AFM investigation

The surface morphology was investigated with a complete system AFM - Atomic Force Microscope and SPM - Scanning Probe Microscope model SOLVER PRO-M, NT-MDT Russia (fig. 1).

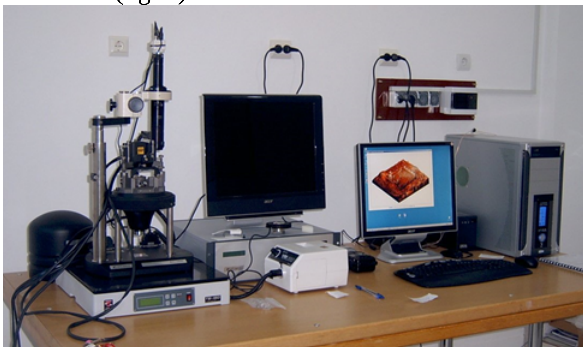

Fig.1. Atomic Force Microscope and Scanning Probe Microscope

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Atomic Force Microscopy (AFM) images were taken in air, at room temperature. The apparatus was operated in semi-contact mode, 256x256 scan point size, at different scan areas (foursquare with the side of 50, 20, 10, 5, 2, 1μm). NSG10/Au Silicon tips with a 10 nm radius of curvature and about 255 kHz oscillation mean frequency was used; the scan velocity wax 1÷ 50nm/s (depends on roughness at different scan areas). To achieve data and process them, Nova _1443 and IA_P9 soft were used.

Also, a Phase Contrast Mode technique was used in order to relieve the existence of two difference phases / materials; this technique is specific for SPM and it is not possible in other instruments who investigate the nanometer architectures (SEM or TEM for example). When there are material heterogeneities on the surface, an AFM technique, namely the phase-contrast imaging method, can analyze them. In this case, simultaneously with the amplitude of the cantilever oscillation, the change in the phase of the oscillation is registered and mapped to obtain the phase-contrast image of the sample.

Before investigation, the teeth have been conditionned by ultrasound treatment in isopropilic alcool, drying and keeping for two hours in preliminary vacuum in order to remove the dust and the monomolecular water layers from the surface.

For every tooth have been analysed three different zones on the surface and for every zone we investigated concentric foursquare areas with the size of 0.5 / 1 / 2 / 5 / 10 / 20 μm. For every area, bi-dimensional-2D and three-dimensional-3D images, phase contrast and statistical parameters have been registered.

There are two possibilities to analyse data: directly, by *the images comparing* and *statistics*. From the *images comparing* one can observe the differences in surface morphology, the nano-arhitectures and their dimmension, the homogeneity or iregularities, phase separation, the roughness, the defects, etc. [12]. *Statistical Analyzis* is another way to compare the surface morphology of scaled teeth [13]. As opposed to inorganic material (for microelectronics, sensors, solar cels, etc.), the *soft* materials (biological, polymers, etc.) have a high roughness and can be different from an area to other. Metodology: every tooth (total number 30, 10 for every scaling technique: G, US, PP) was investigated in three different zones (a, b, c); on each zone, the data from 6 concentric foursquare areas with the size of 0.5 / 1 / 2 / 5 / 10 / 20 μm were registered (totally 540 foursquare). For every foursquare, the statistical parameters of roughness were calculated, totally 540 data batch. For every foursquare, the SPM instrument get vertical data for 256(on X)×256 (on Y) pixeli = 65536 pixeli. Most used roughness parameter is Root Mean Square, Sq, with the next definition:

$$Sq = [1/n\times(x_1^{\,2} + x_2^{\,2} + x_3^{\,2} + \ldots\ldots\ldots\ x_n^{\,2})]^{1/2}$$

where n is the number of investigates points (pixels). For every zone, all the averages values were calculated; then all the means for G, US, PP were computed and ploted.

Results and discussions

The figure 2 shows an example for direct images comparing, for the three teeth prepared by the three methods (G, US, PP). One can see different architectures on surfaces who can be corelated with the scaling technique used. In samples instrumented with Gracey's curette the nano-architecture is rounded but the dimensions of defects is medium, while in smaples instrumented with US the surface morphology looks very sharp and in smaples instrumented with reciprocaing system PP one can see the mildest roughness and nano-architecture.

For all samples the value of phase contrast is about 5°÷10°, that means a good homogeneity and not phase separations. At the samples PP appears a surface texture, with 67.14 degrees orientation (fig. 3) and the *hils* are not simetrical (due to the way Periotor tips mounted into Profin reciprocating handpiece operate).

The statistical parameters of surface roughness for 5 μm section of one sample when ultrasonic scaling technique was used are presented in tabel 1a. The root mean square recorded was 105.26 nm. The calculated average values in the foursquare areas with the size of 0.5, 1, 2, 5, 10 and 20 μm are showed in table 1b.

