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Fractal analyses of osseous healing using Tuned Aperture Computed Tomography images

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Abstract The aim of this study was to evaluate osseous healing in mandibular defects using fractal analyses on conventional radiographs and tuned aperture computed tomography (TACT; OrthoTACT, Instrumentarium Imaging, Helsinki, Finland) images. Eighty test sites on the inferior margins of rabbit mandibles were subject to lesion induction and treated with one of the following: no treatment (controls); osteoblasts only; polymer matrix only; or osteoblast-polymer matrix (OPM) combination. Images were acquired using conventional radiography and TACT, including unprocessed TACT (TACT-U) and iteratively restored TACT (TACT-IR). Healing was followed up over time and images acquired at 3, 6, 9, and 12 weeks post-surgery. Fractal dimension (FD) was computed within regions of interest in the defects using the TACT workbench. Results were analyzed for effects produced by imaging modality, treatment modality, time after surgery and lesion location. Histomorphometric data were available to assess ground truth. Significant differences ($p < 0.0001$) were noted based on imaging modality with TACT-IR recording the highest mean fractal dimension (MFD), followed by TACT-U and conventional images, in that order. Sites treated with OPM recorded the highest MFDs among all treatment modalities ($p < 0.0001$). The highest MFD based on time was recorded at 3 weeks and differed significantly with 12 weeks ($p < 0.035$). Correlation of FD with results of histomorphometric data was high ($r = 0.79$; $p < 0.001$). The FD computed on TACT-IR showed the highest correlation with histomorphometric data, thus establishing the fact TACT is a more efficient and accurate imaging modality for quantification of osseous changes within healing bony defects.

Keywords Computed tomography · Fractal dimension · Digital image processing · Osseous

Introduction

Quantification of osseous changes in healing sites is a complex and challenging procedure. Numerous techniques are employed including digital subtraction using multiple radiographic examinations acquired over a period of time with reproducible projection geometry, advanced imaging modalities, such as, for example, quantitative computed tomography (QCT), MRI, and dual-energy X-ray absorptiometry (DEXA), in order to quantify osseous repair and regeneration. Use of these techniques in a clinical environment is limited owing to numerous limitations posed by each, which includes among other things, the need to stabilize the subject for relatively long periods of time during image acquisition, inability to easily reproduce projection geometry, the relatively high cost of advanced imaging, relative inaccessibility to the general practitioner, increased radiation burden of advanced imaging, and the potential for artifact generation. Acquisition of radiographic information in three-dimensional volume using advanced imaging techniques aids in appropriate non-invasive characterization of the healing process, such as the location, quality, and quantity of osseous tissue which regenerates/remodels [1], but the various drawbacks of these techniques preclude their routine use in clinical and laboratory studies.

Fractal analysis [2, 3] is yet another technique to quantify bone formation through evaluation of the complexity of bone, which in turn serves as an indicator of the level of structural organization. The higher the fractal dimension, the more the morphological complexity at the ultra-structural level [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. Analyses of fractal dimension has been correlated with the strength of bone in previous studies [5, 6, 7, 14, 15, 16, 18], and therefore we decided to evaluate the fractal dimension of healing sites on rabbit mandibles which were subject to destructive bony lesion induction and various treatment modalities, using tuned aperture computed tomography (TACT; OrthoTACT, Instrumentarium Imaging, Helsinki, Finland) images.

The TACT technique is a reconstruction algorithm that utilizes multiple two-dimensional conventional projections acquired with differing geometries, to form three-dimensional image databases which can then be processed to view in static or video mode. The higher diagnostic efficacy of TACT over conventional radiographic techniques, with respect to numerous other clinical applications [1, 19, 20, 21, 22, 23, 24, 25, 26], was the basis for such an investigation. It differs from the existing modalities cited previously in that it computes the 3D representation by using information acquired from known fiducial relationships that exist in the 2D projections themselves. This contrasts with the conventional method wherein a majority of the drawbacks associated with advanced imaging modalities, such as the need to stabilize the region of interest during image acquisition, the prohibitive cost of a QCT examination, and the radiation dose involved, are not issues to contend with while using TACT. Reconstruction is facilitated through the use of known fiduciary relationships that exist within the conventional 2D images. The radiation dose can be prorated among the number of individual 2D projections acquired digitally, to not exceed a comparable dose required to expose a film-based image of the region of interest.

