Influences of Newly Formed Woven Bone on Tissue Stresses in Rat Caudal Vertebrae Subjected to Mechanical Loading* (A Study Based on Morphological Measurement Using a Micro-CT and Computational Stress Analysis)

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Bone tissue stresses in mechanically loaded vertebrae were computationally investigated in order to reveal the influences of adaptive remodeling/modeling on stress distribution. Morphological alteration of the rat fifth caudal vertebrae was periodically and non-invasively measured with a microcomputed tomography (micro-CT). Von Mises stresses were calculated by using a finite element analysis (FEA) together with rigid-body spring models based on the consecutive micro-CT images. Median cross-sectional area periodically increased in the loaded rats depending on the duration of stimuli, which was caused by periosteal woven bone formation. FEA including the newly formed bone demonstrated that the loaded vertebrae showed the lower stress levels compared with non-loaded one. Averaged stress of the offset-loaded rat was markedly symmetry between ventral and dorsal sides under offset loading condition, while that of the non-loaded rat indicated asymmetry. Stress analyses suggested that the loaded vertebrae would adapt to the daily mechanical loading by depositing and calcifying woven bone over periosteum.

Key Words: Biomechanics, Finite Element Method, Adaptive Structure, Bone Remodeling, Mechanical Stimulus, Microcomputed Tomography, Stress Analysis, Stress Distribution

1. Introduction

The interest in the relationship between bone architecture and mechanical behavior has been aroused in the field of the mechanical engineering

because of the clinical needs for implanted bone prostheses and for predicting osteoporotic fractures. Progresses of engineering and computational techniques have made it possible to observe the trabecular architecture of living animals and to estimate mechanical properties of bone. Using noninvasive measuring equipment such as a quantitative computed tomography (QCT)⁽¹⁾, a microcomputed tomography (micro-CT)⁽²⁾⁻⁽⁴⁾, a synchrotron CT^{(5),(6)} and a micro magnetic resonance imaging (micro MRI)^{(7),(8)}, the three-dimensional (3-D) architecture of cancellous bone could have been observed easily and less-invasively compared with observations by traditional tissue sectioning. These equipment could show the differences in 3-D trabecular microarchitectures depending on the types of various osteopenia^{(9),(10)}. Furthermore, combination of the finite element analysis (FEA) and 3-D digitized images produced by a micro-CT allows us to assess

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the material properties of trabecular bone architecture^{(11)–(13)}. Almost all the studies were, however, restricted within *ex vivo* measurements for extracted bone specimens, and could not utilize the advantage of non-invasiveness except for a few works^{(6),(14)}.

Bone tissue can remodel its structure adapted to mechanical environments^{(15),(16)}. Several animal models have been proposed for evaluating alterations of bone morphology when artificial stimuli were quantitatively subjected to bone. Rubin et al. functionally isolated diaphyseal shaft of turkey ulna by surgical treatment⁽¹⁷⁾. The ulna was quantitatively subjected to mechanical stimuli because of using pins inserted into the each end of the preparation. Although it could be exposed to the quantifiable loading regime, there was possibility that bone responses were much influenced by the invasive operation, or by inserting pins into the objective bone itself. Alternative model without any surgical treatment was proposed by Turner et al.⁽¹⁸⁾ Rat tibia was non-invasively applied to bending force through soft tissue by using a four-point loading apparatus in order to completely remove the undesirable factors except for the quantifiable mechanical stimuli; even so, their model had some difficulties in the estimation of the actual stress or strain generated in bone because soft tissue volume around the ulna would largely differ between individuals. Chambers and his coworkers developed a unique experimental model in which the eighth caudal vertebra of rat was subjected to compressive load with using pins inserted into the seventh and ninth caudal vertebrae⁽¹⁹⁾. Fixing of the pins between episodes of compressive loading allowed the eighth caudal vertebra to be isolated from daily mechanical environment. The vertebra to be observed suffered little surgical interference because the loading pins were inserted into the neighboring vertebrae. Additionally, this model has a crucial advantage; remodeling process in cancellous bone can be examined as well as in cortical bone, which lacks in other models. Utilizing these experimental models, mechanical responses of loaded bone have been assessed by gene expression assay and histological analysis based on a soft X-ray measurement or tissue sectioning.

