Injury, Int. J. Care Injured 49S (2018) S3–S10

Contents lists available at ScienceDirect

Injury

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

Assessing bone quality through mechanical properties in postmenopausal trabecular bone

Manuel Toledano[a], Manuel Toledano-Osorio[a], Enrique Guerado[b], Enrique Caso[c], Estrella Osorio[a,*], Raquel Osorio[a]

[a] University of Granada, Faculty of Dentistry, Dental Materials Section, Colegio Máximo de Cartuja s/n, 18071 Granada, Spain
[b] Department of Orthopaedic Surgery and Traumatology, Hospital Universitario Costa del Sol, University of Malaga, Autovía A-7, Km 187, 29603, Marbella, Malaga, Spain
[c] Research Unit, Hospital Universitario Costa del Sol, University of Malaga, Autovía A-7, Km 187, 29603, Marbella. Malaga, Spain

ARTICLE INFO

Article history:
Received 17 May 2018
Received in revised form 26 July 2018
Accepted 27 July 2018

Keywords:
Trabecular bone
Static
Dynamic
Mechanic
Menopause

ABSTRACT

Background: The inner structure of trabecular bone is a result of structural optimization provided by remodeling processes. Changes in hormonal status related to menopause cause bone tissue loss and micro-architectural deterioration with a consequent susceptibility to fracture. Accumulation of micro-damage in bone, as a function of the rate of production and rate of repair, underlies the development of stress fractures, increasing fragility associated to age and osteoporosis, especially in transmenopausal women.

Patients and Methods: Quasi-static and nano-dynamic mechanical characterization were undertaken in trabecular bone from femoral neck biopsies of postmenopausal women. AFM (Atomic Force Microscopy) complementary studies were performed to determine nano-roughness (SRa) and the fibrils width of collagen. Nanoindentations were used to quantify transmenopausal changes in intrinsic mechanical properties of trabecular bone: hardness (Hi), modulus of Young (Ei), complex modulus (E*), tan delta (δ), storage modulus (E') and loss modulus (E'').

Results: As result of the quasi-static measurements, 0.149 (0.036) GPa and 2.95 (0.73) GPa of Hi and Ei were obtained, respectively. As result of the nano-dynamic measurements, 17.94 (3.15), 0.62 (0.10), 13.79 (3.21 and 6.39 (1.28) GPa of E*, tan (δ), E' and E'' were achieved, respectively. 101.07 SRa and 831.28 nm of fibrils width were additionally obtained.

Conclusions: This study poses a first approach to the measurement of bone quality in postmenopausal trabecular bone by combining quasi-static, nano-DMA analysis and tribology of dentin surface through AFM characterization.

Introduction

Bone is a natural composite material consisting primarily of three phases: mineral, organic and water. They are organized into a complex hierarchical structure, and are not independent of each other, but rather work in harmony to determine the biomechanical properties of bone [1]. The macro-scale mechanical properties of the bone are controlled by both the structural organization of the micro and nano-scale constituents as well as the intrinsic mechanical properties of these constituents across the different length scales [2]. There are generally two types of bone tissue: trabecular and cortical. Trabecular bone is a highly porous structure that fills the proximal and distal ends of all bones (*e.g.* femur) and is also present as a filler in other bones (*e.g.* vertebral bodies and both maxillary bones), providing structural support and flexibility [3]. The mechanical properties of the trabecular bone depend on volume fraction, microarchitecture, and trabecular tissue material properties. Characterization of the latter and its role in trabecular bone mechanical behavior has potential clinical and biological importance [4].

Although understanding static mechanical properties is truly beneficial, considering that the majority of bone fractures occur under dynamic conditions such as accidents or sporting activities for the young and falls for the elderly, it is also very important to understand the dynamic mechanical behavior of bone [1]. Atomic force microscopy (AFM) nano-indentation is the most commonly applied means of testing the mechanical properties of materials or substrates [5], and it was deemed to be a suitable method for the

* Corresponding author.
E-mail address: meosorio@ugr.es (E. Osorio).

https://doi.org/10.1016/j.injury.2018.07.035
0020-1383/

determination of the visco-elasticity of hard tissues [6,7], at nanoscale [8]. Clinically, knowledge of the elastic and failure properties of trabecular tissue obtained by nanoindentation could be used to investigate the effects of drugs treatment, aging, and disease at the tissue level. Measurements have high spatial resolution due to small indenter tip radius, are nondestructive on a macroscopic level, and are sensitive to material anisotropy [9–12]. Viscoelastic materials dissipate energy when loaded. In bone, this is important for its fracture resistance under dynamic or impact loading [13]. From composite materials perspectives, the biomechanical properties of bone are dependent on the quality and the spatial arrangement of its constituents [14]. As polymers, bone components exhibit time-dependent behavior representative of non-linearly viscoelastic media. Viscoelasticity is the type of behavior attributed to materials that exhibit both elastic and viscous qualities under deformation. Viscoelastic materials, as bone deform according to a combination of these properties and, as such, exhibit time-dependent strain [15].