This study explored the use of fractal analyses on TACT images, for evaluation of the healing process within regions of interest (ROI) contained by mechanically induced osseous defects in the mandibles of rabbits, which had been subject to different treatment modalities. Pilot studies done at this institution by the same investigators using representative specimen samples drawn from the experimental sample pool had shown promise of an impressive performance of this imaging modality, in comparison with conventional 2D radiographs, with ground truth determined histomorphometrically. Specifically, data computed from fractal analyses of the ROIs were evaluated. The null hypotheses for this study were that: (a) no statistically significant correlation exists between the fractal dimension of the ROI encompassing the defect and osseous integration across all imaging modalities, as determined by a histological examination, and no statistically significant difference exists between the fractal dimensions of the different ROIs, with respect to the treatment modalities; (b) the time elapsed post-surgery; and (c) the location (anterior and posterior) and side (right and left) of the mandible, as evaluated from displays using all imaging modalities.

Materials and methods

Lesion induction

Artificially induced surgical defects were selected over passive models utilizing natural lesions because the former can be standardized and does not vary with respect to etiology. A total of 80 lesions were induced in 20 New Zealand rabbits (Myrtle Rabbitry, Pittsburgh, Pa., USA) along the inferior margin of the mandible. Each rabbit received four defects, two on each side of the mandible, one anteriorly and the other posteriorly. The lesion configuration was standardized across all sites. Previous studies had revealed that two-walled defects in rabbit mandibles, 5 mm or larger in size, failed to heal over a period of 52 weeks post-surgery [29, 30]. Our pilot studies, using a polymer matrix as the scaffolding to induce repair of the defects (Atrix Laboratories, Fort Collins, Colorado), resulted in resolution of even 8-mm (transverse diameter) defects in approximately 12 weeks. Therefore, this study utilized two-walled defects, 8 mm in width and 8–10 mm apart along the inferior mandibular margin, induced with a slow-speed straight fissure bur by one of the investigators. Furthermore, the investigation involved evaluation of the healing process over a relatively shorter period of time (3–12 weeks). Following lesion induction, each experimental site was subject to one of four randomly assigned treatments: (a) no treatment (served as controls); (b) polymer matrix (Atrix Laboratories, Fort Collins, Colorado) alone; (c) polymer matrix with osteoblasts grown in vitro (OPM); and (d) osteoblasts in suspension grown in vitro without the polymer matrix as scaffold. Care was taken not to repeat any single treatment modality per specimen studied. The overlying tissue surrounding each defect was carefully sutured to maintain stability of any graft materials. The rabbits resumed normal life following the surgery. Groups of rabbits were sacrificed following a post-surgical healing period of 3, 6, 9, or 12 weeks, using an overdose of Nembutal. Direct digital radiography was performed on each specimen to serve as diagnostic images for lesion analyses. Ground truth was established using histomorphometric techniques.

Image acquisition

Imaging was accomplished using the X-ray source (OP-100, Instrumentarium Imaging, Finland) of a panoramic imaging unit for ease of use. The specimens were positioned on an imaging platform and imaged using appropriate exposure parameters as determined by pilot studies. The diagnostic task was to visualize and be able to trace the normal anatomic landmarks in the region, which included the inferior margin of the mandible, periodontal ligament space of the teeth in the field of view, the trabecular pattern of normal bone peripheral to the defects, and the margins of the lesion. Exposure parameters used for digital image acquisition were 70 kVp, 15 mA, and 0.13 s at a focal spot to object distance of 40 cm. The projection geometry was varied between 9° and 20° to either side of the orthogonal projection geometry axis [22, 23, 25, 26] to acquire eight more images for TACT reconstruction. A fiduciary reference of 1.5 mm in transverse diameter (Beekley, Bristol, Conn., USA) was placed 3 mm buccal to the outer curvature of the mandible in order for the algorithm to reconstruct the image of the ROI in 3D.