We have already demonstrated the useful method which enable to estimate the stress distribution in cortical and cancellous bone with using FEA based on noninvasive micro-CT measurements⁽¹⁴⁾. Rat caudal vertebrae were externally subjected to cyclic axial- or offset-load by inserted pins according to the experimental model proposed by Chambers et al.⁽¹⁹⁾ Because of few soft tissues around the vertebral body, this model was suited for periodic observations of bone morphological alteration with a micro-CT. The

vertebrae adapted to the external loads not only through the remodeling in trabecular architecture, which was inferred from FEA utilizing micro-CT images, but also through deposition of new bone matrix over the periosteal surface. Our previous study was focused on the functional adaptation in trabecular bone architecture without taking the role of new bone formation into analysis.

Therefore, the goal of the current study was to evaluate the influences of newly formed bone, which was observed over periosteal surfaces of the loaded vertebrae, on tissue stress distribution. Axial- or offset-loads with different duration were daily applied to the rat caudal vertebrae. Morphological measurements by a micro-CT indicated the alteration of bone cross-sectional area depending on the loading duration. FEA under analytical loading was carried out to estimate the stress distribution in cortical and cancellous bone structure including new bone matrix.

2. Materials and Methods

2.1 Animal preparation

Nine Sprague-Dawley female rats, aged 13 weeks, were used (average weight 258 g). They were housed in a room with a 12-hour dark, 12-hour light cycle, and were provided a regular diet and water *ad libitum*. Experimental model was prepared as described previously⁽¹⁴⁾. All rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg body weight). Stainless steel pins with a 1.2-mm diameter were inserted into the fourth and sixth caudal vertebral bodies (C4 and C6) according to the method published by Chambers and his coworkers^{(19),(20)} with some modifications. The positions to be inserted with pins were determined using an X-ray fluoroscopy and marked with thin wires wound on the rat's tail. Pins were driven with a hand-held variable-speed drill and transfixed percutaneously along the transverse axes of the vertebral bodies (Fig. 1). The day of this operation was taken as day 0. The rats were randomly divided into three groups of four axial-loaded rats, four offset-loaded rats and a non-loaded rat as control. The duration of mechanical stimuli for four axial- and offset-loaded rats were 300, 1 200, 2 400 and 3 600 cycles/day, respectively.

2.2 Morphological measurements with a micro-CT and mechanical stimulation to the caudal vertebrae

The rats, which were anesthetized as described above, were secured to a rotating platform of the micro-CT (MCT-12505MF, Hitachi Medico Technology Co., Tokyo, Japan) and were rigidly splinted in order to eliminate motion artifacts. After the length of the fifth caudal vertebra (C5) was measured from

Fig. 1 Schematic representation of mechanical loading to the rat fifth caudal vertebra (C5). Cyclic loading regime was applied to the axial-loaded rat (a) and the offset-loaded rat (b) by using the stainless steel pins inserted into C4 and C6.

cranial intervertebral surface to caudal one by using the X-ray fluoroscopy (Fig. 2), consecutive sections at 400 μm intervals of transverse plane were non-invasively scanned with a spatial resolution of 40 $\mu\text{m}/\text{pixel}$. The scanned region was whole body of the C5. Scanning parameters were 45 kV and 0.04 mA.

Each rat except the control rat was settled on the loading apparatus as described previously⁽¹⁴⁾. The C5 of the axial- and offset-loaded rats were subjected, with pins inserted into C4 and C6, to a daily loading period of sinusoidal-wave-like mechanical stimuli. The maximum magnitude and the frequency of the mechanical stimuli were 30 N and 1.0 Hz, respectively. The duration of mechanical stimuli was 300, 1 200, 2 400 and 3 600 cycles/day as described above. These loading conditions in each cycle were controlled by feedback system consisted of strain gauges, gauge amplifier, personal computer, motor driver and linear actuator. The pin, which was inserted into C4, was moved by 4 mm toward the ventral direction and was reciprocated on each plane for the offset-loaded rats, while pins were loaded normally on the same plane for the axial-loaded rats (Fig. 1).