In AFM measurement, the roughness increases with the dimension of investigated area (not linear), especially at *soft* materials; this situation appears because on extended

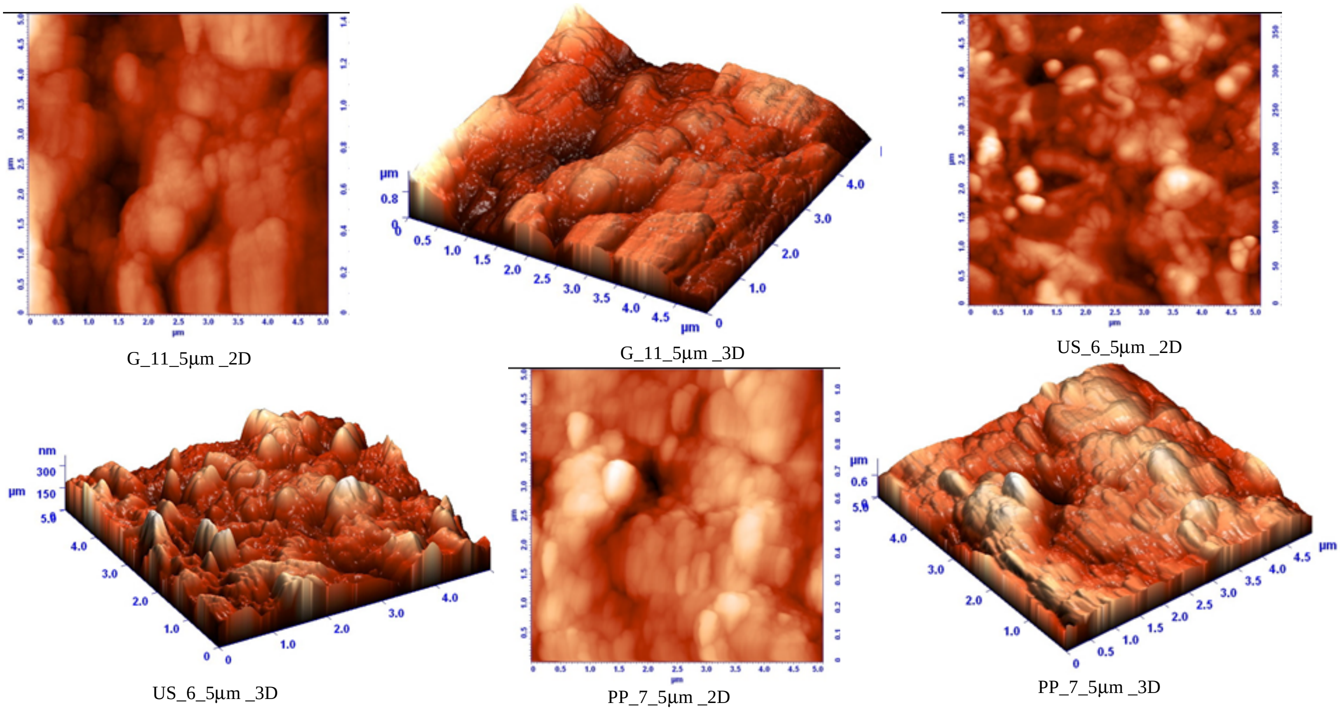
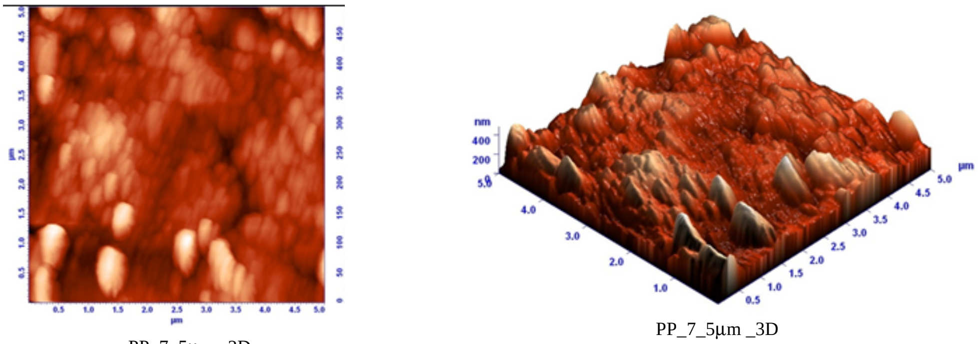


Fig. 2. Images in 2D and 3D of cementum surface instrumented with: Gracey's curette (G), ultrasonic tip (US), and Periotor tip (PP).