All digital images were acquired using the Schick (CDR Computed Dental Radiography) system (Schick Technologies, Long Island City, NY). A no. 2 charge coupled device sensor was mounted on the lingual side of the mandible to be imaged and maintained in position using a precision instrument (Rinn Corporation, Elgin, Ill.). The OP100 unit was equipped with the TACT imaging program (OrthoTACT, Instrumentarium Imaging, Helsinki, Finland) which facilitated controlled exposures using different loci in space along a circular path limited to a range of motion that would optimize complete image capture by the sensor. These images were saved as uncompressed tif (tagged image format) files. Image reconstruction was accomplished using the TACT workbench and the stack of 3D image slices served as the second imaging modality for evaluation. This was called unprocessed TACT (TACT-U). Additionally, iterative restoration (a deblurring technique) was used sequentially to adjacent slices to remove tomosynthetic artifacts. This served as the third imaging modality for purposes of this investigation and was known as iteratively restored TACT images (TACT-IR). Iterative restoration has been shown to improve the diagnostic accuracy of TACT images with respect to numerous other diagnostic tasks [19, 22, 24, 26, 27]. Fractal analyses was carried out on images acquired using three imaging modalities: (a) conventional 2D direct digital images; (b) TACT-U; and (c) TACT-IR.

All TACT images were viewed interactively as a series of slices oriented from buccal to lingual. All images were assigned unique alphanumeric identity numbers by one of the investigators (A.S.) and randomized for viewing. The observer was blinded as to the nature of treatment done at each site, the duration after surgery, and the results of the histomorphometric analyses. Slices of TACT-U and TACT-IR images through comparable planes were used for evaluation across all specimens. All images were histogram equalized using a reference image and an ROI was selected based on the configuration of the defect as it appeared on the images. Fractal analyses were carried out for each of the different ROIs using the power spectrum method employed by the TACT workbench. Thirty-two-bit complex floating point representations of the ROIs (3×32 pixels) that were cropped were subject to 2D fast Fourier transform (FFT), followed by plotting the log of the magnitude vs the log of each frequency component that was generated by the FFT. A regression line was then fitted to this plot. The slope of this line (a negative value) is used to generate a fractal dimension for each of the ROIs. All regression lines had correlation coefficients that exceeded 0.97. The fractal dimension, slope of the regression line, and correlation of the slope to the data were recorded. The entire process was repeated four times in each of the experimental and control sites by the observer and the average of the four readings was used for analyses. This process was repeated after a period of 2 weeks to get a second average measure to test for differences based on the observer sessions separated by a period of time. Intraclass correlation coefficient computed to evaluate intra-observer agreement was 0.86 with respect to the selection of the ROIs on all images indicating good intra-observer reliability.

Histomorphometry

The animals were killed using an overdose of Nembutal, following which the mandibular blocks were removed and fixed in 10% neutral buffered formalin, before decalcifying them in 10% formic acid for histological examination. The tissues were then embedded in paraffin and sectioned sagittally and axially, so that each defect could be visualized in its entirety. Each representative section was 6–8 mm thick and included the lesion in its entirety. Histomorphometric analyses were done in a randomized manner by a single investigator, who was a microbiologist, after staining them with hematoxylin and eosin. The observer was blinded as to the nature of treatment to which each specimen had been subjected and the time elapsed after surgery. The sections were scored on a scale of 0–10, in which 0 represented no bone formation at all and 10 represented complete filling of the defect by osseous tissue.

Statistics

Multifactorial repeated analyses of variance (ANOVA) were conducted with the fractal dimension being the dependent variable. The categorical variables evaluated were treatment modality, imaging modality, side of mandible (left or right), site (anterior or posterior), and time elapsed post-surgery. Alpha was preset at 0.05. Post hoc tests using Tukey's Honestly Significant Difference analyses were further carried out where ANOVA revealed significance.