Micro-CT measurements were carried out every three days (day 1, 4, 7, 10, 13, 16). Mechanical stimulation was daily repeated for 16 days at 24 hours intervals. After the consecutive micro-CT images of transverse plane were thresholded⁽¹⁰⁾, the binary cross-sectional images of cortical bone and trabecular bone were obtained. Vertebral bodies were reconstructed from these binary images by using software of AVS Medical Viewer (KGT Inc., Tokyo, Japan). Periodic changes of bone tissue area in median plane were calculated when the reconstructed bodies were computationally cut at the median plane on the AVS software.

Fig. 2 A radiograph of the rat caudal vertebra measured prior to a consecutive micro-CT scanning. The region for measurement was determined from cranial intervertebral surface to caudal one.

2.3 Stress analysis with a rigid-body spring model and a finite element model

All rats were sacrificed by overdose of pentobarbital sodium on day 16. The C4, C5 and C6 were removed, freed of soft tissues and fixed in 70% alcohol. The transverse sections of whole bodies of the C4 and C5 were scanned by the micro-CT with the resolution of 26 $\mu\text{m}/\text{pixel}$ and the scanning pitch of 50 μm . Images of cortical and trabecular bone were extracted by binary processes with the appropriate threshold⁽¹⁰⁾. The three-dimensional bodies of vertebrae were reconstructed from these consecutive images as described above. Both cortical and trabecular architecture in median plane were obtained by cutting the reconstructed bodies on the AVS software. The features of bone architecture were traced with a digitizer pad linked to a personal computer.

Two-dimensional rigid-body spring models (RBSMs)^{(21),(22)} in median plane were constructed in order to estimate contact pressures acting on the intervertebral joint surface of C5. The vertebral bodies were modeled as rigid bodies (Fig. 3), while the cartilage was represented by a series of compressive and shear springs, with a stiffness ratio of 0.001, normal to the articular surface between C4 and C5⁽¹⁴⁾. Joint ligaments and capsular structure were also modeled as two tensile springs arranged on the dorsal and ventral sides of vertebral bodies. When a unit compressive load was applied to the center of gravity on the longitudinal axis of C4, the reaction forces of each spring were calculated. Contact pressures on the intervertebral joint surface of C5 were obtained by multiplying the reaction forces and 30 N of the applied load together. RBSM analyses were performed fitting

Fig. 3 Schematic representation of two-dimensional rigid-body spring model. The intervertebral joint was modeled as a series of compressive and shear springs, and a pair of tensile springs. Reaction forces of each spring were calculated when the compressive load was applied to the C4.

the longitudinal axis of C4 with that of C5 under the analytical axial loads, while translating the C4 into the ventral direction by 2 mm under the analytical offset loads.

Finite element analysis (FEA) was utilized for evaluating the changes of stress distribution caused by morphological adaptation to daily mechanical stimulation. Two-dimensional (2-D) FE-models with both cortical and trabecular bone were built from the digitized features of C5. These models consisted of about 11200~13500 elements. Von Mises stresses were calculated using the contact pressures from the RBSM analyses for boundary conditions. Young's modulus of 11.0 GPa and Poisson's ratio of 0.3 were assumed. The analyses were performed using MSC/PATRAN Advanced FEA (MSC JAPAN Ltd., Tokyo, Japan).

