Morphological and Mechanical Evaluation of the Cancellous Bone in the Rat Femoral Head after Traumatic Osteonecrosis*

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Abstract

Over 7000 patients are suffering from osteonecrosis of the femoral head annually in Japan. The patients have to undergo surgical treatments if the femoral head collapsed. The mechanism of femoral head collapse is still unclear, although morphological and mechanical changes in the necrotic hard bones or the reparative soft tissues have been believed to cause this collapse. We analyzed three-dimensional morphological and mechanical changes in the metaphyseal cancellous bone during bone regeneration after traumatic osteonecrosis of young rat femoral head using a micro-CT. Morphological indices and apparent modulus were calculated based on parallel plate model and finite element model, respectively. Bone volume, trabecular thickness, and apparent modulus initially decreased on day 7 after osteonecrosis, and they gradually increased during regenerating process. These findings indicate that the structural rigidity of cancellous bone in the regenerative area transiently decreases after osteonecrosis. These changes possibly induce femoral head collapse. The reparative area should be a target to treat osteonecrosis of the femoral head.

Key words: Biomechanics, Osteonecrosis, Collapse, Regeneration, Trabecular Microstructure, Mechanical Property, Histology, Animal Model

1. Introduction

Cancellous bone has load-bearing function and remolds its structure in response to the mechanical environment. Fracture risk increases on femoral neck, spine, and wrist when cancellous bone deteriorates by bone disorders such as osteoporosis or osteonecrosis. To date, there are numerous studies focusing on the relationship between mechanical properties of cancellous bone and its microstructure using a micro computed tomography (micro-CT) (1)(2). Studies to reveal therapeutic effects of medicines, such as bisphosphonates, also focused on changes in trabecular microstructure (3)(4). Therefore, cancellous bone is a target in the orthopedic and bioengineering fields to clarify and treat several bone diseases inducing severe dysfunction of daily life.
Osteonecrosis of the femoral head is one of the bone diseases affecting cancellous bone. Its etiology is still unknown, however, osteonecrosis occurs by deprivation of the blood supply to the femoral head. This bone ischemia leads structural failure of cancellous bone which results in the subsequent collapse of the femoral head as shown in Fig. 1. After the collapse, patients with osteonecrosis have severe pain and their range of motion of hip joints is restricted. Eventually, the femoral head deforms which results in secondary osteoarthritis. Since the success rates of bone and joint preserving surgeries are not acceptable, not a few patients have to undergo total hip arthroplasty (THA), even if patients are still young. THA should be avoided or delayed in especially younger patients because they will need several re-implantations (5). Therefore, to prevent the collapse of the femoral head is essential to treat this bone ischemic disease and we need to understand the mechanism of the collapse.

Using a rat traumatic osteonecrosis model, Norman et al. evaluated histology of the necrotic femoral head after vascular deprivation by striping the periosteum around the femoral neck and cutting the teres ligament (6)(7). They concluded that the revascularization into the ischemic inter-trabecular spaces triggered the following restorative processes, which resulted in anatomical distortion of the femoral head. Using a finite element analysis (FEA), Brown et al. suggested that the femoral head collapse after osteonecrosis was induced by alterations of the mechanical characteristics in the cancellous bone (8). The trabecular microstructure and its mechanical properties seem to change after osteonecrosis, however, these changes in relation to biological repairing process have still remained unclear.

In this study, we evaluated the trabecular microstructure and mechanical properties in the rat femoral head with traumatic osteonecrosis using a micro-CT and a voxel-based FEA. The cancellous bone volume and the apparent modulus of the metaphyseal cancellous bone reduced transiently during the regenerating process after osteonecrosis. These findings indicated one of the mechanisms why the femoral head collapses during the repairing process after osteonecrosis.

2. Materials and Methods

Animal preparation

We used 18 Wister rats (15 week-old, female, 293.5 ± 19.6 g [average weight ± S.D.]) in this study. The circulation of the rat femoral head was surgically disrupted using the method of Norman et al. (6), with some modification in which the femoral neck was burned using a bipolar electro-cautery. This modification is for securely disrupting the blood supply to the femoral head.

