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Fractal Dimension and Lacunarity Analyses of Root Canal Dentin with or without Smear Layer

Smear Tabakası Varlığı veya Yokluğunda Kök Kanal Dentininin Fraktal Boyut ve Lakünarite Analizi

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Abstract

Objective: To investigate the fractal dimension (FD) and lacunarity of dentin in the presence or absence of a smear layer using scanning electron microscopy (SEM) images at various magnifications.

Materials and Methods: Extracted human mandibular premolar teeth were divided into two groups (n=5). After decoronation, the root canals were prepared. While the smear layer was left intact in the first group, it was removed with 5% EDTA and 2.5% NaOCl irrigation in the second group. The roots were split longitudinally and one half was prepared for SEM. Four images at 500 and 1000 magnifications were obtained from the middle thirds of the root canals of each specimen and saved in TIF format. The FD and lacunarity of the SEM images were calculated. Two-way ANOVA and Bonferroni tests were used for statistical analysis (p=0.05).

Results: The FD of dentin surfaces with or without a smear layer did not differ significantly (p>0.05). While magnification was an important factor in the FD of smear-free surfaces (p<0.01), it did not present any significant difference in the presence of a smear layer (p>0.05). Lacunarity showed a significant decrease in the images without a smear layer (p<0.0001). Although it demonstrated a slight increase with magnification, this increase was not significant (p>0.05).

Conclusions: Lacunarity was a differentiating factor in determining the presence or absence of the smear layer regardless of the magnification of the SEM images. FD was affected by magnification and could not discriminate the presence or absence of the smear layer. Lacunarity analysis may be a practical tool for evaluating SEM images of dentinal surfaces.

Keywords: Scanning electron microscopy, smear layer, fractal dimension, lacunarity

Öz

Amaç: Farklı büyütmelerdeki taramalı elektron mikroskop (SEM) görüntülerini kullanarak smear tabakası varlığı veya yokluğunda dentinin fraktal boyutunu (FD) ve lakünaritesini araştırmaktır.

Gereç ve Yöntemler: Çekilmiş mandibular premolar dişler iki gruba ayrıldı (n=5). Kronların uzaklaştırılmasından sonra, kanallar genişletildi. Birinci grupta smear tabakası olduğu gibi bırakılırken, ikinci grupta %5'lik EDTA ve %2,5'lik NaOCl irigasyonu ile uzaklaştırıldı. Kökler uzunlamasına ikiye bölünerek bir yarısı SEM için hazırlandı. Her örneğin kök orta üçlüsünden x500 ve x1000 büyütmede dört adet görüntü elde edilerek TIF formatında kaydedildi. SEM görüntüleri kullanılarak FD ve lakünarite hesaplandı. İstatistiksel analiz için, iki yönlü ANOVA ve Bonferroni testleri kullanıldı (p=0,05).

Bulgular: Smear tabakası olan veya olmayan dentin yüzeylerinin FD'si istatistiksel olarak farklı değildi (p>0,05). Smear tabakası olmayan yüzeylerin FD'sinde büyütme önemli bir faktör iken (p<0,01), smear tabakası varlığında herhangi bir farklılığa rastlanmadı (p>0,05). Smear tabakası olmayan görüntülerde, lakünarite anlamlı bir azalma gösterdi (p<0,0001). Lakünarite, büyütme ile hafif bir artış gösterse de, bu artış anlamlı değildi (p>0,05).

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Sonuç: Lakünarite, SEM görüntülerinin büyütmesinden bağımsız olarak, smear tabakasının varlığı veya yokluğunu saptamada ayırt edici bir faktördü. FD büyütmeden etkilenmekteydi ancak smear tabakası varlığı veya yokluğunu ayırt edemiyordu. Lakünarite analizi, dentin yüzeylerinin SEM görüntülerini değerlendirmek için kullanışlı bir gereç olabilir.