3. Results

3.1 Changes of cross-sectional area in median plane

Figure 4 represents the cross-sectional images in median plane, obtained by computationally cutting the reconstructed vertebral bodies, of the axial- and offset-loaded rats with 300, 3 600 cycles/day and the control rat. Regions with low CT density extended over the periosteal surfaces of the axial- and offset-loaded vertebral bodies (arrowheads). These regions were observed only at the periosteal but endosteal or trabecular surfaces. It would mean the periosteal new bone formation. Area of the new bone increased, depending on the duration of the stimuli. As can be seen in Figs. 5(a) and (b), the cross-sectional areas in median plane of the loaded rats periodically increased in a cycle-dependent manner. Especially, the

Fig. 4 Cross-section of median plane obtained from the reconstructed micro-CT images. Regions with low CT density were observed over the periosteal surfaces in the mechanical loaded vertebrae (arrowheads).

Fig. 5 Periodic changes in cross-sectional area of the vertebrae. The area was calculated for the binary images in median plane obtained from the reconstructed vertebrae, and was normalized by the bone area on day 1. "Axial-300" in the explanatory notes means the axial-loaded rat subjected to the loading regime of 300 cycles/day.

axial- and offset-loaded rats, which were subjected to a daily loading regime of 3 600 cycles, indicated remarkable increases of 27.4 and 34.7 percent on day

Analyses under the axial load

Analyses under the offset load

Fig. 6 Influences of daily mechanical loading on Von Mises stress distribution. Many sites with high stress level were shown in the control rat under the analytical axial load (a), while highly stressed elements were reduced in the axial-loaded rats subjected to the mechanical loading regime of 300 cycles/day (b) and 3600 cycles/day (c). Although the control rat under the analytical offset load (d) indicated asymmetry stress distribution, this asymmetry was rather small in the offset-loaded rats ((e), (f)).

16. The bone tissue area remained steady throughout the experimental period in the control rat.

3.2 Stress analyses

Von Mises stress distribution was calculated for each vertebral body under the analytical axial or offset loading conditions (Fig. 6). The newly formed bone, responded to daily mechanical stimuli, was included in the FE-models in these calculations, and was assumed to have the same material properties as other bone tissues. As can be seen in Fig. 6(a), many sites of cortical and trabecular elements showed high stress levels in the control rat under the analytical axial load. The stress levels were however reduced in the vertebral bodies subjected to the daily axial loading regime (Figs. 6(b) and (c)). Figure 7 represents the Von Mises stresses averaged in ventral and dorsal cortical bones, respectively. The averaged stresses in the axial-loaded rats (Figs. 7(b) and (c)) showed lower values than that in the control (Fig. 7(a)), which indicated that the loaded vertebrae had developed their ability to bear the external loading. When the offset load was analytically applied to the FE-model of the control rat, the highly stressed elements were seen in the ventral part of cortex (Fig. 6(d)). The averaged stress in this part was, more-

over, the highest among the vertebrae (Fig. 7). The center of applied load was moved toward the ventral direction in the analyses under offset load, which caused the asymmetric stress distribution. Daily offset loading could relieve the asymmetry in stress distribution (Figs. 6(e) and (f)) and decrease the stress levels (Figs. 7(e) and (f)).

Additional analyses were performed with altering the Young's modulus of periosteal new bone in order to reveal the influences of new bone formation on the tissue stresses. Boundaries between the new bone and old calcified cortical bone were extracted by manually tracing the gray-scaled images in median plane. Figure 8 demonstrates the results of calculations for the axial-loaded rat with the loading regime of 3600 cycles/day. The ratio of the new bone to the old calcified bone in the Young's modulus was developed to 0.1, 0.5 and 1.0 in these calculations. As the ratio got higher, namely that the calcification developed, the highly stressed elements were disappeared at the periosteal surfaces of cortical bone (Fig. 8(a)). The variation of Von Mises stresses in the cortical bone was shown in histograms (Fig. 8(b)). The majority of highly stressed tissues altered their stress levels to be lower, depending on the Young's modulus in-

Under the axial load

(a) Control rat

(b) Axial-loaded rat with 300 cycles

(c) Axial-loaded rat with 3600 cycles

Under the offset load

(d) Control rat

(e) Offset-loaded rat with 300 cycles

(f) Offset-loaded rat with 3600 cycles

Fig. 7 Von Mises stresses averaged in ventral and dorsal cortical bones, respectively. Stress levels were decreased in the loaded rats compared with the control. Under the analytical offset load, the ventral cortical bone showed higher stress than the dorsal one in the control rat (d). Daily offset loading could relieve the asymmetric distribution and decrease the stress levels ((e), (f)). (Error bars indicate standard deviation. Figure arrangement corresponds to Fig. 6.)