Results

Computation of the ANOVA revealed a statistically significant difference in the fractal dimension based on the treatment modality ($p < 0.0001$), imaging modality ($p < 0.0001$), time after surgery ($p < 0.035$), and location

Table 1 The mean and standard deviation of fractal dimensions based on imaging modality, treatment modality, and time after surgery. *FD* fractal dimension; *TACT-IR* Tuned Aperture Computed Tomography® iteratively restored images; *TACT-U* unprocessed Tuned Aperture Computed Tomography® images

Main effect	Type of effect	Mean FD	SD
Treatment	None	1.991	0.090
	Polymer matrix only	2.039	0.119
	Osteoblasts only	1.992	0.094
	Osteoblast-polymer matrix	2.059	0.114
Image	TACT-U	2.028	0.061
	TACT-IR	2.066	0.132
	2D digital images	1.967	0.091
Location	Anterior	2.0134	0.118
	Posterior	2.0322	0.112
Time	3 weeks	2.046	0.100
	6 weeks	2.014	0.118
	9 weeks	2.024	0.112
	12 weeks	2.000	0.101

($p < 0.05$). No significant higher-order interactions were noted. Post hoc tests of fractal dimensions using Tukey's analyses revealed a statistically significant difference between fractal dimensions measured at the following sites: those treated with the polymer matrix (higher fractal dimension, HFD) and controls ($p < 0.0005$) at all times and across all imaging modalities; polymer matrix (HFD) and osteoblasts alone ($p < 0.002$); and osteoblast-polymer matrix delivery system (HFD) and all other sites except those treated with the polymer scaffolding alone ($p < 0.04$). In addition, a potential effect on the fractal dimension of the ROIs caused by imaging modalities was noted, with TACT-IR images recording a significantly higher fractal dimension (FD) than TACT-U and 2D projections ($p < 0.0001$). Additionally, all TACT images had a higher FD compared with 2D images ($p < 0.0001$). Evaluating the effect of the elapsed time post-surgery on the fractal dimension measures revealed a statistically significant difference in the FD between sites evaluated at 3 and 12 weeks, with a higher FD being recorded at 3 weeks. Location-wise, the mean FD value for all sites located anteriorly recorded a lower value than the posterior sites, across all imaging and treatment modalities.

Table 1 depicts the distribution of the mean fractal dimension for each of the treatment categories, image types, and time after surgery. The mean FD is highest for sites that were treated with a combination of the polymer matrix and osteoblasts, followed by matrix alone, and osteoblasts alone, with the lowest FD recorded for the control sites.

Among the image types (Table 1), the mean fractal dimension (MFD) decreased in the following order: TACT-IR; TACT-U; and 2D digital images. The fractal dimension for the sites evaluated 3 weeks post-surgery recorded the highest mean value with the FD decreasing to 2.0 by the end of week 12.

The highest mean FD was recorded with TACT-IR and came from the polymer matrix-osteoblast treated sites, as was noticed with both of the other imaging modalities also (Table 2). Tables 3 and 4 illustrate the variation of the mean FDs with various pairwise comparisons involving imaging modality, treatment modality and time elapsed post-surgery.

Discussion

This study showed that variations in healing patterns as detected histomorphologically did correlate significantly with observations derived radiographically from in vivo specimens using TACT.

The potential of iteratively restored TACT images to detect and quantify bony changes within ROIs cannot be discounted as the imaging modality uses conventional 2D radiographs to reconstruct images in 3D, thereby permitting examination of the radiographed tissue volume as tomographic slices. The FDs computed from TACT-IR provided the most correlation with the histomorphometric data, followed by TACT-U and conventional radiographs.

