FRACTAL ANALYSIS OF BONE CELL SYNCYTIUM IN NORMAL AND DISEASED BONE

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INTRODUCTION

Fractal analysis is a mathematical technique for quantification of complex structures; the fractal dimension (FD) of a structure is a measure of its geometrical complexity. FD has been used as a mathematical descriptor to differentiate pathologic and normal tissues (e.g. based on bone radiographs[1]) as well as to define cellular structure (e.g. in micrographs of neurons[2]). The diagnostic power of FD to differentiate healthy and diseased bone tissue is unclear. Based on tissue level trabecular geometry, bone radiographs from osteoporotic patients have been assigned high[3] and low[4] FD values. Recent studies[5-7] show that state of the cellular syncytium describes the state of health of bone tissue as a whole. Remarkable changes in interconnectedness and orientation of the lacunocanalicular network are observed in bone specimens from patients with osteoporosis, osteoarthritis, osteonecrosis and osteomalacia[5,6]. The objective of this study was to quantify the changes in interconnectedness and geometrical complexity in micrographs of bone from patients with bone disease. We calculated FD of the lacunocanalicular network and investigated the applicability of FD as a diagnostic tool in orthopaedics.

METHODS

Micrographs of normal and pathologic bone were obtained from a previous study[5,6] investigating cortical bone from human femora that was diagnosed by a pathologist as healthy or overtly osteoporotic, osteoarthritic and osteomalacic. Samples were stained with basic fuchsin. Images were obtained using the laser scanning confocal microscope with excitation/emission spectra comprising 568 nm/580-624 nm.

Image files were converted into binary 8 bit gray scale and FD was calculated based on the Box-Count-Method (ImageJ shareware, NIH, USA), where different sized quadrants are applied as a grid to cover the structure within a given image. For each quadrant size, the number of quadrants intersecting the boundary of the structure is counted and a scatter plot is drawn on a log-log scale based on number and size of the quadrants. Using this procedure, the FD is the negative of the slope of the linear portion of the plot. Quadrant sizes comprising 2, 3, 4, 6, 8, 12, 16, 32, and 64 pixels were used in this analysis.

RESULTS

Three distinct ranges of FD magnitudes were found for normal (N), osteoarthritic (OA), osteoporotic (OP) and osteomalacic (OM) bones (Table 1). Osteoarthritic bone exhibited the lowest range of FD values. Osteoporotic bone also showed a decrease in FD compared to the normal bone samples. In osteomalacic bone, the FD was higher than that of normal bone.

Pathology	N	OA	OP	OM
FD range	1.49–1.60	1.28–1.36	1.25–1.42	1.55–1.65
FD mean	1.54	1.30	1.35	1.60
standard deviation	0.028	0.019	0.057	0.038
standard error	0.008	0.005	0.016	0.010
sample size	13	13	13	13

Table 1. FD ranges for normal and pathologic bone.

DISCUSSION

Based on these preliminary studies of bone micrographs, FD of unhealthy bone may decrease or increase from the normal range depending on the type and degree of pathology present in the tissue. Current studies aim to define the spectrum of FD in cohorts of patients (normal, osteoporotic, osteoarthritic) of the same age and gender. Furthermore, in healthy bone, lacunocanalicular structure appears to

be exemplified by an optimal value of FD, whereby deviation from this value may be indicative of pathology.

To our knowledge, these are the first studies in which fractal analysis has been employed at a cellular level to characterize bone disease. These cortical bone data corroborate previous studies in which a decrease in FD of osteoarthritic and osteoporotic cancellous bone in comparison with normal bone[1] was described. Another study[3] reported higher FD magnitudes in osteoporotic cancellous bone; this discrepancy could be attributable to differences in sampling site (e.g. cortical vs. cancellous bone) and image magnification (cellular vs. tissue level organization). However, the values reported in this study were corroborated in sensitivity measurements using different sized quadrants in different areas of tissue. In general, sharply defined structures tend to have higher magnitude of FD compared to structures with smooth geometry. Normal bone is characterized by high degree of interconnectedness and almost radial orientation of the lacunocanalicular network, resulting in higher FD value. In contrast, osteoarthritic and osteoporotic bone show a marked decrease in interconnectedness and orientation, resulting in a lower FD value than normal bone. Finally, osteomalacic bone shows much higher interconnectedness and sharpness resulting in a higher FD value than normal bone.

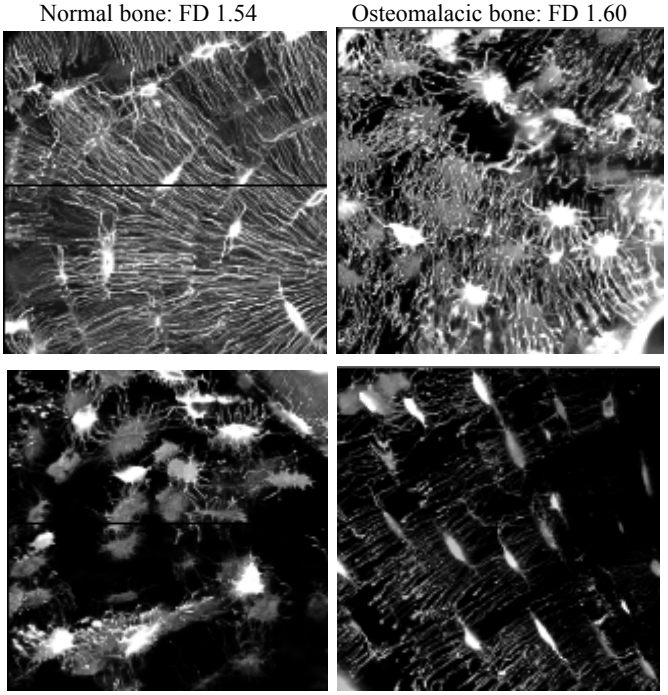

Figure 1. Micrographs exemplifying normal and pathologic bone and their corresponding FD values.

Considering the space filling characteristics of fractal structures in two dimensions, the lower magnitude of FD in osteoarthritic and osteoporotic bone reflects a decrease in the space filling capacity of the bone cell syncytium for these disease states. This may further reflect a reduction in efficiency of transport of metabolites and nutrients from the blood supply to cells[8] in osteoarthritic and osteoporotic bone. Despite the higher space filling capacity of osteomalacic bone, its matrix remains softer than that of normal bone. One explanation may be that the increased tortuosity of the lacunocanalicular network decreases transport efficiency[9] and increases energy requirements for cell survival.

In a parallel study[9] of Haversian canal networks, which represent a hierarchical level of anatomy above the lacunocanalicular system (LCS), we calculated fractal dimensions in human, horse, pig and dog bone specimens; the Haversian system FD ranged from 1.61 to 1.90. Hence, the FD of the Haversian canal system is similar to that of the lacunocanalicular system (LCS); this is referred to as "self-similarity" in fractal structures[10]. Interestingly, in self-similar fractal structures[10] the energy required to distribute resources is minimized. If the Haversian canal and lacunocanalicular systems are organized for efficient transport in normal, healthy bone, a deviation in FD from normal values may be indicative of bone pathology. may also be indicative of bone pathology.

The results of the present study suggest that FD is a useful tool for diagnosis of bone pathology. To our knowledge, this is the first report on FD of the bone cell syncytium. Current studies on cohorts of patients should provide a means to validate the applicability of FD as a diagnostic tool in orthopaedics.

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