Traumatic osteonecrosis was induced surgically as follows. Under anesthesia with Nembutal (4 mg/100 g, i.p.) and after shaving the skin, a proximal longitudinal incision was
made over the greater trochanter. The gluteus maximus muscle was split in the direction of its bundles, the anterior two-thirds of the gluteus medius muscle were detached from the bone, and the joint capsule was transected. The ligamentum teres was cut, and the femoral head was dislocated. The peristeum of the femoral neck was stripped using a surgical blade and was burned with bipolar electrocautery. After reducing the femoral head, the joint capsule and gluteal muscles were sutured, and the skin was closed. After surgery, the animals were housed in spacious cages on a 12-hour dark/light cycle, with access to regular diet and water ad libitum.

On postoperative days 7, 21, and 42 (n=6), perfusion fixation was performed with 4% paraformaldehyde solution (Wako, Osaka, Japan) in 0.1 M phosphate buffer under deep anesthesia for histological observations (hematoxylin eosin [HE] staining and tartrate resistant acid phosphatase [TRAP] staining). Both femora were removed, and the femoral heads proximal to the femoral neck were harvested. The operated and contralateral non-operated femoral heads were treated as osteonecrosis models (ON) and controls, respectively. All animal experiments were conducted according to the “Guidelines for Animal Experimentation” of the Niigata University Graduate School of Medical and Dental Sciences.

**Histological analysis**

Histological observation was performed to characterize the histology of the affected tissue after osteonecrosis and to reveal the alterations of the trabecular bone.

All specimens were fixed in 4% of paraformaldehyde solution in 0.1M phosphate buffer (pH 7.4), decalcified in 0.5M of ethylene diamine tetra-acetate (EDTA) (pH 7.6) solution, and embedded in paraffin wax after scanning on a micro-CT. Serial sagittal sections of 4µm thick were made at the insertion level of the teres ligament using a sliding microtome (Leica, Germany). These sections were stained with HE and TRAP staining.

**Measurement of trabecular microstructure with micro-CT**

Micro-CT was used to evaluate the morphological changes of the cancellous bone during the repairing process after osteonecrosis.

The specimens were secured on a rotating platform, so that the axis of rotation closely matched the bone axis defined as the line connecting the centers of the femoral head and femoral neck. They were then scanned using a cone beam type micro-CT system (SMX-130CT-SV; Shimadzu, Kyoto, Japan) at a tube voltage of 50 kV, tube current of 36 µA, slice thickness of 19 µm, pixel size of 19 µm, and the image matrix of 512×512. A three-dimensional voxel dataset was reconstructed from the multiple slice image data (1 cubic voxel size = 19 µm). The metaphyseal cancellous bone beneath the growth plate was set as the cubic volume of interest (VOI), which consisted of 50×50×50 voxels (Fig. 2 A and B). Since the reconstructed voxel datasets included random noise and some debris, the raw data were purified by using a median filter and removing the clusters consisting of fewer than 20 voxels as noise. The VOIs were binarized using an adequate threshold calculated using the discriminate analysis method in each VOI.

The alterations in the cancellous bone during the repairing process after osteonecrosis were evaluated using conventional morphometric indices of the trabecular microstructure. The morphometric indices were determined from the binarized VOI directly using Parfitt’s definitions, according to the plate model (9). The morphometric indices were as follows: fractional trabecular bone volume (BVF, %), which represents the percentage of trabecular bone volume (BV) relative to the total tissue volume (TV) in the VOI; trabecular thickness (Tb.Th, µm); trabecular separation (Tb.Sp, µm); and trabecular number (Tb.N, 1/mm).

**Voxel-based finite element analysis**
Rat’s femoral head were so small that it was impossible to prepare a precise cubic specimen of the femoral head without any errors in mechanical compressive tests. Therefore, the changes in mechanical properties of the affected cancellous bone were analyzed by a voxel-based FEA (10) in this study.