PP_7_5µm _2D PP_7_5µm _3D

Fig. 3. Example of 2D and 3D aspect of surface texture showing certain orientation, due to instrumentation with Periotor tips mounted in Profin counterangle handpiece (reciprocating system)

Tabel 1
STATISTICAL PARAMETERS OF ROUGHNESS AND THE CALCULATED AVERAGE VALUES PER ZONE FOR ONE SAMPLE - ULTRASONIC SCALING TECHNIQUE

Tabel 1a
Statistical parameters of roughness

US-5_a_5µm	
Amount of sampling	65536
Max	795.253 nm
Min	0 nm
Peak-to-peak, Sy	795.253 nm
Ten point height, Sz	399.756 nm
Average	453,.392 nm
Average Roughness, Sa	79,0924 nm
Second moment	465.45
Root Mean Square, Sq	105.26 nm
Surface skewness, Ssk	-0.129816
Coefficient of kurtosis, Ska	0.769674
Entropy	12.5921
Redundance	-0.30712

Tabel 1b
The calculated average values per zone for one sample number 16 – ultrasonic scaling techinque

l (µm)	Root Mean Square, Sq (nm)			
	US-16_a	US-16_b	US-16_c	media
0.5	7=.23921	7.39341	10.6479	8.43
1	14.67	11.95	22.4846	16.37
2	36.7635	25.6608	29.8687	30.76
5	67.3703	52.0857	55.7551	58.40
10	72.2807	72.2358	77.6623	74.06
20	88.7042	114.261	132.082	111.68

Tabel 2
THE AVERAGE ROOT MEAN SQUARE OF SURFACE RUGOSITY FOR ALL ANALISED FOURSQUARES

	Root Mean Square, Sq(nm)		
l (µm)	US sr1	PP sr2	G sr3
0.5	14.95	12.43	12.78
1	27.50	36.77	39.45
2	49.41	60.70	67.71
5	106.12	122.00	135.71
10	152.09	180.32	203.04
20	191.92	269.93	290.32

areas increases the probabillity to have defects and nano-structures with large dimensions. In figure 2, the diferences in surface morphology appears very clear.

Evaluation of root surface roughness is one of the most important criteria to explain the biocompatibility and the cell response of different periodontal treatments [14]. In many studies determination of the surface roughness was performed using profilometer device. In the present study, AFM evaluation was used, which is a modern and precise method. AFM investigation can give information related to the surface morphology of dental hard tissues by assessing their roughness [15]. The mandatory flat surface of the samples in profilometric measurements was no longer needed.

A lack of common opinion regarding the effect of different type of instrumentation on root surface is still in the literature. Some researches pointed that manual instrumentation might lead to massive root surface removal, while other researches reported same effects when using ultrasonic scalers [16, 17]. It has been reported also that rotating instruments increased the risk of damaging the root surface and the adjacent soft tissues [18].

When the root surface texture was evaluated in previous studies, it was showed that rotating instruments leaded to numerous scratches, while Gracey scalers determined a different texture of the surface, with a more important roughness. However, the surface roughness measurements revealed no differences between the tested instruments [19]. These controversial results might be explained by the shorter distance in evaluation the surface roughness using the profilometer.

Tabel 2 shows the averages for root mean squares of surface rugosity for the three consiedered scaling techniques for al foursquares that have been analized.

In our study Periotor device and Gracey scalers conducted to almost similar roughness of the root surface (fig. 4). The same Periotor efficiency in planing root surface and in causing abrasion was reported by other researcher [20]. The results of our research are in contradiction with other studies which have showed that the hand instruments

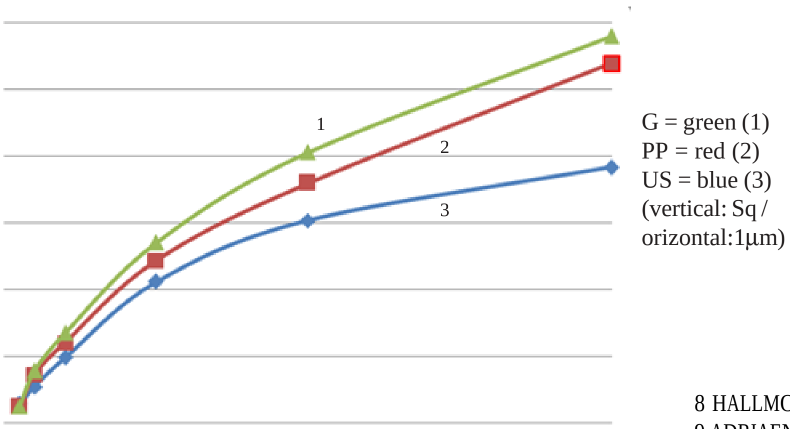


Fig. 4. Distribution curve of the means average values for the three scaling techniques: G, US, PP

produced smoother surface when compared to ultrasonic instrumentation [21- 25].

Conclusions

Surface morphology obtained after instrumentation with Periotor inserts is similar to that obtained by instrumentation with Gracey curette, and caused a “deletion” of the relief in the direction of instruments action.

Our AFM study of the root surface morpholgy consecutive three scaling techniques put in first place the ultrasonic techinque, which determined surfaces with the lowest average of root means square.

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