(a)

(b)

Fig. 8 Influences of new bone formation on Von Mises stress distribution: (a) As the ratio of the new bone (E_n) to the old calcified bone (E_o) in the Young's modulus was developed to 0.1, 0.5 and 1.0, the highly stressed elements were reduced over the periosteal surfaces (Refer to contour rank in Fig. 6). (b) Histograms of Von Mises stress in the cortical bone. The elements showing high stress level were decreased as the Young's modulus increased in the new bone.

creased, which indicated that the caudal vertebra reduced its stress level by forming new bone in response to mechanical stimuli.

4. Discussion

We carried out the periodic measurements for externally loaded rat caudal vertebrae with utilizing micro-CT, which indicated that daily mechanical stimuli increased bone cross-sectional area depending on the loading duration. The detailed *ex vivo* measurements after experimental period made it clear that new bone formation was remarkably induced over periosteal surfaces. The newly formed matrix is considered to be woven bone. Woven bone is a spinate organization that is fabricated by depositing hydroxyapatite on the bundles of irregularly oriented collagen fiber. It has only low mechanical strength, but can rapidly deposit over bone surfaces. According to a histological examination by Chambers et al., the periosteal woven bone formation was strikingly increased in rat caudal vertebral bodies mechanically compressed⁽¹⁹⁾. Furthermore, our previous study revealed that the matrix with low elastic modulus and low hardness was formed on vertebral periosteal surfaces after mechanical loading, which implied the deposition of immature bone tissues over periosteum⁽²³⁾. These previous findings would support our measurements, in other words, drastic increases of periosteal woven bone formation.

In order to demonstrate the role of newly formed woven bone in bone adaptation, tissue stress distribution was estimated by FEA based on the micro-CT images. Young's modulus of the new bone was gradually increased in the analyses, which roughly mimics the processes of maturation and calcification in woven bone. The ratio of elements showing high stress level was decreased as the Young's modulus increased in the new bone (Fig. 8(b)). It should be noted that highly stressed tissues in the cortical bone could be relieved by immature bone even if its elastic modulus was only one-tenth as large as cortical bone. The deposition of new bone matrix, which drops the stress level in cortex, would be responsible for the activity of osteocytes and bone-lining cells. Both cells are considered providing cellular basis for mechanosensing in bone, and transmitting signals through the 3-D cellular networks for recruiting osteoblasts^{(16),(24)}. In the mechanically loaded vertebrae, the unexpected increases in stress/strain level should be informed to the osteocytes in bone matrix and/or bone-lining cells over bone surface, which induces the osteoblastic differentiation and activation of matrix synthesizing functions. Stress analyses we performed would indicate that the loaded vertebrae adapted to the external

loading with appositional modeling of periosteal woven bone, by which they attempted to reduce the high stresses unexpectedly acted in bone tissue. We have only considered the periosteal new bone that was measured with the micro-CT as low CT-valued area. There is, however, a possibility that bone formation at endosteal and trabecular surfaces failed to be detected in the current study because it might be too little to be extracted from micro-CT images by the threshold processes we used. Histological examinations by Chambers et al., in fact, showed the formation of lamellar bone on trabecular surfaces in the mechanically loaded vertebrae⁽¹⁹⁾. Although it might be true that the undetected bone formation in our measurements would have some influences on stress distribution, its contribution is likely to be small compared with the volume of periosteal woven bone.

The rat caudal vertebrae were subjected to the cyclic axial loads as well as offset loads in our study, because we intended to examine whether they could adapt to the unphysiological environments under the asymmetric loading condition. Analytical offset load to the control rat induced asymmetric average stress between ventral and dorsal cortical bone (Fig. 7(d)). This asymmetry was disappeared in the offset-loaded rats under analytical offset load (Figs. 7(e) and (f)), implying that the offset-loaded vertebrae adapted to the daily mechanical loading. These suggestions were, however, restricted within 2-D analytical models. Rat caudal vertebra has a complicated morphology, and does not show the symmetric feature between ventral and dorsal side. 3-D analysis is thus necessary for further discussion.