Anahtar Kelimeler: Taramalı elektron mikroskobu, smear tabakası, fraktal boyut, lakünarite

Introduction

Digital images are widely used to represent data in all fields of science. In order to extract subtle information from digital images, which are indiscernible to the naked eye, various image processing and texture analyses techniques are required (1-3). These techniques are used both in medical and dental radiology to supplement diagnosis of numerous pathologies (1-3). Other than the radiographic images, the use of microscopic images in medical and dental diagnostics has also its fundamental aim in the discernment of potential abnormalities. Recently, fractal and self-similarity properties have attracted substantial attention to represent the texture and physical properties of two-dimensional digital images (4,5).

Fractal analysis is a method for quantitative evaluation of complex geometric structures that exhibit patterns throughout the image. The fractal dimension (FD), which is calculated with a computer algorithm describes the complexity of the structure and is represented by a single number (6,7). FD is described as a measure of irregularity. However, the fact that fractals with identical dimensions can have greatly different appearances, the term lacunarity has been introduced to describe the characteristics of fractals of the same dimension with different textures (8). Lacunarity is considered as a scale dependent measure of heterogenity of texture (9). In dentistry, FD calculation has been performed on radiographs for the assessment of diagnosis of many systemic pathologies, periapical lesions and evaluation of osseointegration (4,10).

The quantification of surface topography of dentine is frequently done using SEM. Characterization of dentine surface using SEM can provide an understanding of the relationship between the surface topography and the microstructure, the mechanical properties as well as the surface generation process (e.g., coating & cutting process, wear). By quantifying the surface topography, it may be possible to obtain information for the development of new materials, the understanding of material's properties and for quality assurance. Quantification is also necessary for comparison of surface qualities. Even though SEM has been prevalently used for the interpretation of dentine surfaces due to its convenience, the qualitative nature of visual topography has created a need for more quantitative methods. For this purpose, some authors have recommended use of fractal analysis and lacunarity evaluation (11).

Therefore, the aim of this study was to investigate the FD and lacunarity of dentine in presence or absence of smear layer using scanning electron microscopy (SEM) images at x500 and x1000 magnifications.

Materials and Methods

Archives of SEM images obtained from the middle third of the root canal acquired from previous studies were examined and digital photographs of root canal dentine with smear layer present (n=40) or absent (n=40) at x500 and x1000 magnifications were used for the study. The selected images were saved in TIF format (Figures 1, 2). The FD and lacunarity were calculated on four SEM images of each tooth.

Fractal Dimension Calculation

A public domain Image J software and FracLac plug-in was used for all image processing and analysis using a differential box-counting method (NIH Image software (Image J version 1.34s software, National Institutes of Health, Bethesda, MD) (http://rsbweb.nih.gov/ij/). Rectangular regions of interests were created (ROIs) and selected comprising the whole SEM image. Identical ROI sizes were used for images with and without smear layer.

The box-counting method described by White and Rudolph (12) was used for the calculation of FD. Overall aim of the method was to remove large-scale differences in brightness of the images. For this purpose, Gaussian filter with a diameter of 35 pixels was used to duplicate and blur ROIs. This procedure leaves only large variations in density by removing all fine-and medium-scale structures. The blurred area was subtracted from the original image, and 128 were added to each pixel location. This step produces an image with a mean gray value of 128. The result is an image in which individual variations in the image reflect particular types of features with different brightness (i.e., trabeculae and marrow spaces). The image was then made binary and inverted (Figures 3, 4). After eroding and dilating once, it was skeletonized to reveal features that can be seen and measured and FD was calculated using the abovementioned software (Figures 3, 4). FD for each image was calculated by obtaining the mean of the two ROIs (13).

Lacunarity Calculation

Same ROIs were used for lacunarity calculations. According to the Plotnick et al. (9) lacunarity can be defined in terms of local means and variance measured for different neighborhood sizes for each pixel in an image. Images with high lacunarity values indicate wider range of sizes of structures (9). Lacunarity was calculated using FracLac plugin that compares digital images for many morphometrics including lacunarity (13).

Statistical Analysis

The FDs of ROIs from the images with and without smear layer were compared using two-way ANOVA and Bonferroni tests (p=0.05).

