JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 20 (2013) 6–18

ELSEVIER

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jmbbm

Research Paper

Novel method to analyze post-yield mechanical properties at trabecular bone tissue level

Roberto Carretta[a], Benoit Luisier[a], Daniel Bernoulli[b], Edgar Stüssi[a], Ralph Müller[a], Silvio Lorenzetti[a,*]

[a]Institute for Biomechanics, ETH Zürich, Zürich, Switzerland
[b]Laboratory for Nanometallurgy, ETH Zürich, Zürich, Switzerland

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form
1 December 2012
Accepted 3 December 2012
Available online 21 December 2012

Keywords:
Trabecular bone
Bone mechanics
Elastic modulus
Non linear mechanics
Finite element method
Bone quality

ABSTRACT

Tissue level mechanics is a key factor to be investigated to improve the knowledge of how the overall trabecular structure reacts to loading and overloading. The aim of this study was to develop a new device for measuring the mechanical competence of single trabeculae in the post-yield region for both tensile and bending tests, characterized by high accuracy and precision, and to assess the effect of testing mode, donor age and material composition. A novel approach for measuring the displacement and deformation was developed (accuracy error of 0.3% and a precision of 2.7%). A total of 30 samples from two bovine femora of different ages (from <3-year-old and 14-year-old cows) were tested in tension or bending, while average material properties have been acquired by means of Raman spectroscopy. A group of trabeculae was tested in bending after treatment for collagen degradation. As a result, a complete set of post-yield properties has been reported. The results highlight significant differences between tensile and bending groups, with higher values for the bending test mode for yield strain, ultimate strain and post-yield work and lower for the elastic modulus. Significant higher values were found for the old donor (differences in the range of 30–60%) for elastic modulus, yield stress and ultimate stress as well as for material properties measured by Raman spectroscopy. We quantified that changes in materials properties induced by collagen degradation corresponded to a substantial decrease (up to 120% for post-yield work) of mechanical competence, both in the elastic and inelastic region.

1. Introduction

Trabecular bone is a hierarchical material; its overall mechanical response is influenced by the interplay of its structure and material composition at different scales. At the macroscale, trabecular bone is a network of rod- and plate-like elements, called trabeculae, and its material composition and morphology are defined by adaptation to internal and external stimuli, such as the load distribution. Both the morphology and the material composition change and

*Correspondence to: Institute for Biomechanics, ETH Zürich, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland. Tel.: +41 44 633 61 95; fax: +41 44 633 14 53.
E-mail addresses: rcarretta@ethz.ch (R. Carretta), lbenoit@ethz.ch (B. Luisier), daniel.bernoulli@mat.ethz.ch (D. Bernoulli), estussi@ethz.ch (E. Stüssi), ram@ethz.ch (R. Müller), slorenzetti@ethz.ch (S. Lorenzetti).

1751-6161/$ - see front matter
http://dx.doi.org/10.1016/j.jmbbm.2012.12.003

degrade due to aging and disease (Djuric et al., 2010; Saito and Marumo, 2010; Stauber and Muller, 2006). Investigation of these aspects is, therefore, pivotal in terms of mechanical competence evaluations and in determining fracture risk in the patient population. The analysis of trabecular bone morphology has become feasible with the advent of micro-computed tomography (microCT) (Borah et al., 2001; Muller et al., 1994). The investigation of the mechanical response of the bone tissue, defined as the intermediate organizational level where bone is spatially continuous (Carretta et al., in press), can be performed at the macro- or microscale.

In the first case, the main issue arises from the effect of the trabecular architecture and tissue anatomical heterogeneity at the macroscale. To overcome these limitations, mechanical testing at the macroscale is combined with microCT geometry acquisition and finite element simulation or strain mapping. Using an indirect approach, a mechanical model of the trabecular bone tissue can be iteratively estimated (Van Rietbergen et al., 1995; Verhulp et al., 2008b). This indirect approach provides results in terms of the average tissue elastic modulus in the range of 2–10 GPa. It has also been shown that large differences occur between different samples in terms of their inelastic behavior (Van Rietbergen et al., 1995; Verhulp et al., 2008a). Macroscale testing has been used as reference data to develop ad-hoc finite element models. In this case, measurable variables such as morphology, fabric or bone density are used as input parameters to predict the macroscopic mechanical behavior of trabecular bone (Zysset and Curnier, 1996). The main advantages of these approaches are the simplification of the testing procedure and the use of a small number of important factors that describe the overall experimental output. However, a major drawback arises from intrinsic averaging across the macroscopic sample of the underlying material properties, which remain unknown at the local level. Knowledge about the local mechanical behavior is therefore needed to improve the understanding of failure behavior at the tissue level and the prediction of local crack generation and, eventually, overall trabecular bone failure.

