

Evaluation of Surface Topography Changes in Three NiTi File Systems Using Rotary and Reciprocal Motion: An Atomic Force Microscopy Study

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KEY WORDS atomic force microscope; reciprocal motion; rotary motion; endodontics

ABSTRACT Aim: To evaluate the surface topography changes in three nickel-titanium (NiTi) file systems using either rotary or reciprocal motion using atomic force microscopy (AFM), and to determine the effect of scanning area on the AFM results in this study. Methodology: Five points on a F2 Protaper file, R25 Reciproc file, and a Primary file from WaveOne systems were scanned preoperatively in 1×1 and $5 \times 5 \mu\text{m}^2$ with an AFM device that can scan an intact (not sectioned) file. One standardized resin block was used for each instrument, according to the manufacturer's recommendations. Points were re-scanned postoperatively using the same AFM and settings. Root-mean-square (RMS) and roughness average (Ra) values were obtained. The preoperative and postoperative surface topographies were compared separately in terms of RMS and Ra values. The surface topography change scores were analyzed using Kruskal–Wallis and Mann–Whitney *U* tests using a 0.10 significance level. Results: There were no significant differences preoperatively among the NiTi file systems in 1×1 or $5 \times 5 \mu\text{m}^2$ areas. Postoperatively, the WaveOne Primary had more surface irregularities (significant for $5 \times 5 \mu\text{m}^2$ scan in Ra evaluation). Conclusions: Three-dimensional AFM images of instrument surfaces showed topographic irregularities preoperatively and postoperatively. AFM results differ depending on the scanning area and file used. *Microsc. Res. Tech.* 77:177–182, 2014. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Rotary nickel-titanium (NiTi) endodontic file systems are popular because of their superior elasticity and resistance to torsional fracture versus stainless steel hand files (Thompson, 2000). However, NiTi instrument fracture can affect the success of endodontic therapy. Used files demonstrate greater surface deformation and wear than new instruments. In NiTi files, cracks arising from surface micro-scale defects may result in unexpected fracture (Inan et al., 2007; Tripi et al., 2001; Yamazaki-Arasaki et al., 2012). Thus, the surface of NiTi instruments has been examined using various microscopic methods (Ametrano et al., 2011; Martins et al., 2002; Wei et al., 2007); e.g., atomic force microscopy (AFM). AFM has become increasingly popular for imaging the surfaces and interfaces of dental biomaterials. Using AFM, Ametrano et al. (2011) evaluated the effects of two different irrigation solutions (NaOCl and EDTA) on the surface characteristics of NiTi instruments, de Assis et al. (2012) investigated the adhesion force between root canal sealers and root canal obturation cones (Gutta Percha and Resilon) following different disinfection protocols, Huang et al. (2012) evaluated the effect of intracanal dentine wettability on human dental pulp cell attachment after application of endodontic irrigants, Valois et al. (2008) evaluated the surface of rotary Ni-Ti files after multiple autoclave cycles, Salerno et al. (2010) investigated the surface damage of different conditions of air-polishing performed in vitro on

a dental restorative composite, Salerno et al. (2011) evaluated the surface morphology and the elastic properties of four dental restorative flowable composites, Salerno et al. (2012) investigated the elastic properties of one dental restoration resin composite of common use, at submicrometer spatial resolution.

AFM is a scanning-probe microscopy technique used to reconstruct three-dimensional surface topography images in real time. The sample surface is probed with a sharp tip attached to a flexible cantilever that deflects in the *z*-direction due to the surface topography. This deflection is detected by a differential photodiode. Data are recorded in digital form as *x*, *y*, and *z* values, allowing surface topography to be examined using vertical topographic parameters (Siedlecki and Marchant, 1998). The effects of various factors on instrument surfaces, such as irrigation and disinfection solutions (Ametrano et al., 2011; Topuz et al., 2008), sterilization (Valois et al., 2008), and preparation processes (Inan et al., 2007; Yamazaki-Arasaki et al., 2012), have been evaluated using AFM.

