Bone 64 (2014) 82–87

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

# Trabecular microfractures in the femoral head with osteoporosis: Analysis of microcallus formations by synchrotron radiation micro CT

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ARTICLE INFO

*Article history:*
Received 16 October 2013
Revised 7 March 2014
Accepted 18 March 2014
Available online 2 April 2014

Edited by: David Fyhrie

*Keywords:*
Microcallus
Subchondral bone
Femoral head
Osteoporosis
Synchrotron radiation micro CT

ABSTRACT

Trabecular bone microfracture pathogenesis and associated healing processes are not well understood. We analyzed the microcalluses that form subsequent to microfractures in patients with osteoporosis (OP) using synchrotron radiation micro CT (SRCT).
Subchondral bone columns were extracted from the femoral heads of 11 female patients with a femoral neck fracture. SRCT scanning was performed with $5.9 \times 5.9 \times 5.9\ \mu m^3$ voxel size and the microcallus number was measured in a 5-mm cubic subchondral bone region. The trabecular bone microstructure was measured and its relationship to the microcallus number was analyzed. In addition, the degree of mineralization of the microcallus region and that of the rest of the trabecular bone were measured and compared.
Microcallus formations were detected in all cases, with a mean microcallus number of 4.9 (range, 2–11). The microcallus number had a significantly negative correlation with bone volume fraction (BV/TV), trabecular thickness (Tb.Th), and degree of mineralization, and had a positive correlation with specific bone surface (BS/BV). The degree of mineralization of the microcallus region was lower than that of the rest of the trabecular bone and had a wider range of values.
Microcallus formations were frequently detected in patients with OP, and more prevalent in the bone with thinner trabeculae, suggesting microfractures might occur due to activities of daily living as the OP progresses. The degree of mineralization of microcallus might represent the process of bone healing from immature woven bone to mature trabecular bone.



## Introduction

Patients with bone fragility due to bone diseases such as osteoporosis (OP), rheumatoid arthritis, and hyperparathyroidism sometimes have bone fractures by normal impacts of daily living, which is called insufficiency fracture. Insufficiency fractures usually occur at the vertebral body, pelvis, and femoral neck. Generally, the fracture line or deformation is not clearly visible by plain radiography, and can only be detected by computed tomography (CT) or magnetic resonance imaging (MRI). Such fractures sometimes occur in the subchondral bone of the femoral head, and this is now receiving attention as a "subchondral insufficiency fracture of the femoral head" [1–3]. This condition can result in femoral head collapse, producing a rapidly progressing deformity [4,5].

Fractures that occur in trabecular bone at a microscopic level are referred to as microfractures. A callus that subsequently forms at a microfracture site as part of the healing process is referred to as a microcallus [6,7]. Microfractures and microcalluses cannot be detected by conventional clinical imaging equipment. It is postulated that microfractures occur and are repaired by microcalluses even in normal bones without any symptoms. If this bone healing is not successful, the trabecular bone connectivity will be lost at the site of the microfracture, resulting in trabecular structural weakness. If such microfractures occur extensively, it can eventually cause clinical signs, which can be related to osteoporotic pain and insufficiency fracture.

Synchrotron radiation micro CT (SRCT) has significant advantages when compared with standard micro CT. The SRCT possesses a high resolution and a high quantitative capability and can identify three-dimensional (3D) trabecular bone mineralization in addition to the bone microstructure [8–10]. Synchrotron radiation is generated by passing electrons accelerated to the speed of light in a magnetic field. The resulting X-ray beam is highly intense, strongly collimated, and practically monochromatic. Artifacts such as beam hardening and noise rarely occur with the synchrotron radiation X-rays used for micro CT, whereas they are certainly present in conventional micro CT. Therefore, a high quantitative performance can be achieved with SRCT. It can be used to identify the 3D distribution of trabecular mineralization and to visualize the trabecular structure [11–13].

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http://dx.doi.org/10.1016/j.bone.2014.03.039
8756-3282/

The degree of trabecular mineralization is equivalent to the density of the mineral deposited in collagen, and is regarded as an index of bone turnover as well as an index of the mechanical property of the bone [8,9]. Highly mineralized bone generally means that the bone is mature and aged, and has low bone turnover with hard material. Poorly mineralized bone indicates that there is high bone turnover and soft material. Therefore, SRCT is one of the few ways to perform 3D analysis of the trabecular bone microstructure and metabolism at the same time.

Whereas microcalluses have been detected using optical microscopy, electron microscopy, histological techniques, and radiographic techniques, there has been no published report of 3D analysis using SRCT. Although the degree of mineralization of microcallus has been analyzed histologically [14], there has been no study which analyzed microcallus mineralization quantitatively using SRCT. It might be possible to understand more about microfracture pathogenesis and their healing processes by performing such an analysis. A study of microcalluses in the subchondral bone of the OP femoral head might also lead to greater understanding of subchondral insufficiency fractures of the femoral head.