Results

Table 1 shows the mean and standard deviation (SD) values of FD and lacunarity for the groups with and without smear layer. Samples with smear layer and with x500 magnification had a mean FD of 1.79 (± SD, 0.02) while samples with smear layer at x1000 magnification showed 1.78 mean FD (± SD, 0.03). On the other hand samples without smear layer had a mean FD of 1.80 (± SD, 0.02) and 1.78 (± SD, 0.02) consecutively at x500 and x1000 magnifications (Table 1).

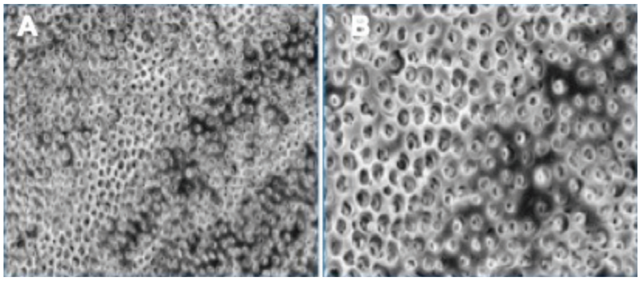

Figure 1. Smear-free root canal dentine at x500 (A) and x1000 (B) magnifications. Sample image showing the region of interest used for FD analysis

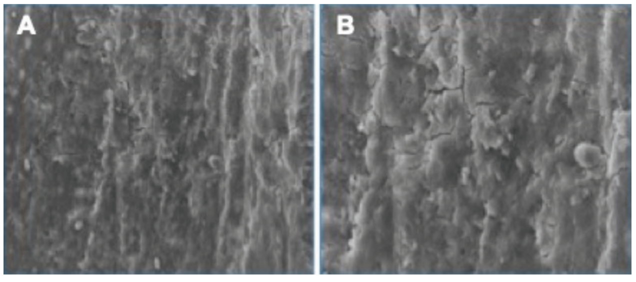

Figure 2. Root canal dentine with smear layer at x500 (A) and x1000 (B) magnifications. Sample image showing the region of interest used for FD analysis

Mean lacunarity measurements for samples with smear layer were 0.66 (±0.13) and 0.71 (±0.24) simultaneously at x500 and x1000 magnifications. While samples without smear layer and with x500 magnification had a mean lacunarity of 0.50 (±0.15), samples without smear layer at x1000 magnification showed mean lacunarity of 0.53 (± 0.11).

The FDs of dentin surfaces with or without smear layer were not significantly different (p>0.05). While magnification was an important factor in FD of smear-free surfaces (p<0.01), it did not present any significant difference in presence of smear layer (p>0.05). However, the lacunarity properties of dentin presented different results. Lacunarity showed a significant decrease in images without smear layer (p<0.0001). On the other hand, it demonstrated a slight increase with magnification; however, this increase was not significant (p>0.05).

Discussion

FD uses a statistical surface examination to delineate surface microarchitecture and gives the numerical value of the complexity as demonstrated on an image (13). It is a precise, intact and effectively accessible method. Even though, fractal geometry has contributed to the description of complexity (14) computation of FD alone cannot always provide unequivocal descriptions. In order to provide unique description, further concepts such as lacunarity have

Table 1. FD and lacunarity values ± SD of dentin images with (smear +) or without (smear -) smear layer at x500 and x1000 magnifications

	FD ± SD	Lacunarity ± SD
Smear (+) x500	1,794±0.02	0.662±0.13
Smear (-) x500	1,803±0.02	0.503±0.15
Smear (+) x1000	1,777±0.03	0.709±0.24
Smear (-) x1000	1,778±0.02	0.526±0.11