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In previous studies, files were sectioned to fit the sample holder of the AFM before scanning; therefore, two files of the same type were used for preoperative and postoperative evaluations (Inan et al., 2007; Yamazaki-Arasaki et al., 2012). Thus, evaluating the same instrument before and after usage and determining whether surface irregularities detected postoperatively, caused by usage or during manufacturing, were not possible. Comparing surface characteristics using different files preoperatively and postoperatively is unreliable. However, an AFM device, the High-Performance AFM (HP AFM, Nanomagnetics Instruments, Ankara, Turkey), can accommodate a whole file. Thus, the same file can be evaluated preoperatively and postoperatively.

Additionally, a single scanning area was used in previous studies (1×1 , 5×5 , 15×15 , or $20 \times 20 \mu\text{m}^2$) (Saglam et al., 2012; Spagnuolo et al., 2012; Topuz et al., 2011; Valois et al., 2005; Yamazaki-Arasaki et al., 2012), and the effect of scanning area was not evaluated.

NiTi root-canal preparation systems use two motions, rotary and reciprocating. Rotary motion involves full-cycle (360°) rotation in one direction; reciprocating motion involves oscillation; i.e., the instrument rotates in one direction and then reverses direction before completing a full rotary cycle (Wan et al., 2011). Protaper (Dentsply Maillefer, Ballaigues, Switzerland) is a popular rotary NiTi system; its surface topography under different conditions was evaluated using AFM (Ametrano et al., 2011; Inan et al., 2007; Saglam et al., 2012; Spagnuolo et al., 2012; Yamazaki-Arasaki et al., 2012). The surface topography of file systems using reciprocating motion has not been evaluated yet. Two reciprocating systems were introduced recently: the Reciproc (VDW, Munich, Germany) and WaveOne (Dentsply Maillefer).

Thus, we evaluated (i) surface topography changes in three NiTi file systems using rotary and reciprocal motion by AFM and (ii) the effect of scanning area size on AFM results.

MATERIALS AND METHODS

Preoperative AFM Scanning

An F2 file from the Protaper System, a R25 file from the Reciproc system, and a Primary file from the WaveOne system were examined. All three instruments were scanned with HP AFM. The handle of each instrument was marked with a reference point using permanent ink; this was used to place the same side of the rotary instruments in preoperative and postoperative scanning processes. The file was positioned in the AFM using this reference mark. Then, five points were determined on a 4-mm section of the tip of each file randomly, and their coordinates were detected using the tip of the file as a reference. The coordinates of the five points were recorded for postoperative AFM scanning. The accuracy of the repositioning is better than $1 \mu\text{m}$ under the guidance of optical microscope with $0.5 \mu\text{m}$ resolution as well as an integrated Motorized XY stage with 43 nm resolution. The five points were scanned using a cantilever with force constant of $20\text{--}100 \text{ N/m}$, resonance frequency of $130\text{--}250 \text{ kHz}$, and tip height of $15\text{--}19 \mu\text{m}$. AFM was used in tapping mode, with a $1 \mu\text{m/s}$ scanning speed. Three-dimensional images (256×256 pixels) were processed using the NMI SPM software (ver.

2.0.23; Nanomagnetics Instruments). 1×1 and $5 \times 5 \mu\text{m}^2$ areas were scanned at each point.

Operation Process

After preoperative scanning, each instrument was used on a single $10 \times 10 \times 30\text{-mm}$ resin block (VDW, Munich, Germany), with a simulated canal of 19 mm length, ISO size 15 apical foramen diameter, $44\text{--}45^\circ$ canal curvature, and 0.02 taper. In the Protaper system, Sx, S1, S2, F1, and F2 instruments were used with an X-Smart endodontic motor and handpiece (Dentsply Maillefer) at 300-rpm rotary motion. Reciproc R25 and WaveOne Primary instruments were used with the VDW Silver Reciproc motor (VDW) with the 'RECIPROC ALL' and 'WAVEONE ALL' programs, with reciprocating motion. FileCare EDTA (VDW) was used as a lubricant. All three systems were used according to the manufacturer's recommendations. The rubber stop of the files was adjusted to 18 mm from the tip and the preparations were finished when the stopper touched the flat surface of the resin block. Distilled water (10 mL) was used for irrigation.

After preparation, files were placed in an ultrasonic cleaner (SONICA Sweep System, Soltec Technology, Milan, Italy) for 5 min , filled with distilled water. The files were then removed and dried with a soft cotton swab.