Our stress analyses had some simplifications and assumption to be noted. First, these analyses have performed only for the 2-D analytical models as mentioned above. It would seem that analyses in 2-D models were too simplified to evaluate the stresses in vertebral body. Some researchers calculated the tissue stresses in detailed 3-D architecture of bone samples although their FE-models only represented the small samples of trabecular bone but not the large or whole bone^{(11),(12)}. Large-scale 3-D models of the whole bone have some difficulties in analysis, that is, the uncertainty in the determination of boundary conditions, or the limitation of computing ability. Even in the 2-D analysis, it was qualitatively indicated that the highly stressed tissues tended to be relieved because of the adaptive remodeling/modeling in the stimulated vertebrae. Second, a homogeneous Young's modulus was used for all tissues. Many researchers reported that the Young's modulus in trabecular bone was less than that in cortical bone. The reported Young's moduli for cortical bone have

been shown to be about 20~22 GPa along the axis of long bone and about 12~14 GPa transversely^{(25),(26)}, while they range from 1 to over 20 GPa in trabecular bone⁽²⁷⁾. Turner et al. recently indicated that these lower values in material properties resulted from the mechanical testing methods, which suffered from considerable artifacts due to small specimen and irregular geometry⁽²⁸⁾. They obtained the elasticity of trabecular tissue within the range of that of cortical bone tissue by using a new mechanical testing technique, nanoindentation method. Third, our current study could not perform any stress analysis for the bone architecture prior to the mechanical loading regime. The longitudinal measurement *in vivo* did not provide the spatial resolution enough to construct the FE-model with trabecular architecture because of the issue of the animal size to be fitted on the rotating platform in the micro-CT. The trabecular architecture could be described in case of transverse measurement; however, it takes quite long time to obtain the median plane feature by transversely serial measurements. These measurements would be difficult for the anesthetized rats. Although it was impossible to compare the identical vertebra before and after the experiment, the comparison between the loaded rats and control rat in stress distribution suggested that the tissue stress levels were decreased by appositional new bone formation after mechanical loading regime. Finally, only one rat was used in each mechanical loading condition. It was certain that the each loaded vertebra showed gradual increases in bone cross-sectional area because changes in each area were calculated from the morphological measurements that were periodically performed for the same vertebra through the experimental period; even so, more samples would be necessary to examine the reproducibility of bone alteration in response to mechanical loading.

Notwithstanding its preliminary experiment, it was shown in this study that the computational analyses based on the consecutive micro-CT images could evaluate the alteration of the stress distribution in the rat caudal vertebra when it was subjected to the daily cyclic loading regime. These techniques would be useful for assessing the alteration of stress distribution and morphological changes related to mechanical environments, and provide a better understanding of the adaptive remodeling/modeling processes in bone.

5. Conclusions

We examined the alteration of tissue stresses influenced by periosteal bone formation with utilizing FEA based on micro-CT measurements. When the rat caudal vertebrae were subjected to daily cyclic loading regime, a remarkable increase in cross-sectional area was observed, which would be caused by the newly formed woven bone over periosteum. Stress analyses indicated that the woven bone would reduce the highly stressed tissues at the periosteal surfaces as depositing and calcifying.

The axial- or offset-loaded rats under analytical loading indicated the lower stress levels compared with the non-loaded control rat. The control rat showed asymmetric average stress between ventral and dorsal sides. This asymmetry was disappeared in the offset-loaded rats under analytical offset load. These analyses suggested that the vertebral body would rapidly deposit the woven bone over periosteum in order to adapt to the daily mechanical stimuli.