Histogram equalization of all images used in the study ensured that any variables that may have confounded the analyses were considered. In any case, it seems apparent that detectable healing effects are determined equally by the experimental conditions underlying lesion management and the period over which healing was evaluated under the experimental conditions imposed by this investigation. The differences in

Table 2 Interaction between imaging modality and treatment modality: mean FD values (standard deviations in parentheses)

Imaging vs treatment modality	Controls	Polymer matrix	Osteoblasts	Polymer matrix-osteoblasts
2D digital radiographs	1.951 (0.058)	1.993 (0.076)	1.940 (0.055)	1.996 (0.055)
TACT-IR	2.048 (0.117)	2.119 (0.138)	2.030 (0.113)	2.168 (0.160)
TACT-U	1.972 (0.063)	2.066 (0.102)	2.010 (0.084)	2.066 (0.082)

Table 3 Interaction between imaging modality and time after surgery: mean FD values (standard deviations in parentheses)

Time after surgery vs imaging modality	3 weeks	6 weeks	9 weeks	12 weeks
2D digital radiographs	1.970 (0.049)	1.965 (0.069)	1.965 (0.057)	1.971 (0.083)
TACT-IR	2.133 (0.090)	2.066 (0.158)	2.074 (0.143)	2.002 (0.105)
TACT-U	2.036 (0.082)	2.012 (0.090)	2.038 (0.083)	2.028 (0.110)

Table 4 Interaction between treatment modality and time after surgery: mean FD values (standard deviations in parentheses)

Time after surgery vs treatment modality	3 weeks	6 weeks	9 weeks	12 weeks
Controls	1.986 (0.087)	2.018 (0.127)	2.006 (0.087)	1.950 (0.054)
Polymer matrix	2.079 (0.120)	2.032 (0.072)	2.032 (0.039)	2.015 (0.122)
Osteoblasts	2.030 (0.058)	1.960 (0.099)	2.005 (0.116)	1.978 (0.075)
Polymer matrix-osteoblasts	2.090 (0.012)	2.048 (0.152)	2.055 (0.116)	2.059 (0.113)

fractal dimension computed based on treatment modalities are attributed to the presence/absence of a scaffolding to hold the osteoblasts in place. The scaffolding provided by the polymer matrix appears to enhance bridging of the defect in some way as was indicated by the relatively high FD values noted in instances where the matrix was employed. There was increased bone formation in these areas as was confirmed histomorphometrically even at the 3-week post-surgery period. Osteoblasts by themselves could not effectively enhance the structural integrity of the healing osseous tissue appreciably, but in conjunction with good scaffolding, the best results were obtained.

The relatively high MFD recorded at 3 weeks post-surgery probably indicates that the greatest amount of structural complexity was evident at 3 weeks, and that remodeling probably resulted in a loss of FD subsequently. The higher fractal dimension recorded in the posterior mandibular defects could probably be attributed to the masticatory pattern seen in these rabbits, with more bone complexity seen in areas of increased bone stimulation.

New bone formation detected as part of the healing and remodeling process at all sites by histomorphometric analyses correlated with the computation of the FD of the same regions in radiometric studies using TACT. Fractal dimension and the results of the histomorphometric analyses were positively correlated indicating that a healing site exhibiting a high FD was likely to have a higher osseous content with better structural integrity (Pearson's correlation coefficient: $r = 0.79$; $p < 0.001$).

We believe that this study establishes the potential of TACT to quantify healing in osseous defects, thus providing alternatives to QCT and other advanced imaging methods [9, 10, 11, 12, 13, 14, 15, 18, 19, 20, 21]. The TACT technique does not require expensive equipment and can be easily employed in routine clinical and laboratory settings without having to standardize or maintain rigid projection geometry. Studies using conventional film-based 2D radiographs as the basis images for 3D reconstruction using TACT have produced impressive results, too [1, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28].

In conclusion, TACT has been shown to be a reliable and accurate method for facilitating noninvasive quantitative assessment of osseous healing in rabbit mandibles and warrants more studies comparing it with other advanced imaging modalities routinely in use for this purpose. The TACT technique can thus be used in the clinical setting to monitor and quantify osseous healing in patients, without having to resort to expensive and relatively inaccessible advanced imaging modalities that require immobilization of the ROI, and the potential for artifact generation.

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