Postoperative AFM Scanning

The same AFM-operating conditions were used as for the preoperative scans. The mark on the handle was used for instrument re-placing, and the recorded coordinates were used to identify the points scanned preoperatively. 1×1 or $5 \times 5 \mu\text{m}^2$ areas were scanned at each of the five points, as performed preoperatively.

Image Analyses

Even though there are roughly 20 parameters that could have been analyzed, only the roughness average (Ra) and root-mean-square (RMS) of the scanned surface areas were calculated from the AFM data. These parameters belong to the class of amplitude parameters quantifying the properties of technical surfaces (Löberg et al., 2010) we are just interested in the wear and damage on the files not the texture.

We used, [Post-Preoperative (RMS or Ra)]/Preoperative (RMS or Ra) formulation for the 'surface topography change' calculations, to eliminate the possible bias, which could be based on different starting quality of brand-new files.

These scores were analyzed for statistically significant difference by Kruskal-Wallis and Mann-Whitney *U* tests using a 0.10 significance level.

RESULTS

Three-dimensional surface topography images of the same points were obtained both preoperatively and postoperatively (Fig. 1). All the RMS and Ra values, preoperative and postoperative, as both scan sizes, and the respective post-pre difference scores, are reported in Figures 2 and 3.

In preoperative 1×1 and $5 \times 5 \mu\text{m}^2$ scans, there were no significant differences among the three instruments' RMS values ($P = 0.335$ and 0.459 , respectively).

