FRACTAL ANALYSIS OF HEALTHY HUMAN PERIODONTIUM

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Summary
The objective of this study is to perform a study of complex morphological features that characterise cells and tissues of healthy human periodontium using the fractal geometry. The histological and histomorphometric analyses were performed at different locations around the gingival tissues. A set of forty digital images corresponding for the granular, spinous and basal layers of healthy human periodontium, randomly selected from a database, were evaluated. The fractal analysis of digital images was performed with the Image J fractal analysis software and the fractal dimensions were calculated using the standard box-counting method. We found that the healthy human periodontium has fractal properties. The central tendency and dispersion measure of the fractal dimensions were expressed by the mean value and standard deviation. The mean fractal dimensions were for: a) granular layer: 1.0639 ± 0.0011 for cell membrane, 1.0503 ± 0.0012 for nuclear membrane of cell, 1.0453 ± 0.0014 for nucleolus membrane of cell; b) spinous layer: 1.0938 ± 0.0014 for cell membrane, 1.0651 ± 0.0012 for nuclear membrane of cell, 1.0511 ± 0.0011 for nucleolus membrane of cell; c) basal layer: 1.0798 ± 0.0012 for cell membrane, 1.0562 ± 0.0011 for nuclear membrane of cell, 1.0504 ± 0.0014 for nucleolus membrane of cell. The fractal analyses were in agreement with the histological observations. Fractal analysis of the human periodontium structure using the fractal geometry is an efficient noninvasive prediction tool for early detection of patients with different periodontal diseases.

Keywords: image analysis, fractal, fractal dimension, human gingival layers, periodontium, shape

Introduction
Mandelbrot's fractal geometry provides a mathematical model for the description of many complex biological geometric structures (Mandelbrot, 1982; Kenkel and Walker 1996; Losa et al., 2005).

Over the last decades, in the field of dentistry, different methods to analyze and predict the structure of cells and tissues of healthy human periodontium for the evaluation of healthy or the detection of the periodontal disease were performed (Xiang, 2007; Parvu et al., 2011).

These methods and measurements prove sufficient for some studies (Xiang, 2007; Parvu et al., 2011), however they are less well suited for quantifying changes in the morphology of human periodontium (normal and pathological) cell shape, size and tissue hierarchical structure.

Complex anatomical systems admit many descriptions (Grizzi and Chiriva-Internati, 2005). Shape descriptors have proven to be a useful tool with a great
potential for medical image processing applications.

Various shape descriptors exist in the literature for 2D and 3D images, mainly categorised into two groups: contour-based shape descriptors and region-based shape descriptors (Laitakari, 2003; Zhang and Lu, 2004; Pincus and Theriot, 2007; Martinez-Ortiz, 2010).

Fractal theory offers methods for describing the complexity and irregularity of anatomic structures that comprise organs, tissues and cells considered as fractals objects.

Fractal objects have properties that include self-similarity, scale independence, complexity, and infinite length or detail (Lopes and Betrouni, 2009).

The fractal dimension, very often non-integer, can be viewed as a relative measure of complexity, or as an index of the scale-dependency of a pattern and it determines how the fractal object differs from Euclidean objects (Reljin and Reljin 2002; Nailon, 2010; Haidekker 2011).

Biofractals are the fractal textures/contours in biology whose properties aid in the classification of biological and medical data and images (Sztójajnov et al., 2009).

Fractal analysis is a useful method for quantifying the complexity of the cells architecture (Smith et al., 1996; Ichim, 2007; McNally and Mazza, 2010).

The fractal dimension depends on the methodological and experimental parameters involved as: diversity of subjects, image acquisition, type of image, image quality, its processing, fractal analysis methods, including the algorithm and specific calculation used (Reljin and Reljin 2002; Lindström, 2008; Talu and Giovanzana, 2011; Ivanovici and Richard, 2011; Talu and Giovanzana, 2012; Talu, 2012).

In our study we have investigated the cells and tissues microstructure in healthy human periodontium using fractal dimensions.

### Fractal analysis

The fractal analysis of binary images was made using one of the simplest and most intuitive algorithm - the standard box-counting algorithm (Falconer, 2003).

#### Fractal method

Let’s consider a fractal object recorded into a digital image.

Let A be any nonempty bounded subset of $R^n$ ($n$-dimensional Euclidian space $R^n$). The fractal dimension gives the scaling between the smallest number of $n$-dimensional $\varepsilon$ boxes needed to cover the set A completely, and the boxes’ size $\varepsilon$.

The box-counting fractal dimension of $A$ is expressed by (Falconer, 2003):

$$D_B(A) = \lim_{\varepsilon \to 0} \frac{\log N_\varepsilon(A)}{\log(1/\varepsilon)}$$  \hspace{1cm} (1)

In equation (1) the zero limit cannot be applied to biological images (Grizzi et al., 2005) and $D_B(A)$ can be estimated by means of the equation:

$$D_B(A) = D$$  \hspace{1cm} (2)

where $D$ is the slope of the regression line for the log-log plot of the scanning box size and the count from a box counting scan.

The “count” usually refers to the number of grid boxes that contained pixels in a box counting scan. The slope of the linear region of the plot is $(-D)$, where $D$ is the box-counting dimension that corresponds to the fractal dimension.