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References

- (1) Müller, R. and Rüegsegger, P., Analysis of Mechanical Properties of Cancellous Bone under Conditions of Simulated Bone Atrophy, *J. Biomech*, Vol. 29 (1996), pp. 1053-1060.
- (2) Feldkamp, L.A., Goldstein, S.A., Parfitt, A.M., Jesion, G. and Kleerekoper, M., The Direct Examination of Three-Dimensional Bone Architecture *in Vitro* by Computed Tomography, *J. Bone Miner. Res.*, Vol. 4 (1989), pp. 3-11.
- (3) Kuhn, J.L., Goldstein, S.A., Feldkamp, L.A., Goulet, R.W. and Jesion, G., Evaluation of a Microcomputed Tomography System to Study Trabecular Bone Structure, *J. Orthop. Res.*, Vol. 8 (1990), pp. 833-842.
- (4) Ciarelli, M.J., Goldstein, S.A., Kuhn, J.L., Cody, D. D. and Brown, M.B., Evaluation of Orthogonal Mechanical Properties and Density of Human Trabecular Bone from the Major Metaphyseal Regions with Materials Testing and Computed Tomography, *J. Orthop. Res.*, Vol. 9 (1991), pp. 672-682.
- (5) Bonse, U., Busch, F., Gunnewig, O., Beckmann, F., Pahl, R., Delling, G., Hahn, M. and Graeff, W., 3D Computed X-Ray Tomography of Human Cancellous Bone at 8 Microns Spatial and 10⁻⁴ Energy Resolution, *Bone Miner.*, Vol. 25 (1994), pp. 25-38.
- (6) Kinney, J.H., Lane, N.E. and Haupt, D.L., *In Vivo*, Three-Dimensional Microscopy of Trabecular Bone, *J. Bone Miner. Res.*, Vol. 10 (1995), pp. 264-270.
- (7) Majumdar, S., Newitt, D., Jergas, M., Gies, A., Chiu, E., Osman, D., Keltner, J., Keyak, J. and Genant, H., Evaluation of Technical Factors Affecting the Quantification of Trabecular Bone Structure Using Magnetic Resonance Imaging,