Representative Point

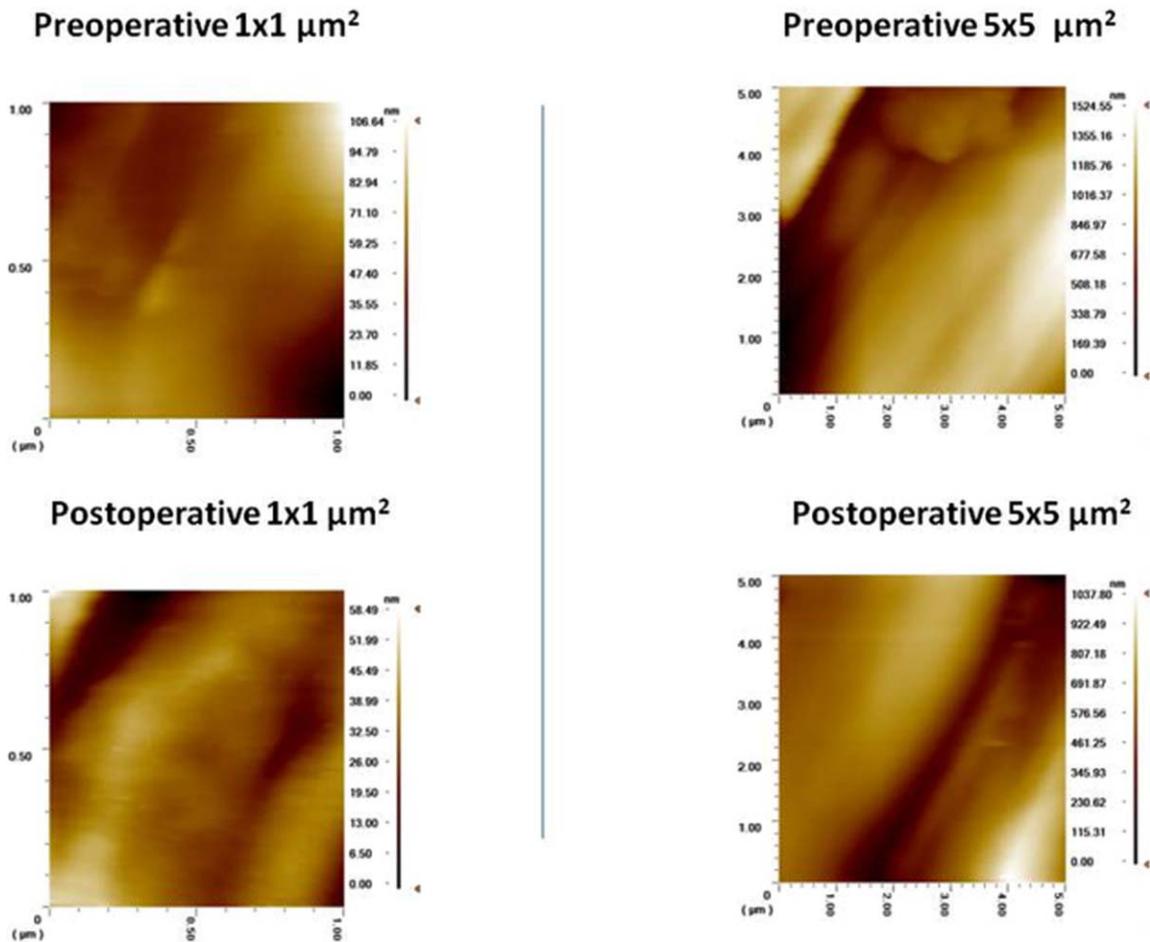


Fig. 1. Three-dimensional AFM images of a representative point on a WaveOne Primary file obtained from preoperative and postoperative 1×1 and $5 \times 5 \mu\text{m}^2$ scanning processes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In postoperative 1×1 and $5 \times 5 \mu\text{m}^2$ scans, there were no significant differences among the three instruments' RMS values ($P = 0.166$ and 0.127 , respectively).

In surface topography change scores in $1 \times 1 \mu\text{m}^2$ scans, there were no significant differences among the three instruments' RMS values ($P = 0.252$). In $5 \times 5 \mu\text{m}^2$ scans, there were no significant differences among the three instruments' RMS values ($P = 0.564$).

In preoperative 1×1 and $5 \times 5 \mu\text{m}^2$ scans, there were no significant differences among the three instruments' Ra values ($P = 0.331$ and 0.459 , respectively).

In postoperative $1 \times 1 \mu\text{m}^2$ scans, there were no significant differences ($P = 0.154$) among the three instruments' Ra values. However, in $5 \times 5 \mu\text{m}^2$ scans, the WaveOne Primary had more surface irregularities than the other two ($P = 0.084$). There was no significant difference between the Protaper and Reciproc 25 files' Ra values, and Waveone Primary and Reciproc 25 ($P = 0.460$, 0.151).

In the surface topography change scores in $1 \times 1 \mu\text{m}^2$ scans, there were no significant differences among

the three instruments' Ra values. ($P = 0.233$). And also in the $5 \times 5 \mu\text{m}^2$ scans; there were no significant differences among the three instruments' Ra values. ($P = 0.564$).

DISCUSSION

Generally, individual root canals are not anatomically identical and the hardness of dentin may also differ even in different teeth of the same type (Hülsmann et al., 2005). These factors can affect the surface irregularities of files. Thus, our use of simulated canals in resin blocks allowed standardization of results. The results may differ if extracted teeth were used because the microhardness of dentin differs from that of resin ($35\text{--}40 \text{ kg/mm}^2$ for dentin and $20\text{--}22 \text{ kg/mm}^2$ for resin) (Hülsmann et al., 2005). For removal of natural dentin, a force twice than that for resin is typically necessary (Lim and Webber, 1985).

NiTi files are exposed to irrigation solutions during root canal preparation, the effects of which, especially NaOCl, on the surface of instruments should be

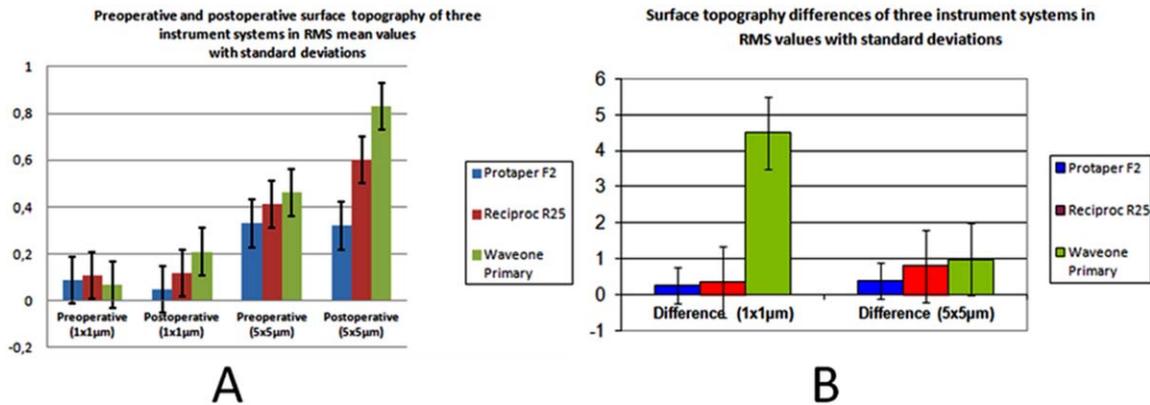


Fig. 2. A: Preoperative and postoperative surface topography and (B) surface topography change scores of three instrument systems: mean RMS scores with SDs ($P < 0.1$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