Trabecular tissue properties are of great interest in the etiology of osteoporosis since it is widely speculated that this aspect of bone "quality" does affect fracture risk [4]. Lower heterogeneity of trabecular bone of the osteoporotic patients may contribute to fracture susceptibility due to a lowered ability to prevent crack propagation [16]. The importance of assessment of mechanical properties at the tissue level, *i.e.*, at the level of single trabeculae can be demonstrated using indirect determination of trabecular bone mechanical properties from high-resolution images of its viscoelastic performance. These models can be used to study the relation between the microstructure and overall properties at the tissue levels. Moreover, nanoindentation, as a measurement tool for very local mechanical properties is able to distinguish this variation in different content (interstitial bone has a larger mineral content compared to the bone on the trabecular surface). Thus, modulus mapping shows the trend of larger stiffness in core, meanwhile smaller values are measured in superficial areas [3]. Scanning probe microscopes and, in particular, the AFM have facilitated the imaging and analysis of biological surfaces with little or no sample preparations [17]. AFM operates in a near field with a sharp probe by scanning, enabling characterization of three-dimensional surface morphology with minimal sample preparation and high resolution. AFM has been widely used to visualize the bone matrix and to determine the spatial relationship between mineral and collagen and their morphology/topology as well [8]. By integrating AFM and nano-dynamic mechanical analyzer (nano-DMA), both morpho and nanomechanical properties can be obtained. In particular, biological sample systems resemble complex biochemical and biophysic architectures [16].

At present, studies addressing the association between quasi-static and dynamic nano-indentation with surface roughness and fibril diameter in human trabecular bone are lacking. The goal of the present study was to propose a study protocol on trabecular bone, based on nanoindentation and AFM characterization, in order to assess degenerative bone diseases and bone quality with the aim of evaluating new therapies.

Material and methods

Femoral neck biopsies

Study subjects were hip fracture patients undergoing surgery for total hip replacement, and the specimens were retrieved from the base of the femoral neck. Subjects consented to undergo a femoral neck biopsy. All protocols were approved by the Institutional Review Board, prior to obtaining biopsy samples. These samples were then kept immersed in a phosphate -buffered saline (PBS) solution (pH 7.4) and stored frozen at −20 °C [18]. The biopsy specimens were processed in epoxy-resin [19,20].

Nanoindentation

To prepare the specimens for dynamic nanoindentation (nano-DMA), the epoxy-resin-embedded biopsy specimens that were used to generate sections, were further polished with different diamond pastes, from 10 μm to 0.1 μm in diameter, to obtain smooth surfaces. Measurements were performed on randomly selected femoral specimens and ~4–5 different trabeculae from each sample [21,22].

Three sections of epoxy-resin-embedded bone were submitted to nano-DMA to perform the modulus mapping. Property mappings were conducted using a HysitronTi 950 nanoindenter (Hysitron, Inc., Minneapolis, MN) equipped with a commercial nano-DMA package. The nanoindenter was a Berkovich (three-sided pyramidal) diamond indenter tip (tip radius ~20 nm). The nanoindenter tip was calibrated against a fused quartz sample using a quasistatic force setpoint of 5 μN to maintain contact between the tip and the sample surface. Based on a calibration-reduced modulus value of 1.1400E + 03 N/mm^2 for the fused quartz, the best-fit spherical radius approximation for tip was found to be 150 nm, for the selected nano-DMA scanning parameters. Trabecular struts near the centre of the biopsy were chosen from the optical image for testing. Modulus mapping of our samples was conducted by imposing a quasistatic force setpoint, F_q = 5 μN, to which it was superimposed a sinusoidal force of amplitude F_A = 1.8 μN and frequency f = 200 Hz. The resulting displacement (deformation) at the site of indentation was monitored as a function of time. Data from regions approximately 30 x 30 μm in size were collected using a scanning frequency of 0.2 Hz. Specimens were scanned in a hydrated state.

Under steady conditions (application of a quasistatic force) the indentation modulus of the tested sample, E, was obtained by application of different models that relate the indentation force, F, and depth, D. [23]. Most of these theories assumed proportionality between the force and the indentation modulus:

$$F = g(D)E \Rightarrow E = \frac{F}{g(D)} \tag{1}$$

where $g(D)$ is a function on the indentation depth, which depends on the geometry of the probe of the indenter. For example, for a spherical probe, the Hertzian contact theory predicts [24,25]:

$$g(D) = \frac{4R^{\frac{1}{2}}D^{\frac{3}{2}}}{3(1-\nu^2)} \tag{2}$$

In this equation R is the radius of the spherical probe and ν is the Poisson's ratio of the tested sample. As mentioned above, in nano-DMA experiments an oscillatory force is superimposed to a quasistatic force:

$$F = F_q + F_A \sin(2\pi f t), \tag{3}$$

with t being the time. Under this imposed force, the indentation depth takes the following form:

$$D = D_q + D_A \sin(2\pi f t - \delta). \tag{4}$$

This means that the indentation depth also oscillates around a quasistatic value, with the same frequency that the oscillating force and delayed by a phase lag δ. In the limit of $F_A << F_q$ we can expand Eq. (1) to a first order Taylor approximation, to obtain:

$$F_q + F_A \sin(2\pi f t) = g(D_q)E + g'(D_q)||E^*|)D_A \sin(2\pi f t - \delta). \tag{5}$$

In this equation, g' is the first derivative of g, and E^* is the complex dynamic indentation modulus. Now, it can be equaled the time-