Quantification of voxel values in micro computed tomography using multiple porosity hydroxyapatite blocks

By

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Summary: Microfocus X-ray computed tomography (micro-CT) has been applied as a method for the nondestructive and detailed assessment of trabecular bone patterns and tooth structure. Voxel values obtained from micro-CT are not absolute values. Therefore, voxel values were assessed using hydroxyapatite (HA) blocks with a different vesicle rate to quantify voxel values of micro-CT images in the present investigation.

HA blocks with 4 levels of porosity and a block with a soft tissue-equivalent density were used, and the voxel values of each block were measured. Correlations between voxel values of micro-CT and HA densities were analyzed. Also, black and white binary images were produced, and the ratios of white pixels to pixels in regions of interest (ROIs) were calculated. The relationship between voxel values of micro-CT and HA densities could be regressed using a linear equation, and the correlation coefficient was high. Also, there were no significant differences in the regression equations between the first and second times. Voxel values of micro-CT might be convertible to HA densities using a regression equation.

Introduction

Microfocus X-ray computed tomography (micro-CT) has been applied as a method for the nondestructive and detailed assessment of trabecular bone patterns and tooth structure. The accuracy of micro-CT images was reported to be high for linear measurement and shape reproduction¹⁻³.

Voxel values obtained from computed tomography using cone beams of X-rays like micro-CT and cone beam CT for dental use (CBCT for dental use) are not absolute values⁴. The bone fraction measurements of a CT phantom from global and local algorithms to determine the threshold in micro-CT were compared with histomorphometry, and a local threshold-determining algorithm has been suggested for examining inflammation-induced bone loss⁵. However, the quantification of voxel values in micro-CT was not assessed.

In CBCT for dental use, various methods have been proposed to evaluate the bone density⁵, ⁶. In previous studies, blocks with a range between 0 to 400 mg/cm³ of hydroxyapatite (HA) were used for the evaluation of cancellous bone densities. Bone mineral densities (BMDs) of mandibular cancellous bones in humans were beyond the range of HA blocks in certain cases and sites. Also, BMDs of cortical bones were higher than 400 mg/cm³ of HA.

In the present investigation, voxel values were assessed using HA blocks with a different vesicle rate to quantify voxel values including BMDs of cortical bones and densities of dentin and enamel in micro-CT.

Materials and Methods

Hydroxyapatite (HA) blocks

HA blocks with 4 levels of porosity (0, 30, 55, and 85%, APACERAM, Hoya Technosurgical Co., Tokyo, Japan) and a block with a soft tissue-equivalent density (SZ-207, Kyotokagaku Co., Kyoto, Japan) were used in
the study (Fig. 1). The HA density of the block with 0% porosity was \(3.16 \times 10^3\) mg/cm\(^3\), 30% porosity \(2.21 \times 10^3\) mg/cm\(^3\), 55% porosity \(1.42 \times 10^3\) mg/cm\(^3\), and 85% porosity \(0.47 \times 10^3\) mg/cm\(^3\) are shown from the left side. Each block was 10 × 10 mm in height and width and 4 mm in length. They were joined and set in the center of a polypropylene test tube (diameter: 30 mm), which was filled with water.

**Micro computed tomography (Micro-CT)**

A micro-CT unit (CosmoScan GX, Rigaku Co., Tokyo, Japan) was used. The exposure volume was set at 36 mm in diameter and 20 mm in length. The voxel size was 0.072 × 0.072 × 0.072 mm. The scan was set at 90 kV and 88 μA, as recommended by the manufacturer. Micro-CT scans were performed and voxel values were standardized from original attenuation values using the air and water portions. Then, the DICOM files on 14-bit scales of the axial images were saved. Micro-CT was repeated three times in the same way.

**Relationship between voxel values in micro-CT and HA densities**

The thickness of axial images was set at 0.504 mm on a computer (MacBook Pro, Apple Computer Inc., USA) using 3-D visualization and measurement software (OsiriX Imaging Software, version 3.9: The OsiriX Foundation, Geneva, Switzerland, http://www.osirix-viewer.com) (Fig. 2). Subsequently, circular regions of interest (ROIs) (0.385 mm\(^2\) in area) were set on the images of each block. Each measurement was performed five times. In micro-CT performed three times, measurements were repeated in the same way. Correlations between voxel values of micro-CT and HA densities were analyzed. Moreover, micro-CT performance and measurements were repeated in the same way after 1 month to evaluate the stability of voxel values with the passage of time. Analysis of covariance (ANCOVA) was used to analyze the differences in the regression equations between the first and second times. Values were considered significant at \(p < 0.05\).