In this study, we analyzed microcallus formations that occurred in subchondral trabecular bones of OP femoral heads using SRCT to investigate: (1) the prevalence and number of microcallus formations; (2) the relationship between microcalluses and the trabecular bone microstructure; and (3) the characteristics of the degree of mineralization of microcallus.

## Materials and methods

### *Subjects*

The subjects were 11 patients with femoral neck fracture who had undergone bipolar hip arthroplasty at Nishiisahaya Hospital (mean age, 76.2 ± 7.7; range, 65–92 years, all female). Those who were male, patients with any symptoms in the hip joints or bone and joint diseases such as rheumatoid arthritis, or patients with medications that affect bone metabolism such as bisphosphonate were excluded. Eleven femoral heads were obtained in surgery, and subchondral bone samples were extracted from the loading area of each femoral head (Fig. 1). Subchondral bone columns (10 mm in diameter and 10 mm in height) were extracted using a coring reamer at a position 15 mm outside the lateral edge of the fovea of the capitis femoris. The samples were preserved in an acrylic case filled with physiological salt solution, and maintained in a freezer.

The ethics review board of our institute approved the study protocol, and all patients were provided informed consent to participate. The study protocol complied with the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects.

### *Imaging*

SRCT scanning was performed at beamline BL20B2 in the synchrotron radiation facility SPring-8 (Hyogo, Japan). A 30-keV X-ray beam and a 4000 × 2624-pixel charge-coupled device (CCD) camera were used. The voxel size was 5.9 × 5.9 × 5.9 $\mu m^3$. The samples were thawed gradually at room temperature, and maintained at the same temperature while being scanned. Each sample was placed on the table, and a 1-s exposure was performed every 0.1° of rotation for a total of 180° and 1800 radiographic projections. The total scanning time for each sample was approximately 2.5 h. The raw data were reconstructed as Tag Image File Format (TIFF) images.

### *Image analysis*

The (1) microcallus number, (2) trabecular bone microstructure, and (3) degree of mineralization of the microcallus region were measured using the bone microstructure measurement software TRI/3D-

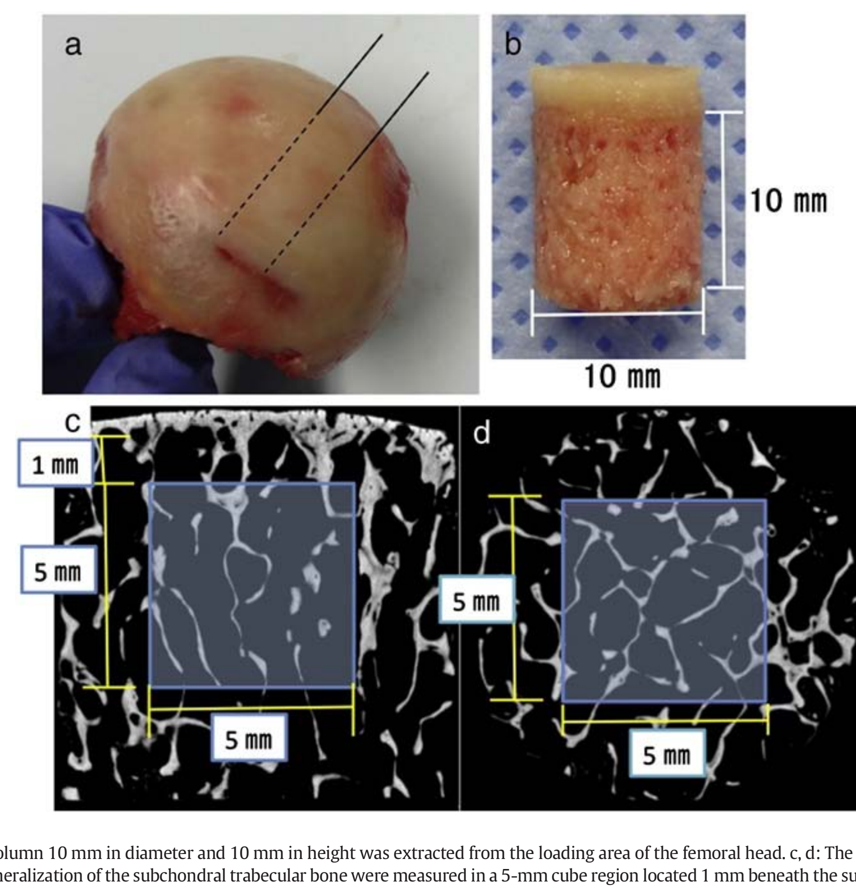


**Fig. 1.** a, b: A subchondral bone column 10 mm in diameter and 10 mm in height was extracted from the loading area of the femoral head. c, d: The microcallus number, microstructural parameters, and the degree of mineralization of the subchondral trabecular bone were measured in a 5-mm cube region located 1 mm beneath the subchondral bone plate at the center of the column.