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Age-related regional deterioration patterns and changes in nanoscale characterizations of trabeculae in the femoral head

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ABSTRACT

This study aimed to investigate the mechanical properties and features of bone materials at the nanoscale level in different regions of the femoral head in elderly patients with femoral neck fracture. Ten femoral heads from female patients with femoral neck fractures were extracted during surgery (five for the Aged group, aged 65–66 years; five for the Advanced aged group, aged 85–95 years). The femoral head was divided into three equal layers (anterior, central, and posterior) in the coronal view, and each layer was segmented into five regions (superior, central, inferior, medial, and lateral). Nanoindentation testing and atomic force microscopy imaging were used to study the mechanical properties and surface morphology of the specimens. No statistical differences in grain size were found between age groups, which suggested that the nanostructure of trabeculae in the femoral heads of postmenopausal women cannot be used to predict age-related bone loss and fracture risk. Mechanical properties in the longitudinal direction deteriorated more quickly than those in the transverse direction for the whole femoral head. Comparisons between layers showed a higher deterioration rate with aging in the anterior layer than in other layers. In different regions, mechanical properties of the medial and lateral regions deteriorated more quickly than those in the three other regions, and deterioration in the longitudinal direction was more serious than that in the transverse direction. The regional deterioration patterns and material properties with aging observed in this study contribute to an understanding of the age-related fracture mechanism and provide a basis for predicting age-related fracture risk and decreasing early fixation failure in the proximal femur.

1. Introduction

Aging is an important factor leading to enhanced bone loss. Advanced age is associated with decreased bone quality and fractures. Fragility fractures, especially hip fractures, may be caused even after minimal trauma in the case of low bone mass (Milovanovic et al., 2012a). Femoral neck fractures in the elderly population are caused by numerous factors, such as osteoporosis, degeneration of supporting muscles in the hip joint, delayed responses, or serious injury. Femoral neck fractures conventionally lead to increased numbers of deaths and substantial disability. High rates of nonunion and avascular necrosis occur during the healing process, and the increased morbidity renders femoral neck fractures an enormous social burden (Melton, 1993).

As a common treatment for femoral neck fractures, internal fixation (IF) has numerous advantages, such as minimal blood loss, short operating time, and low infection rates (Viberg et al., 2014), and it is considered to be a better choice for elderly patients (Schep et al., 2004). However, deterioration of structure and mechanical properties of bone material in the femoral head during aging may lead to changes in stresses and strains of trabeculae, this may cause a failure of implant (Goffin et al., 2014). Hence, understanding the material properties of trabeculae in the femoral head is important in improving design criteria and surgical methods during the treatment of fractures in the proximal femur.

Nanoindentation testing is a useful technique for measuring the intrinsic mechanical properties of materials. This technique has been widely used in exploring the mechanical properties of bone tissues, such as osteons, woven bone, and trabeculae (Fan et al., 2006; Hengsberger et al., 2001; Ishimoto et al., 2011; Milovanovic et al., 2011, 2012a; Rho et al., 1999). Investigating the mechanical properties of bone materials could provide more information for understanding the relationship between bone quantity and fractures. Atomic force microscopy (AFM) is a high-resolution [$<$0.1 nm in depth, typically $<$5 nm on x–y plane (Wallace, 2012)] scanning probe microscopic technique that can show the surface morphological features of materials at the molecular and atomic levels (Milovanovic et al., 2011; Hassenkam et al., 2005; Chappard et al., 2011; Thurner et al., 2005). The combination of nanoindentation testing and AFM imaging has been proven to be a powerful tool for exploring the mechanical properties and features of the surface of bone materials at the nanoscale level (Hengsberger et al., 2001; Milovanovic et al., 2012a) as well as evaluating the

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relationship between bone strength and morphological features (Milovanovic et al., 2011, 2012a,c). This strategy offers an improved understanding of the intrinsic mechanism of bone deterioration with aging (Chappard et al., 2011; Hassenkam et al., 2005; Li et al., 2012).

With increasing age, trabecular microstructure in the proximal femur deteriorates significantly (Djuric et al., 2010; Cui et al., 2008; Lochmüller et al., 2008), especially in elderly patients with hip fractures (Milovanovic et al., 2012b). Age-related bone loss and trabecular microstructure changes within the proximal femur are not uniform (Cui et al., 2008; Djuric et al., 2010; Milovanovic et al., 2011), which means deterioration follows different patterns among regions. Therefore, identifying regional variations of morphological and mechanical properties in femoral heads with femoral neck fracture could offer new insights into bone quality. Results will be of great significance for evaluating femoral fracture risk and failure of IF by understanding regional bone material characteristics and deterioration in different directions of trabeculae with aging. Exploring the bone quality of patients with fractures may also contribute to the understanding of the fracture mechanism and provide a theoretical basis for improving IF techniques in the proximal femur.

In the present study, femoral heads from senile female patients with femoral neck fractures were extracted. Mechanical properties and surface morphology of trabeculae in different regions were investigated at the nanoscale level by nanoindentation testing and AFM imaging. The deterioration pattern with aging was explored to evaluate the age-dependent fracture mechanism more extensively. This study may provide a theoretical basis for preventing age-related fragility fractures and decreasing early fixation failure and offer a guide for IF in elderly patients.