**Evaluation of binary micro-CT images**

Black and white binary images were produced using 0.072-mm-thick micro-CT images and the above-mentioned software (Fig. 3). The threshold of the black and white binary image was set at average voxel values in blocks of 3.16 × 10\(^3\) mg/cm\(^3\) and 0 mg/cm\(^3\) of HA. Subsequently, the region of interest (ROI) was set in each block using image-editing software (Photoshop CS, Adobe Systems, San Jose, CA, USA) and the total number of pixels in the ROI and white pixels was measured. Then, the ratios of white pixels to ROI pixels were calculated. Moreover, in micro-CT performed three times, calculations were repeated in the same way.

**Results**

**Relationship between voxel values of micro-CT and HA densities**

The relationship between voxel values of micro-CT and HA densities in a range between 0 mg/cm\(^3\) and 3.16 × 10\(^3\) mg/cm\(^3\) were calculated. The measurements were repeated in the same way after 1 month to evaluate the stability of voxel values with the passage of time. Analysis of covariance (ANCOVA) was used to analyze the differences in the regression equations between the first and second times. Values were considered significant at \(p < 0.05\).
Quantification of voxel values in micro-CT using HA blocks

$10^3$ mg/cm$^3$ of HA could be regressed using a linear equation ($y = 1.3344x + 279.19$) (Fig. 4), and the correlation coefficient ($r^2$) was 0.980. Also, the relationship between voxel values of micro-CT and HA densities in a range between 0 mg/cm$^3$ and $2.21 \times 10^3$ mg/cm$^3$ of HA, i.e., a range of BMDs, could be regressed using a linear equation ($y = 1.5078x + 160.71$) (Fig. 4), and the correlation coefficient ($r^2$) was 0.988.

In micro-CT after 1 month, the regression equation was $y = 1.3418x + 249.47$, and the correlation coefficient ($r^2$) was 0.984 in densities in a range between 0 mg/cm$^3$ and $3.16 \times 10^3$ mg/cm$^3$ of HA (Fig. 5). Also, the regression equation was $y = 1.4898 + 148.34$, and the correlation coefficient ($r^2$) was 0.988 in a range between 0 mg/cm$^3$ and $2.21 \times 10^3$ mg/cm$^3$ of HA, i.e., BMDs (Fig. 5).

There were no significant differences in the regression equations between the first and second times ($P = 0.9521$ in a range between 0 mg/cm$^3$ of HA and $3.16 \times 10^3$ mg/cm$^3$ of HA, and $P = 0.6413$ in a range between 0 mg/cm$^3$ of HA and $2.21 \times 10^3$ mg/cm$^3$ of HA, respectively).

Evaluation in binary micro-CT images

The mean rate of white pixels to ROI pixels was 100% in the $3.16 \times 10^3$ mg/cm$^3$ HA block, 99.7% in the $2.21 \times 10^3$ mg/cm$^3$ HA block, 61.4% in the $1.42 \times 10^3$ mg/cm$^3$ HA block, and 0% in the $0.47 \times 10^3$ mg/cm$^3$ HA block, (Table 1).

Fig. 3. Binary micro-CT images

Binary micro-CT images of hydroxyapatite blocks with 0% porosity ($3.16 \times 10^3$ mg/cm$^3$ HA), 30% porosity ($2.21 \times 10^3$ mg/cm$^3$ HA), 55% porosity ($1.42 \times 10^3$ mg/cm$^3$ HA), and 85% porosity ($0.47 \times 10^3$ mg/cm$^3$ HA) are shown from the left side.

Fig. 4. Correlation between voxel values of micro-CT and HA densities in first micro-CT

The linear equation was $y = 1.3344x + 279.19$, and the correlation coefficient ($r^2$) was 0.980 for a range including high-density measurements.

The linear equation was $y = 1.5078x + 160.71$, and the correlation coefficient ($r^2$) was 0.988 for a range of bone mineral density measurements.