Bone tissue voxels in the cubic VOI were directly converted into 8-node brick elements (1 cubic element size = 19 µm) in the FE model, as shown in Fig. 2 C. The tissue elastic properties were set as linear elastic and isotropic, with a Young’s modulus of 10 GPa and a Poisson’s ratio of 0.3 (4). The boundary condition of this FE model represented the situation in a compressive test. In the direction of the bone axis, which was defined as a line connecting the centers of the femoral head and femoral neck, a displacement of -0.0095 mm was assigned to the top face (beneath the growth plate), which is equivalent to 1% strain on the cube as a whole (10). At the bottom face, the displacement was constrained in the direction of the bone axis. All other faces of the cube were unconstrained. This boundary condition simulates a compression test on the cube, with zero friction between the test plates and bone. Therefore, the cube is in a state of uniaxial stress at the apparent level. The apparent Young’s modulus in the direction of the bone axis, for the specimen as a whole, was calculated from the apparent stress and apparent strain to evaluate the rigidity of the cancellous bone in the VOI. The apparent strain was 1%, and the apparent stress was defined as the reaction force on the top face divided by the area of the top face. All linear finite element analyses were performed using ABAQUS software (Version 6.5, ABAQUS, RI).

**Statistical analyses**

All data obtained from above analyses were statistically analyzed with STATISTICA version 6 (StatSoft, USA), using unpaired t-tests to reveal differences between normal cancellous bone and bone with osteonecrosis. P-value less than 0.05 was considered to be significant.

**3. Results**

**Histological analyses**

The metaphyseal bone marrows were occupied intact bone marrow cells in the femoral head of the control group (Fig. 3A). In contrast, the osteocytic lacunae were partially empty and fibroblastic cells occupied the metaphyseal marrow spaces of the osteonecrosis group.
Fig. 3  Histology of the metaphyseal region in the femoral head with HE staining. Magnification × 100
A: A metaphyseal trabecular region in the normal femoral head (Control) showed intact trabecular bone and marrow cavities with hematopoietic cells.
B: A metaphyseal trabecular region in the necrotic femoral head (ON) on day 7. The osteocytic lacunae were partially empty. The inter-trabecular marrow spaces were occupied by fibroblastic cells, indicating bone regenerating process after osteonecrosis.

Fig. 4  Histologies of the necrotic femoral heads of young and adult rats with TRAP staining. A: The metaphyseal trabecular bones of the intact femoral head in the control group. The inter-trabecular spaces were occupied with intact marrow cells.
B: The metaphyseal trabecular bones of the femoral head with osteonecrosis on day 7 after the operation. Many osteoclasts (arrows) lined trabecular bones. C: The repair region between the necrotic and viable tissues in mature rat with osteonecrosis. There were also many osteoclasts (positive for TRAP staining) along the trabecular bones. A, Magnification × 100; B, × 100, C, × 200.
on day 7 (Fig. 3B). There were many osteoclasts, which were multinuclear cells and positive for TRAP staining, lining the metaphyseal trabeculae (Fig. 4B). This histology was similar to that in the femoral heads of mature rats with surgical osteonecrosis on day 7 shown in Fig. 4C, in which many osteoclasts lined the trabecular bony surfaces at the interval between ischemic and intact area. This finding also corresponded to the previous histological study in the traumatic osteonecrosis model of immature rats created by Norman et al (6).

Fig. 5 Sagittal section of the micro-CT images of the rat femoral head bisected at the insertion of the teres ligament. The femoral head is about 4.0 mm in diameter. A: The intact femoral head of the control group, B: The femoral head with traumatic osteonecrosis on day 42. The metaphyseal cancellous bone region lost bone volume and connectivity. The femoral head demonstrated deformity indicating osteoarthritis after osteonecrosis.

Fig. 6 Morphometry for the trabecular microstructure in the metaphyseal cancellous bone of the femoral head on days 7, 21, and 42 after operation. A: Bone volume fraction [BVF, %], B: Trabecular thickness [Tb.Th, µm], C: Trabecular space [Tb.Sp, µm], D: Trabecular number [Tb.N, 1/mm]. BVF, Tb.Th, and Tb.N decreased and Tb.Sp increased on day 7 in the group with osteonecrosis and Tb.Sp was still wider in the femoral head on day 21.
Typical sagittal micro-CT images of the femoral head on day 42 are shown in Fig. 5. Bone volume and connectivity lost in the metaphyseal cancellous bones of the osteonecrosis group (Fig. 5B) compared with that of the control group. The femoral head surface was irregular, indicating osteoarthritis.