#### Fractal and statistical analysis

In our study, fractal analysis of images corresponding to the silhouettes or the outlines of the fractal objects were computed applying the standard box-counting algorithm to the digitized data, using the Image J software (Wayne Rasband, National Institutes of Health, in Bethesda, Maryland, USA) (http://imagej.nih.gov/ij) together with the FracLac plug-in (A. Karperien – Charles Sturt University, Australia) (http://rsbweb.nih.gov/ij/plugins/frac lac/FLHelp/Introduction.htm). Lastly, all the raw data were exported and analyzed in Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, Washington, USA).
Descriptive statistics were calculated for gingival cells in each group and the obtained average results were expressed as mean value and standard deviation. It was found that fractal dimensions $D$ of the granular, spinous and basal cells followed a normal distribution.

The average values of the fractal dimensions presented are statistically highly significant ($p < 0.01$).

**Material and methods**

The protocol was approved by the Ethics Committee of University of Medicine and Pharmacy, Iuliu Hatieganu Cluj-Napoca, Romania. All subjects gave informed consent to participate.

**Collection of gingival tissues**

Biopsy specimens of gingival mucosa were harvested from healthy subjects with normal mucosa. The biopsies were taken under local anesthesia (1 % Lidocain).

**Light and electron microscopy**

Immediately after excision, all specimens were transferred to a 2.7 % glutaraldehyde solution in phosphate buffered saline (PBS) 0.1M, pH 7.2 for 90 min at 4°C for pre-fixation. Than they were washed in four successive baths with PBS 0.15M pH 7.2 for 4h at 4°C.

Biopsies were post-fixed with 2 % osmic acid in PBS 0.15M pH 7.2 for 75 min at 4 °C. Dehydration was performed in a graded acetone series (50 %, 70 %, 80 %, 90 %, absolute) at room temperature, 30min in each bath. The samples were infiltrated and embedded with Epon 812.

Sections were cut on a Leica UC 6 ultramicrotome (DDK diamond wheel (Craciun and Horobin, 1989; Hayat, 2000; Toader, 1996).

Semi-thin sections of 200-400 nm thick were stained with Epoxy tissue stain for light microscopy (LM) and orientation purposes. For light microscopy were used an Olympus BX 51 microscope, a CCD Media Cibernetics camera, and Image Pro Plus software (Kuo, 2007; Kay, 1967).

Ultra-thin sections of 20-40 nm were cut and stained with uranyl acetate and lead citrate and examined on a JEOL JEM 1010 transmission electron microscope (Japan Electron Optical, Ltd., Tokyo, Japan). For TEM were used a Megaview III camera and Soft Imaging Analysis software (Pavelka and Roth, 2005; Ploaie and Petre, 1979).

Let us consider a set of fourty digital images corresponding to the tissues of healthy human periodontium, selected from the database, for the granular, spinous and basal layers (Parvu et al., 2011).

![Fig. 1](image-url)
After correction of the digital images, by contrast adjustment and spatial filtering, the binary skeletal patterns randomly chosen were extracted, using the segmentation and morphological operations, from the original digitalized images with the structuring elements.

The algorithm for fractal analysis was applied with the following options: a) Grid positions – 12; b) Calculating of grid calibers – use default box sizes.

**Results and discussions**

A summary of the obtained results is presented in the table given below.

<table>
<thead>
<tr>
<th>Type</th>
<th>((D_{m1}))</th>
<th>((D_{m2}))</th>
<th>((D_{m3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granular cells</td>
<td>1.0639 ± 0.0011</td>
<td>1.0503 ± 0.0012</td>
<td>1.0453 ± 0.0014</td>
</tr>
<tr>
<td>Spinous cells</td>
<td>1.0938 ± 0.0014</td>
<td>1.0651 ± 0.0012</td>
<td>1.0511 ± 0.0011</td>
</tr>
<tr>
<td>Basal cells</td>
<td>1.0798 ± 0.0012</td>
<td>1.0562 ± 0.0011</td>
<td>1.0504 ± 0.0014</td>
</tr>
</tbody>
</table>

Table 1. Results of the fractal dimensions for the analyzed cells (average ± standard deviation).

Note:
- \((D_{m1})\) = monofractal dimension for cell membrane;
- \((D_{m2})\) = monofractal dimension for nuclear membrane of cell;
- \((D_{m3})\) = monofractal dimension for nucleolus membrane of cell.

The obtained average results were expressed as (average ± standard deviation).

For all analyzed cases (Table 1), the coefficients of correlation \((R^2)\), that characterizes the goodness-of-fit of the regression line, were more than 0.9965 representing a good linear correlation. An \((R^2)\) of 1.0 indicates that the regression line perfectly fits the data.

The real fractals have \((R^2)\) values smaller than 1.0. The digital images of human gingival cells and tissues microstructure exhibit fractal properties.

The fractal analyses were in agreement with the histological observations.

**Conclusions**

The fractal analysis of digital images of healthy human gingival cells and tissues, using the standard box-counting method was presented.

Fractal analysis provides a deeper insight and can detect subtle morphologic changes in human gingival cells and tissues and can provide detailed information for investigation of healthy and diseased gingival patients.

This method is simple, reproducible and inexpensive permitting retrospective studies without any additional costs.

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