considered (Saglam et al., 2012; Topuz et al., 2008). Thus, we used distilled water for irrigation and an ultrasonic bath to prevent such artifacts due to sticking solution residues.

Alterations in a metal surface can be evaluated using either SEM or AFM. However, SEM does not provide quantitative data, while AFM reconstructs a three-dimensional image of the surface topography in real time (Siedlecki and Marchant, 1998). The peculiar features of the used HP AFM, such as a z scanner independent of (x , y) ones and its large room for sample placement in z (height of up to 5 cm) made the placement of a complete root canal instrument without sectioning and scanning of the same points preoperatively and postoperatively. In most previous studies, AFM in contact mode was used to evaluate the surface topography of root canal instruments (Inan et al., 2007; Spagnuolo et al., 2012; Valois et al., 2008), whereas here, the dynamic mode (tapping) of HpAFM was used. This mode prevents damaging the surface and the scanning tip. Using the tapping mode of HP AFM, it was possible to protect both contacting bodies (i.e., tip and sample) from lateral forces and to obtain height measurements not affected by contact side-effects such as adhesion and friction.

Preoperatively, the ProTaper, Waveone, and Reciproc instruments showed similar structural defects. These results confirmed previous reports that the manufacturing process of NiTi instruments often results in an irregular surface, characterized by milling grooves, multiple cracks, and pits (Pirani et al., 2011; Spagnuolo et al., 2012; Thompson, 2000; Valois et al., 2005). Preoperative RMS and RA values showed that the manufacturing-related surface irregularities were not significantly different among the three files.

Other reasons for surface irregularities and deformations may be the shear stresses of torsional load (Parashos and Messer, 2006) and the hardness of the contacted surface in preparation procedures. Postoperatively, excluding the Ra scores for $5 \times 5 \mu\text{m}^2$ areas, there were no significant differences in the surface irregularities of all three files.

It was not possible to determine the exact reason(s) for the postoperative topography changes. However, because we scanned the same points on the same files preoperatively and postoperatively, the surface topography changes were the result of usage. Regardless of the cause, these irregular sites may lead to stress concentration and crack initiation (Parashos and Messer, 2006).

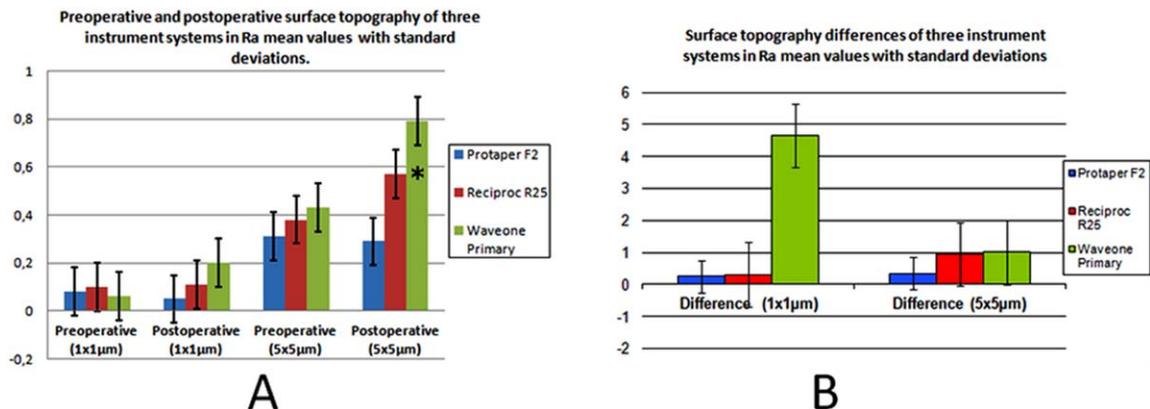


Fig. 3. A: Preoperative and postoperative surface topography and (B) surface topography change scores of three instrument systems: mean Ra scores with SDs ($P < 0.1$), * means the statistically difference. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

It is known that regardless of the instrument brand, type of NiTi alloy, or testing mode, the fatigue life is significantly higher when instruments were driven by reciprocating movement versus continuous rotation (Pedulla et al., 2013; Plotino et al., 2012). During continuous rotation, tensile stresses are concentrated in one area, whereas during reciprocating motion the tensile stresses are distributed to three points. The three instrumentation systems evaluated here used rotary and reciprocating motions.

