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Effect of estrogen deficiency on regional variation of a viscoelastic tissue property of bone

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ABSTRACT

Estrogen deficiency changes the regional distribution of tissue mineral density leading to alteration of the mechanical properties of bone at the tissue level. Direct measurement of the regional variation of elastic modulus and viscosity, which is the capacity to resist time-dependent viscoelastic deformation, will aid in our understanding of how estrogen deficiency alters bone quality. It was observed that, compared to bone from other anatomical sites, the jaw bone is less sensitive to estrogen deficiency. Thus, the objective of this study was to examine the effect of estrogen deficiency on (1) the regional variations of tissue modulus and viscosity of bone using nanoindentation, and (2) the modulus–viscosity relationships in jaw and vertebral bones for comparison between different anatomical sites. Mandibular and vertebral bone specimens of sham surgery and ovariectomized (OVX) rat groups were subject to nanoindentation in hydration. Indentation modulus and viscosity were measured at relatively new (less mineralized) tissue regions and at the corresponding pre-existing old (more mineralized) tissue regions of mandibular and vertebral bones. In the mandibular bones, significant regional variations of indentation modulus and viscosity were observed ($p < 0.039$) and OVX increased the indentation viscosity. While significant positive correlations were found between indentation modulus and viscosity ($p < 0.001$), the correlation slopes for the mandibular and vertebral bones were significant different ($p < 0.001$). The current results indicated that changes in viscoelastic property and its regional variation should be examined to obtain a better understanding of estrogen deficiency-dependent alteration of bone quality.

1. Introduction

Estrogen deficiency-dependent active bone remodeling causes unbalanced bone turnover, resulting in bone loss in postmenopausal patients (Lerner, 2006). In addition to this alteration of bone quantity, active bone remodeling can increase resorption of old bone, which is in turn followed by the formation of new bone tissue. This process changes the quality of the bone by amplifying the variability of mineral density at the tissue level, as observed in postmenopausal human and animal bones (Ames et al., 2010; Busse et al., 2009; Deguchi et al., 2008). Recently, it was found that the variability of tissue mineral density determines unrecoverable residual deformation at the macro level of human and animal vertebral bones, resulting from a time-dependent viscoelastic creep deformation (Kim et al., 2011, 2012). It was also found that viscosity, which is a capacity to resist time-dependent viscoelastic deformation, has a strong linear relationship with elastic modulus (Kim et al., 2010), which is linearly correlated with tissue mineral density (Mulder et al., 2008). These findings suggest that the estrogen deficiency-dependent changes of tissue mineral density distribution may alter the distribution of the viscoelastic properties, including viscosity, at the tissue level resulting in the high risk of time-dependent deformation of postmenopausal osteoporotic bone at the organ level. However, the effects of estrogen deficiency on elastic modulus and viscosity of bone at the tissue level have not been fully examined.

The elastic and viscoelastic mechanical properties of bone tissue are mainly determined by the interaction between collagen fibrils and crystalline mineral components, for both human and animal bone (Pathak et al., 2011; Ruppel et al., 2008; Wu et al., 2012). Bone tissue maturity (mineral-to-matrix ratio) was used to reflect bone quality (Pathak et al., 2011; Ruppel et al., 2008). It was indicated that the mineral-to-matrix ratio was lower in newly-formed bone tissue regions than in pre-existing old bone tissue regions when mouse femurs were observed under Raman

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spectroscopy (Pathak et al., 2011). The mineral-to-matrix ratio of bone tissue also varies depending on anatomical site (Fu et al., 2012; Otomo et al., 2004). Pathak et al. (2011) found a larger viscoelastic response in a region of tissue with a smaller mineral-to-matrix ratio using nanoindentation-based dynamic mechanical analysis. However, a lack of knowledge exists about the effects of estrogen deficiency on the regional variation of the mechanical properties of bone at the tissue level.

Obtaining more information about the mechanical properties of bone at the tissue level can provide baseline knowledge to better understand how the load bearing ability of bone changes with disease, including estrogen deficiency-induced postmenopausal osteoporosis. In this study, we hypothesized that direct measurement of the regional variation of elastic modulus and viscosity at the tissue level will help us to understand how estrogen deficiency-dependent abnormal bone remodeling alters bone quality differently between bone regions and anatomical sites. The objective of this study was to examine the effect of estrogen deficiency on (1) the regional variations of tissue modulus and viscosity of bone using nanoindentation, and (2) the modulus–viscosity relationships in jaw and vertebral bones for comparison between different anatomical sites.

2. Materials and methods

2.1. Specimen preparation

Sprague-Dawley female rats (6 months old) were obtained following experimental protocol approval by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University. A group of rats had a sham operation (sham) and the other group of rats underwent a bilateral ovariectomy (OVX) operation at Harlan Sprague Dawley, Inc (Indianapolis, IN). The rats were fed a normal diet for 2 months post-surgery and were then euthanized. Tooth-bearing 5 mm mandibular bone sections were made in the bucco-lingual direction using a low speed saw with two parallel diamond blades under water irrigation (Fig. 1a). Three lumbar vertebrae (L3, L4 and L5) were also obtained from each rat (Fig. 1b). A total of 20 mandibular specimens (sham: 6 left and 4 right sides, and OVX: 4 left and 6 right sides) and 12 vertebral specimens (sham: 2 L3, 3 L4, 1 L5; and OVX: 2 L3, 3 L4, 1 L5) were prepared from randomly selected rats and subject to nanoindentation. Each specimen was obtained from an individual rat. The fresh specimens were stored −20 °C until nanoindentation.