Fig. 5. Correlation between voxel values of micro-CT and HA densities in micro-CT after 1 month

The linear equation was $y = 1.3418x + 249.47$, and the correlation coefficient ($r^2$) was 0.984 for a range including high-density measurements.

The linear equation was $y = 1.4898x + 148.34$, and the correlation coefficient ($r^2$) was 0.988 for a range of bone mineral density measurements.
Discussion

CT values (Hounsfield units: HU) obtained by multislice CT were absolute values, set as -1,000 HU in air and 0 HU in water. The mean CT value in the molar region of mandibular cancellous bone was reported to be $4.5 \times 10^2$ HU\(^9\). Voxel values of the micro-CT scanner used in the study were decided using air and water; however, tube voltages differed between micro-CT and multislice CT. It is known that CT values vary at different multislice CT X-ray tube voltages\(^9\).

In the present investigation, voxel values in micro-CT images were assessed using HA blocks with a different vesicle rate to quantify voxel values including BMDs of cortical bones and densities of dentin and enamel in micro-CT.

Measurements of voxel values were performed on 0.504-mm-thick axial images to reduce the influence of noise. The relationship between voxel values of micro-CT and HA densities could be highly regressed using a linear equation. In a previous study, the relationship between CT values in multislice CT with a tube voltage of 120 kV and voxel values in cone beam CT with a tube voltage of 80 kV could be regressed using a linear equation within a range of BMD measurements. However, the relationship within a range including a high density could be regressed using a quadratic equation\(^10\). In the study, the tube voltage of micro-CT (88 kV) was close to that of CBCT. The total filter thickness and influence of scatter radiation and beam hardening might differ between micro-CT and CBCT. Also, the correlation coefficient between voxel values and HA densities within $2.21 \times 10^3$ mg/cm\(^3\) of HA were slightly higher than those within $3.16 \times 10^3$ mg/cm\(^3\) of HA. It was considered that the beam-hardening effect affected the results. In animal studies, when voxel values in micro-CT are regressed using HA densities, different regression equations should be applied between measurements of BMDs and densities including dentin and enamel. Moreover, regressed equations were similar between the first micro-CT and that after 1 month, and there were no significant differences between them.

Micro-CT of HA blocks with a different vesicle rate was performed before and after animal examination, and voxel values of micro-CT images could be converted to HA densities using the average regressed equation between before and after animal examination.

In binary micro-CT images, the mean rate obtained from micro-CT images agreed with a porosity rate of 0% porosity in the HA block. However, the mean rate did not agree with a porosity rate of 30, 55, or 85% in HA blocks. The substance rate of the HA block with 30% porosity was 70%, and that with 55% porosity was 45%. The mean rate obtained from micro-CT images was higher than the substance rate. The substance rate of the HA block with 85% porosity was 15%, and the mean rate obtained from micro-CT images was lower than the substance rate. Uchiyama et al.\(^11\) reported that the difference in correlation coefficients between micro-CT image analysis and conventional histomorphometry could be caused by three factors: the resolution of micro-CT, the threshold values in micro-CT, and the shrinkage of tissue during cutting and biologic processing. The results of the investigation might be associated with the resolution of micro-CT images, i.e., the voxel size of micro-CT images. The voxel size of micro-CT images might be large compared with the substance of HA blocks corresponding to trabecular bone of cancellous bone at low HA densities ($0.47 \times 10^3$ mg/cm\(^3\) of HA). Also, the size might be large compared with the vesicle of HA blocks corresponding to the bone marrow space of cancellous bone at high HA densities ($2.21 \times 10^3$ and $1.42 \times 10^3$ mg/cm\(^3\) of HA). So, it was considered that the method with the ratios of pixels could not be applied to quantify voxel values in micro-CT images under the present conditions. Vesicles had two sizes of a few microns and a few hundred microns in the HA blocks, and they were connected based on the manufacturer’s specification. The sizes of substances and vesicles in HA blocks need to be consistent with those of trabecular bones and bone marrow spaces in the mandible.

Conclusion

In the present investigation, voxel values were quantified using HA blocks with a different vesicle rate in micro-CT. The relationship between voxel values of micro-CT and HA densities could be regressed using a linear equation, and the correlation coefficient was high. Voxel values of micro-CT might be convertible to HA densities using a regression equation.

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