When operating within a curved canal, the rotary instrument experiences alternating tensile and compressive stresses, which may lead to deformations and breakage (Pedulla et al., 2013; Plotino et al., 2012). An instrument subjected to reciprocating movement is also subjected to these alternating stresses, although at lower magnitudes because of the shorter angular distance traversed by the instrument (Pedulla et al., 2013; Plotino et al., 2012). Thus, greater surface deformation of a file using rotary motion was expected. The three file systems used here have different angles of rotation and speed. Protaper F2 was used with continuous rotation at 300 rpm, Reciproc 25 was used in "RECIPROC ALL" mode at 300 rpm, and the Waveone Primary was used in "WAVEONE ALL" mode at 350 rpm. The rotation type and rate may affect fatigue resistance (Gavini et al., 2012; Kim et al., 2012; Pedulla et al., 2013) and the surface changes in the instruments (Arias et al., 2012; Kim et al., 2012). Nonetheless, surface topography change was less marked in the Protaper system, which uses continuous motion. However, it was not possible to determine the effects of motion type and speed on surface irregularities.

The concepts underlying the three systems also differ. The Reciproc and Waveone systems are 'one-file' systems. The same file is exposed to the preparation procedure and so contact between the file surface and the canal increases. These factors should result in more significant changes in the surfaces of files. However, Protaper is a 'multi-file' system. Three shaping files (Sx, S1, S2) and a finishing file (F1) are used before the F2 file is used to work on the root canal. Thus, the F2 file might be subject to less stress from the preparation procedures than the Reciproc 25 and WaveOne Primary files. As expected, our data showed that the Protaper 'multi file' had fewer surface irregularities than the 'one-file' systems (though not statistically significantly so).

Differences in surface topography among endodontic instruments may be related to use of different metal alloys or design features (Castello-Escriva et al., 2012). The NiTi alloy M-Wire exhibits increased resistance to cyclic fatigue (Johnson et al., 2008). Both the Reciproc and WaveOne files use M-Wire. However, in the present study, the WaveOne Primary showed more surface irregularities than the Protaper system (though not statistically significantly so), which is manufactured of a regular NiTi alloy.

To our knowledge, there is no report evaluating the surface topography of the Reciproc and Waveone systems with AFM; therefore, we cannot compare the results here with other work. One article evaluated surface changes in the Protaper system preoperatively and postoperatively (Inan et al., 2007); our results are similar.

In the former studies, only one scanning area was used in most AFM evaluations of instrument surfaces. In the present study, 1×1 and $5 \times 5 \mu\text{m}^2$ areas at the same point were scanned to evaluate the effect of scanning area size. We identified that results may differ when a 1×1 or $5 \times 5 \mu\text{m}^2$ scanning area is used for analyses. Thus, use of one type of scanning area may provide misleading results. Further studies should determine the area size most suitable for AFM scanning of root canal instruments. Also, use of RMS and Ra scores to evaluate surface irregularities led to different results at the same points with scanning areas of identical size. There is no clear guidance in the literature as to whether RMS or Ra provides a more accurate surface roughness analysis. The most appropriate roughness parameter chosen, Ra or RMS roughness should probably depend on the specific application. For example, when considering bacterial adhesion, the spatial texture parameter of spacing of the height features should also be considered, if fitting to the size of bacterial cells; or, in case of electrochemical or surface reactions at the material, the hybrid parameter of actual interface area ratio (Loeberg, et al. 2012) should be considered.

CONCLUSION

In this study, three-dimensional AFM images of the surfaces of three files showed topographic irregularities preoperatively and postoperatively. Also, the results differed depending on the scanning area size and file used, although the same points were analyzed and the same experimental design used.

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