Figure 6 shows the changes in the morphometrical indices of the metaphyseal cancellous bone on days 7, 21, and 42, which demonstrating micro-structural deterioration. BVF, Tb.Th and Tb.N significantly decreased, and Tb.Sp significantly increased in the osteonecrosis group on day 7 compared to those in the control group. Then, BVF, Tb.Th and Tb.N increased and recovered to the control level after day 7 during the repairing phase.

Analysis of apparent modulus

The apparent modulus represents the structural rigidity of the cancellous bone in the cubic VOI. The apparent modulus decreased 44% and 19% on day 7 and 21, respectively, compared to that of the control as shown in Fig. 7A. After day 7, the apparent modulus increased. There was no significant difference between the osteonecrosis and control groups on day 42.

4. Discussion

In our young rat osteonecrosis model, we could not detect complete osteonecrosis in every trabecular bones in the femoral head on day 7 after disruption of circulation in the femoral head. There were mixtures of necrotic and viable bones in the ischemic lesions. Proliferation of fibroblasts and multi-nuclear TRAP positive cells (osteoclasts) were also observed in the inter-trabecular spaces. These histological findings were very similar to the histology observed between the osteonecrotic and intact area in our matured rat osteonecrosis model and in the previous reports by Norman et al. Therefore, the following micro-structural and mechanical changes in the femoral head seemed to be changes in the reparative tissues observed in the human femoral head with osteonecrosis. The results of our morphological analysis showed transient decreases in the bone volume and thickness on day 7 after osteonecrosis, and subsequent increases in these indices during regenerating process. According to these deteriorative changes of the trabecular microstructure at early repairing phase, the apparent modulus was decreased. Bone resorption and formation shows coupling in intact bone turnover. However, bone regeneration after osteonecrosis initiated by bone resorption between the necrotic and viable
area, which was followed by fibroblast proliferation with vascular regeneration in the inter-trabecular spaces. New bones formed by replacing the fibrous tissues or directly on the existing dead bones. This uncoupling of bone formation and resorption deteriorates the apparent modulus of the regenerating cancellous bone. From our speculation, it appears that the deterioration on the structural rigidity of the regenerating cancellous bone alters the stress distribution in the femoral head, which results in stress concentration at the subchondral bone. Eventually, the femoral head collapses as shown in Fig. 1. This speculation is supported by previous studies using numerical analyses (8)(11). To our knowledge, this is a first report to describe 3-dimensional biomechanical deterioration in the regenerating cancellous bone during the repairing process after osteonecrosis.

Previous clinical studies indicated that the extent of osteonecrosis affected the development of the collapse of the femoral head (12)-(16). From our findings, the extent and site of the regenerating region would have effect on the collapse. This regenerating cancellous bone lesion could be detected precisely as a low signal intensity band in T1-weighted images using 3D-MRI (17). Therefore, to detect the volume and location of the regenerating tissues would be an important parameter to predict the collapse.

Our results also suggest that maintaining the mechanical properties in the repairing cancellous bone region between necrotic and viable bone tissues will be a therapeutic target to prevent collapse. Recently, Alendronate was clinically used to treat osteonecrosis and showed a short-term clinical effect to prevent the collapse (18)(19). Alendronate, one of the most potent bisphosphonates, inhibits osteoclastic bone resorption and is widely used for osteoporosis (20). Our results support the hypothesis that if the osteoclastic bone resorption is suppressed at initial phase after osteonecrosis, mechanical properties of the cancellous bone will be maintained and the collapse can be prevented or delayed. However, Alendronates suppresses the bone turn over, we have to follow up the clinical results carefully.

A weak point of this study was that the effect of bone mineralization was not analyzed and the same homogeneous Young's modulus was used for all bony tissues. The apparent modulus was determined based on the trabecular microstructure, not on the bone mineralization. Therefore, we focused on the relative structural rigidity between normal and necrotic cancellous bone. We have conducted additional study to develop a measurement system of bone mineralization utilizing micro-CT.

In conclusion, our results demonstrated transient deterioration and subsequent regeneration in the cancellous bone during repairing process after osteonecrosis. The uncoupling of bone resorption and bone formation seems to cause these disproportional changes in the trabecular microstructure and apparent modulus, which probably a reason why the femoral head collapses in patients with osteonecrosis of the femoral head.

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References