The sliced surfaces of the bone specimens were polished under wet conditions. The polished specimens were glued onto a polycarbonate holder that has a fluid drainage system as previously introduced (Huja et al., 2006) and mounted on a nanoindenter (Nano-XP, MTS, Oakridge, TN). Indentation regions were determined using the nanoindenter microscope (Fig. 1a and b). Two paired regions in the mandibular bone and one paired region in the vertebral bone were identified. One of the mandibular bone paired regions was the alveolar region (AB), in which rapid bone turnover is stimulated by mastication, and the other was the cortical basal (CB) region, in which routine bone turnover is anticipated (Mavropoulos et al., 2004; Renders et al., 2006). Other paired regions of interest were marginal (TBM) and inner (TB) regions of mandibular trabecular bone, and marginal (VBM) and inner (VB) regions of vertebral trabecular bone. These regions were examined because the alveolar and marginal regions (AB, TBM and VBM) under rapid bone turnover have newer bone tissue relative to the basal and inner regions (Renders et al., 2006). Inter-indent locations were at least 50 μm apart to prevent any interruptions from the adjacent indents. A total of 579 indentations were successfully performed. The 302 indents from the sham group (71 indents from AB, 41 from CB, 42 from TBM, 38 from TB, 62 from VBM and 48 from VB) and the 277 indents from the OVX group (74 indents from AB, 41 from CB, 28 from TBM, 28 from TB, 54 from VBM and 52 from VB) were analyzed.

2.2. Nanoindentation

All nanoindentations were performed under wet conditions by dripping 0.5 mg/ml solution of gentamicin sulfate (Sigma, St. Louis, MO) solution to maintain wet specimens (Huja et al., 2007). All indentations were conducted under load control with indentation depth equivalent to 500 nm, with corresponding displacement rates of 50 nm/s for the mandibular specimens and 100 nm/s for the vertebral specimens. After a 30-s hold period under a constant peak load, the indenter was unloaded with the corresponding displacement rates of 50 nm/s for the mandibular specimens and 10 nm/s for the vertebral specimens. Nanoindentation modulus was measured using Eq. (1).

$$\frac{1}{E_r} = \frac{(1-\nu_s^2)}{E_s} + \frac{(1-\nu_i^2)}{E_i} \tag{1}$$

where E_r (reduced modulus) is measured as the unloading slope from the displacement-force curve. The indices s and i refer to the specimen and the indenter material, respectively, and ν is Poisson's ratio. For diamond, values of E_i=1141 GPa and ν_i=0.07 are typically used. Poisson's ratio for bone was assumed to be 0.3 following a previous study (Huja et al., 2007).

Creep was measured as the displacement during the 30-s hold period at peak load (Fig. 2). The creep displacement-time curve of each indentation was fitted using the following Voigt model (Eq.2).

$$h^2(t) = \frac{\pi}{2} p_{\max} \cot\alpha \left[\frac{1}{E_2}\left(1-e^{-tE_2/\eta}\right)\right] \tag{2}$$

where $h(t)$ is creep displacement (nm) as a function of time, $P_{\max}$ is the peak load during the hold period, α is an equivalent cone semi-angle (70.3°) to the face angle of the Berkovich indenter (65.27°) (Fischer-Cripps, 2004), E_2 is an elastic element of the Voigt model (GPa) and η is the indentation viscosity (GPaS) term.

2.3. Statistical analysis

A Student's t-test was performed to compare the BV/TV and BMD between the sham and OVX groups. Analysis of variance (ANOVA), followed by Fisher's PLSD post hoc test, was utilized to compare the regional variations of nanoindentation

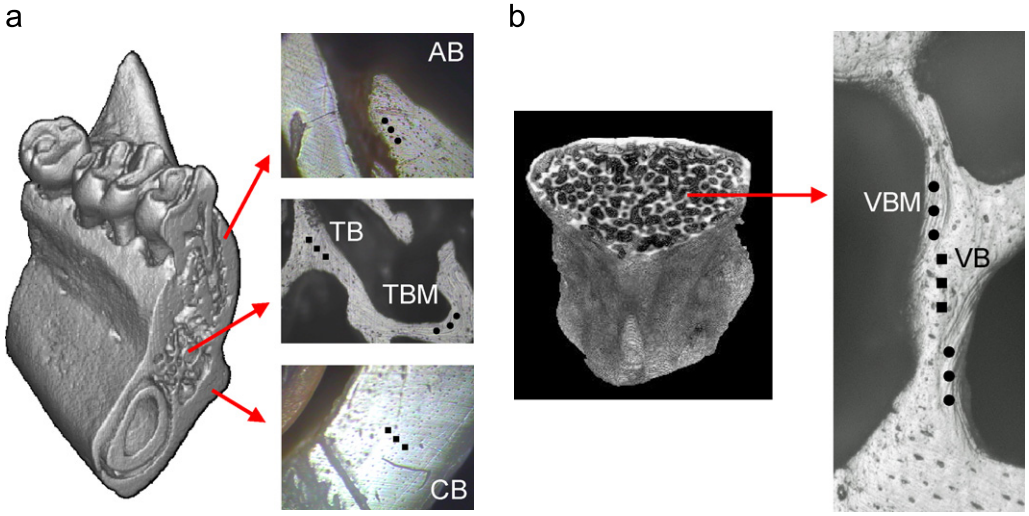

Fig. 1. Descriptive regions for nanoindentation in 3D micro-CT images and under an indenter microscope; (a) Four mandibular regions (AB, alveolar bone; CB, cortical basal bone; TBM, marginal region of trabecular bone (black dots); TB, inner region of trabecular bone (black squares)) and (b) two vertebral regions (VBM, marginal region of vertebral bone (black dots) and VB, inner region of vertebral bone (black squares)).