FD: Fractal dimension, SD: Standard deviations

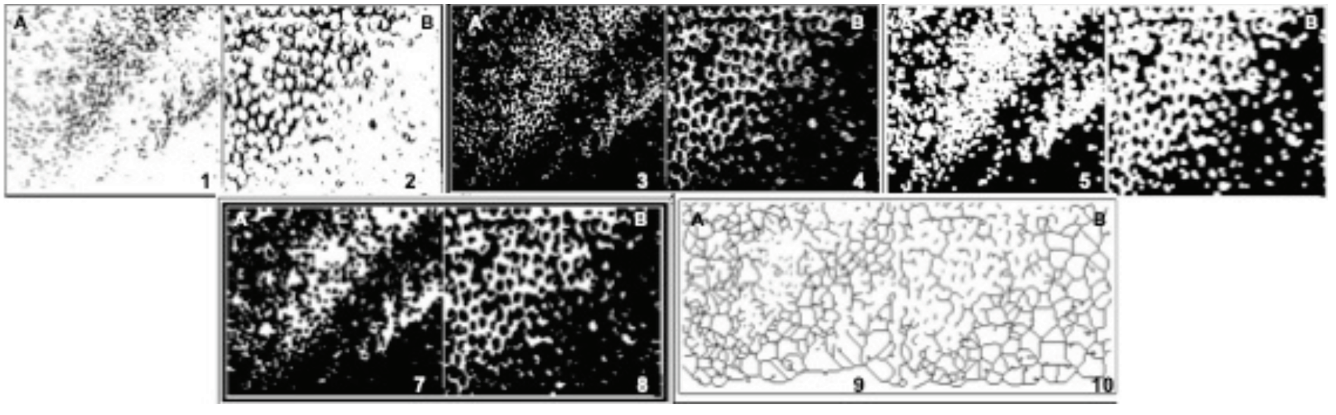

Figure 3. Binarized (1-2), inverted (3-4), eroded (5-6), dilated (7-8) and skeletonized (9-10) sample

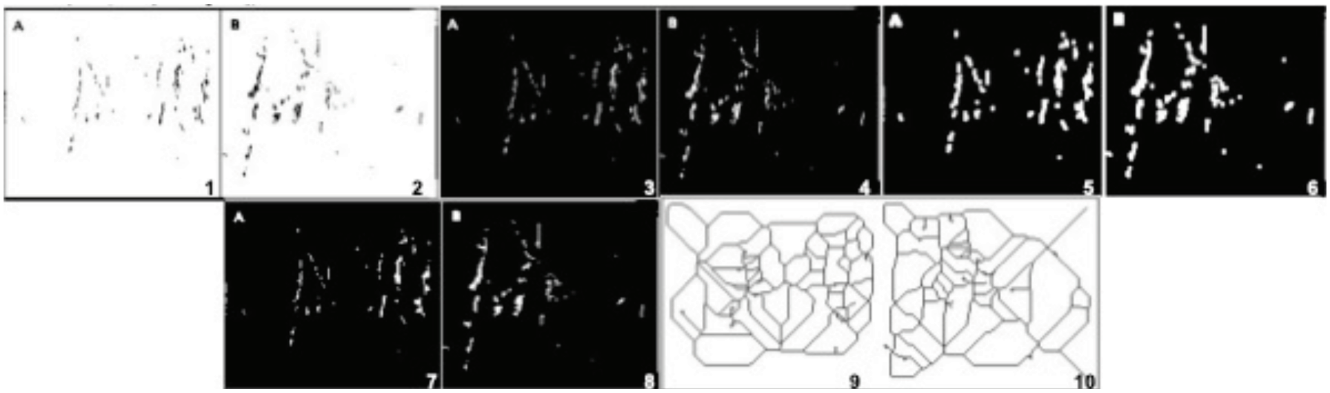

Figure 4. Binarized (1-2), inverted (3-4), eroded (5-6), dilated (7-8) and skeletonized (9-10) sample images of the region of interest of root canal dentine with smear layer at x500 (A) and x1000 (B) magnifications

been recommended to discriminate complex structures demonstrating similar FDs, while looking totally different. One of the individual depictions of lacunarity is scale-dependent measure of heterogeneity however; it can be also characterized as the distribution of voids in the organized series (14). It has been advocated that lacunarity can be used to disentangle patterns in every kind of images including computer graphics (11,15).