- Bone, Vol. 17 (1995), pp. 417-430.
- (8) Hipp, J.A., Jansujwicz, A., Simmons, C.A. and Snyder, B.D., Trabecular Bone Morphology from Micro-Magnetic Resonance Imaging, *J. Bone Miner. Res.*, Vol. 11 (1996), pp. 286-297.
 - (9) Barbier, A., Martel, C., De Vernejoul, M.C., Tirode, F., Nys, M., Mocaer, G., Morieux, C., Murakami, H. and Lacheretz, F., The Visualization and Evaluation of Bone Architecture in the Rat Using Three-Dimensional X-Ray Microcomputed Tomography, *J. Bone Miner. Metabol.*, Vol. 17 (1999), pp. 37-44.
 - (10) Mawatari, T., Miura, H., Higaki, H., Kurata, K., Moro-oka, T., Murakami, T. and Iwamoto, Y., Quantitative Analysis of Three-Dimensional Complexity and Connectivity Changes in Trabecular Microarchitecture in Relation to Aging, Menopause, and Inflammation, *J. Orthop. Sci.*, Vol. 4 (1999), pp. 431-438.
 - (11) Hollister, S.J., Brennan, J.M. and Kikuchi, N., A Homogenization Sampling Procedure for Calculating Trabecular Bone Effective Stiffness and Tissue Level Stress, *J. Biomech.*, Vol. 27 (1994), pp. 433-444.
 - (12) Müller, R. and Rügsegger, P., Three-dimensional Finite Element Modelling of Non-Invasively Assessed Trabecular Bone Structures, *Med. Eng. Phys.*, Vol. 17 (1995), pp. 126-133.
 - (13) Van Rietbergen, B., Müller, R., Ulrich, D., Rügsegger, P. and Huiskes, R., Tissue Stresses and Strain in Trabeculae of a Canine Proximal Femur Can Be Quantified from Computer Reconstructions, *J. Biomech.*, Vol. 32 (1999), pp. 165-173.
 - (14) Kurata, K., Higaki, H., Miura, H., Mawatari, T., Murakami, T. and Iwamoto, Y., The Morphological Measurements with a Micro CT and the Stress Analyses of the Adaptive Remodeling by Applied Mechanical Stimuli in Rat Caudal Vertebrae, *JSME Int. J., Ser. C*, Vol. 42 (1999), pp. 492-500.
 - (15) Cowin, S.C., Wolff's Law of Trabecular Architecture at Remodeling Equilibrium, *J. Biomech. Eng.*, Vol. 108 (1986), pp. 83-88.
 - (16) Cowin, S.C., Moss-Salentijn, L. and Moss, M.L., Candidates for the Mechanosensory System in Bone, *J. Biomech. Eng.*, Vol. 113 (1991), pp. 191-197.
 - (17) Rubin, C.T. and Lanyon, L.E., Regulation of Bone Formation by Applied Dynamic Loads, *J. Bone Joint Surg. [Am]*, Vol. 66 (1984), pp. 397-402.
 - (18) Turner, C.H., Akhter, M.P., Raab, D.M., Kimmel, D.B. and Recker, R.R., A Noninvasive, *in Vivo* Model for Studying Strain Adaptive Bone Remodeling, *Bone*, Vol. 12 (1991), pp. 73-79.
 - (19) Chambers, T.J., Evans, M., Gardner, T.N., Turner-Smith, A. and Chow, J.W.M., Induction of Bone Formation in Rat Tail Vertebrae by Mechanical Loading, *Bone Miner.*, Vol. 20 (1993), pp. 167-178.
 - (20) Chow, J.W.M., Jagger, C.J. and Chambers, T.J., Characterization of Osteogenic Response to Mechanical Stimulation in Cancellous Bone of Rat Caudal Vertebrae, *Am. J. Physiol.*, Vol. 265 (1993), pp. E340-E347.
 - (21) Kawai, T. and Takeuchi, N., A Discrete Method of Limit Analysis with Simplified Elements, *ASCE Proc. Int. Conf. on Computing in Civil Engineering*, (1981), pp. 1-16.
 - (22) Schuind, F., Cooney, W.P., Linscheid, R.L., An, K.N. and Chao, E.Y.S., Force and Pressure Transmission through the Normal Wrist: A Theoretical Two-Dimensional Study in the Posteroanterior Plane, *J. Biomech.*, Vol. 28 (1995), pp. 587-601.
 - (23) Kurata, K., Higaki, H., Miura, H., Murakami, T. and Iwamoto, Y., Alteration of Mechanical Properties of Remodeling Bone Adapted to Mechanical Stimuli, *JSME Int. J., Ser. C*, Vol. 43 (2000), pp. 822-829.
 - (24) Burger, E.H. and Klein-Nulend, J., Responses of Bone Cells to Biomechanical Forces *in Vitro*, *Adv. Dent. Res.*, Vol. 13 (1999), pp. 93-98.
 - (25) Yoon, H.S. and Katz, J.L., Ultrasonic Wave Propagation in Human Cortical Bone-II: Measurements of Elastic Properties and Microhardness, *J. Biomech.*, Vol. 9 (1976), pp. 459-464.
 - (26) Katz, J.L., Yoon, H.S., Lipson, S., Maharidge, R., Meunier, A. and Christel, P., The Effects of Remodeling on the Elastic Properties of Bone, *Calcif. Tissue Int.*, Vol. 36 (1984), pp. S31-S36.
 - (27) Rice, J.C., Cowin, S.C. and Bowman, J.A., On the Dependence of the Elasticity and Strength of Cancellous Bone on Apparent Density, *J. Biomech.*, Vol. 21 (1988) pp. 155-168.
 - (28) Turner, C.H., Rho, J., Takano, Y., Tsui, T.Y. and Pharr, G.M., The Elastic Properties of Trabecular and Cortical Bone Tissues Are Similar: Results from Two Microscopic Measurement Techniques, *J. Biomech.*, Vol. 32 (1999), pp. 437-441.