2. Materials and methods

2.1. Sample preparation

Informed consent forms were signed by the patients, and the study was approved by the Ethics Committee of The First Hospital of Jilin University (no. 2012-064).

A total of 31 cases of femoral heads were collected in this study with the help of Department of Orthopedic Trauma, the First Hospital of Jilin University. After a rigorous screening process, 10 cases were selected finally (all females). Patients with a history of smoking, drinking, and drug use, as well as those who took medicines that may affect bone metabolism, were excluded (patient-related information, inclusion and exclusion criteria were shown in Appendix A).

Femoral heads were extracted from ten female patients with femoral neck fractures (five for the Aged group, aged 65–66 years; five for the Advanced aged group, aged 85–95 years). The samples were cleaned of adherent soft tissue and maintained in 70% alcohol prior to further experimentation.

For easy observation of the orientation of trabeculae, the femoral head was divided into 3 equal layers [i.e., anterior (Ant), central (Cen), and posterior (Pos) layers] in coronal view by a low-speed diamond saw (Fig. 1A). Each layer was then segmented into 5 regions [i.e. superior (S), central (C), inferior (I), medial (M), and lateral (L) regions, Fig. 1B].

The axis of the trabecular was defined as the longitudinal direction, and the direction perpendicular to the axis was defined as the transverse direction (Rho et al., 1999) (Fig. 2). Four samples from each region were cut as described above (two samples in the transverse direction: one for AFM imaging and the other for nanoindentation testing; two samples in the longitudinal direction: one for AFM imaging and the other for nanoindentation testing). Trabecular bone specimens were cleaned ultrasonically in alcohol for 5 min and dried naturally at room temperature. Fig. 3 shows typical "preliminary" samples of different directions. To avoid the influence of edges between regions, the samples we used for analysis were obtained from these "preliminary" samples by preserving only the trabeculae at the center of each specimen. All specimens were handled in the same manner to ensure the validity of inter-specimen comparison.

2.2. AFM imaging of bone materials

Bone samples were placed horizontally onto the sample disk and imaged by a Multimode quadrex SPM with a Nanoscope IIIe controller (Veeco Instruments, USA). Imaging was performed under ambient conditions in standard AFM tapping mode using a commercial Silicon AFM probe (Tap300Al-G, Budget Sensors Instruments, Bulgaria) with a 125 μm cantilever length, a 40 Nm^{-1} constant force, a 300 kHz resonant frequency, and a tip radius lower than 10 nm. A minimum of ten images were obtained from different locations in each sample, from which six images with better representativeness of the morphological features were selected for analysis. The size of mineral grains was measured using NanoScope Analysis version 1.4.0 (Milovanovic et al., 2011).

2.3. Nanoindentation testing of bone materials

For the same position, the specimens were dehydrated in a series of alcohol baths (70%, 80%, 90%, 100%, 24–48 h for each period) and embedded in epoxy resin at room temperature (Fan et al., 2006; Rho et al., 2002; Zhang et al., 2014). All samples were metallographically polished using silicon carbide abrasive papers of decreasing grit sizes (600, 800, 1500, and 2000 grit) and finally on microcloths with finer grades of diamond suspensions to the finest, 0.05 μm grit, to produce

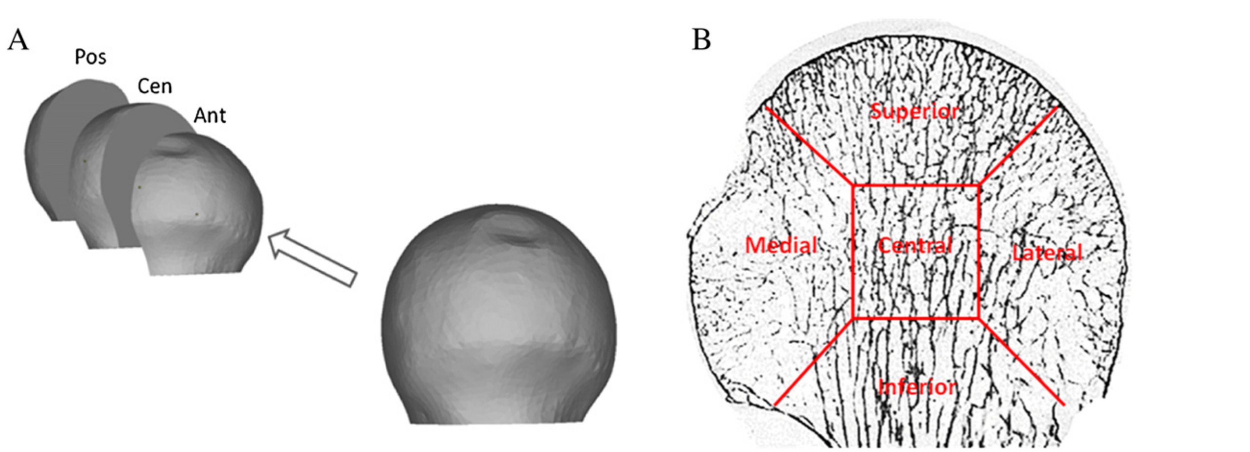

Fig. 1. Schematic diagram of partition. (A) Definition of layers. (B) Regions on the layer.