Different methodologies have been used to experimentally calculate bulk mechanical properties at the microscale level. At this scale, a single trabecula is composed of multiple bone packets with different mineralization densities and orientations. Axial tension and compression tests (Bini et al., 2002; Hernandez et al., 2005; Rho et al., 1993; Ryan and Williams, 1989), as well as bending and buckling tests (Busse et al., 2009; Choi and Goldstein, 1992; Kuhn et al., 1989; Lorenzetti et al., 2011) have been used to assess the average tissue mechanical properties of single trabecula (Lucchinetti et al., 2000); local surface measurements such as nanoindentation (Lewis and Nyman, 2008) and acoustic microscopy (Litniewski, 2005) have been used on the bone packet level (Carretta et al., in press). Elastic bulk measurements provide a wide range of values for the tissue elastic modulus, with an average of a few GPa up to 14 GPa (Lucchinetti et al., 2000), which is lower than the elastic moduli reported by local surface experiments (in the range of 15–25 GPa) (Lewis and Nyman, 2008). Possible causes of this discrepancy are compliance of the instrumentation, errors in the estimation of mechanical parameters that result from irregular geometry, local anisotropy of the samples or the generation of small defects during preparation, which are not accounted for in surface tests such as nanoindentation and may reduce the elastic modulus in bulk trabecula tests.

Recently, inelastic mechanical properties have also been investigated at the tissue level on single trabeculae. An estimation of yield-ultimate parameters based on a simplified geometrical model has been reported (Busse et al., 2009). Among the results, it was shown that the mechanical properties are significantly degraded in osteoporotic bone. Yielding appears to occur at (30 ± 10) vs. (40 ± 10) MPa, and the ultimate stress was estimated at (40 ± 10) vs. (50 ± 10) MPa. Nevertheless, the absolute values were extremely low compared with those for cortical bone (Reilly and Burstein, 1975). Strain mapping of a single trabecula from a bovine femur during a three-point bending test has also been performed by means of an ink pattern and high-speed photography (Jungmann et al., 2011). It was also shown that strain is associated with whitening of the material (Thurner et al., 2007), which was indicated to be an estimator of microdamage. The strain at whitening onset and the ultimate strain were found to be (1.6 ± 0.9) % and (12 ± 4) %, respectively (Jungmann et al., 2011). Tensile tests were also applied to single trabecula beyond the elastic limit, yielding a wide range of ultimate strain values from 1.8% to 20.2% (Hernandez et al., 2005), while ultimate stress and toughness values have not been reported.

The aim of this study was to develop a new device for measuring the mechanical competence of single trabeculae in the post-yield region of both tensile and bending tests, characterized by high accuracy and precision. Given the small dimensions of the samples, a novel approach for measuring the displacement and deformation was developed to gain the required high accuracy, especially at low strains. It has been reported that yielding may start at strains as low as 0.5% (Keaveny et al., 1994). Hence, we aimed for an accuracy of 5% at a 0.4% strain, which requires a resolution of 0.1 μm using a field of view (FOV) larger than 0.5 mm. We chose an optical acquisition system to exclude errors associated with the relatively high and non-reproducible compliance at the boundaries of the sample, which significantly affects the measurements, given the small range of displacements that would occur in the sample. Various approaches have been investigated. Electronic Speckle Pattern Interferometry (ESPI) (Barak et al., 2010) has good resolution but the setup for single trabecula testing did not allows us for its use. Standard digital image correlation (DIC) based on reflected light could not be used because of variations in the reflected light intensity caused by the irregular three-dimensional geometry of the trabecula. Classical bright field microscopy was not useful because it only allows for a maximal theoretical resolution in the range of 1 μm with a FOV > 0.5 mm. The required resolution was achieved with a novel approach, which consisted of a point-detection technique combined with confocal laser scanning microscopy (CLSM). Optical displacement measurements were combined with real geometry acquisition by means of a microCT and finite element (FE) simulations to calculate the mechanical properties using a reverse engineering approach. Finally, average materials properties for each single trabecula have been acquired by means of Raman