SEM is the most frequently used technique for the delineation and characterization of surface topography giving details regarding the surface features. However, the major shortcoming with SEM is that it solely shows the qualitative characteristics of the surface texture. It is very difficult to match or classify SEM images of surface topographies with regard to visual inspection since it is usually performed subjectively and superficially. Accordingly, many studies have included lacunarity analysis along with FD to elucidate information from electron microscopy images (11,16). Nevertheless, this is the first study using both FD and lacunarity to compare the changes in dentine heterogeneity in presence and absence of smear layer as measured on SEM images. Once SEM images of dentine surfaces treated with different materials have been quantified using FD and/or lacunarity analyses, they can be compared and categorized in an objective and standard manner. Simultaneous use of FD and lacunarity determines surface topography changes with respect to changes in treatment materials, methods and/or parameters.

The FD values found in the presented study indicate that FD is (moderately) reduced after EDTA and NAOCl treatment. However, in terms of overall complexity, dentine with and without smear layer showed rather similar FD values. In other words, FD was not able to discriminate presence or absence of smear layer in SEM images and was affected by magnification of the images.

The so-called box-counting method gives the estimate of the box numbers that are needed to cover the grid of particular size at various scales. Box-counting, is the most frequently used method for the calculation of FD (17). The box-counting method pretends that the image under observation has only white pixels (1's) and black pixels (0's), which are the foreground and background respectively and thus a binary image. Calculating FD can quantitatively compare inherent roughness and derangement of different images. However, it was already proved that FD is not an individual and adequate measure, i.e., two images that look quite different may generate the same FD due to similarities in roughness (9,18-20). Furthermore, it was demonstrated that FD is a poor descriptor to quantify and compare SEM images of surface topographies and FD values depend on the microscopic magnification and on the algorithm used to compute these values (21). Complexity and surface characteristics are profoundly scale dependent. Surfaces with same topography may appear coarse and very fine depending on the magnification of the image (22). Due to the above-mentioned factors, FD analysis of SEM images of dentine could not discriminate the presence and absence of smear layer in the present study.

The automated image analysis particularly deals with particle shape characterization. Geometrical information that is not effected by scale, rotation and translation was defined as the shape. Several descriptors such as circularity and shape factor have been used to characterize the shape of objects independent of their size both of which requires the boundary perimeters. However, even though the shapes look very much alike to the naked eye basically the same the divergence in shape descriptor values could be very large due to the difference in perimeter, which is majorly dependent on scale of observation. Therefore, difference in FD could be expected for the same image at different magnifications. It was previously proved that shape descriptors of fractal objects involving boundary lengths are scale variant (14).

While the FD is a poor descriptor of surface complexity, lacunarity has been claimed to be a feasible technique for the analysis of SEM images for surface texture analysis (11). The results obtained in this study also substantiated the previous results that lacunarity is in fact sensitive to the treatment applied to dentine surfaces. Lacunarity of the SEM images of dentine decreased significantly with the removal of the smear layer and this decrease was not affected by the magnification of the images. It has been described that lacunarity is the measure of the discrepancy of an object or fractal from translational uniformity (9). Objects with high and low lacunarity values were characterized as coarse and fine texture respectively (11). The results found in this study verify this definition too, since dentine with smear layer demonstrates rough and bold surface pattern while clean dentine surface free of smear will show more delicate and organized surface characteristics. Ling et al. (11) have reported similar results that lacunarity analysis is a powerful tool that can be used to characterize surface characteristics and contours of SEM images. Furthermore, its use is not limited to this task. It was previously proved that lacunarity could also be used to assess osteoporosis, to differentiate benign from malignant tumors, to analyze the behavior of prostate and breast cancer and microvascular morphology (2).

Conclusion

Finally, this study employs the box-counting FD and lacunarity from SEM images with and without smear layer at two different magnifications. Results show that lacunarity can serve as a potential tool in determining the presence or absence of smear layer regardless of the magnification of SEM images. FD was affected by magnification and was not able to discriminate presence or absence of smear layer in SEM images. Lacunarity analysis may be a practical tool to evaluate SEM images of dentinal surfaces.

Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.G.B., B.H.Ş., Design: B.G.B., B.H.Ş., Data Collection or Processing: B.G.B., Analysis or Interpretation: B.G.B., B.H.Ş., Literature Search: B.G.B., Writing: B.G.B., B.H.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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