JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 52 (2015) 51–62

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm

Research paper

Extracellular bone matrix exhibits hardening elastoplasticity and more than double cortical strength: Evidence from homogeneous compression of non-tapered single micron-sized pillars welded to a rigid substrate

Krzysztof W. Luczynski[a], *Andreas Steiger-Thirsfeld*[b], *Johannes Bernardi*[b], *Josef Eberhardsteiner*[a], *Christian Hellmich*[a,*]

[a]*Institute for Mechanics of Materials and Structures, Vienna University of Technology (TU Wien), Karlsplatz 13/202, 1040 Vienna, Austria*

[b]*University Service Center for Transmission Electron Microscopy, Vienna University of Technology (TU Wien), Wiedner Hauptstrasse 8-10, 1040 Vienna, Austria*

ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form
13 February 2015
Accepted 1 March 2015
Available online 7 March 2015

Keywords:
Bone
Focused ion beam milling
Homogeneous strain state
Micropillars
Unloading
Elastoplasticity

ABSTRACT

We here report an improved experimental technique for the determination of Young's modulus and uniaxial strength of extracellular bone matrix at the single micrometer scale, giving direct access to the (homogeneous) deformation (or strain) states of the tested samples and to the corresponding mechanically recoverable energy, called potential or elastic energy. Therefore, a new protocol for Focused Ion Beam milling of prismatic non-tapered micropillars, and attaching them to a rigid substrate, was developed. Uniaxial strength turns out as at least twice that measured macroscopically, and respective ultimate stresses are preceded by hardening elastoplastic states, already at very low load levels. The unloading portion of quasi-static load–displacement curves revealed Young's modulus of 29 GPa in bovine extracellular bone matrix. This value is impressively confirmed by the corresponding prediction of a multiscale mechanics model for bone, which has been comprehensively validated at various other observation scales, across tissues from the entire vertebrate animal kingdom.

1. Introduction

Inelastic deformation of bone material is often associated with the formation and evolution of microcracks, exhibiting a characteristic size of some tens of micrometers (Schaffler et al., 1994, 1995; Wenzel et al., 1996; O'Brien et al., 2000; Chapurlat et al., 2007). These microcracks are already found in bone samples harvested from specific anatomical sites, as was evidenced for human adult ribs (Schaffler et al., 1994; O'Brien et al., 2000), human femur (Schaffler et al., 1995), human vertebral bone

*Corresponding author.
E-mail address: christian.hellmich@tuwien.ac.at (C. Hellmich).

http://dx.doi.org/10.1016/j.jmbbm.2015.03.001

(Wenzel et al., 1996), postmenopausal human transiliac bone (Chapurlat et al., 2007); and they grow under homogeneous loading of millimeter-sized bone samples in fatigue tests (Reilly and Currey, 1999; Akkus and Rimnac, 2001; O'Brien et al., 2003; Nicolella et al., 2011). The aforementioned microcracks are thought to interact with tens-of-micrometers-sized objects, such as lacunar pores (Reilly and Currey, 1999; Hamed and Jasiuk, 2013) or cement lines around osteons (Hamed and Jasiuk, 2013; Katsamenis et al., 2013; Nobakhti et al., 2014). It is theoretically well accepted that no cracks occur at the single nanometer scale (Gao et al., 2003), and this is in accordance with transmission electron micrographs of single bone mineral crystals (Ziv and Weiner, 1994; Kim et al., 1995, 1996; Zylberberg et al., 1998; Su et al., 2003). Comparatively, the situation at the single-micron scale is only vaguely investigated, and therefore, rather poorly understood. The reason for this is twofold: (i) a purely theoretical investigation as in the case of hydroxyapatite is a tremendous challenge due to the complex micro-heterogeneous nature of the material at the single microns-scale; (ii) experimental approaches undertaken at that scale, such as the many nanoindentation campaigns during the last two decades (Ko et al., 1995; Roy et al., 1996; Rho et al., 1997, 1999a,b; Hoffler et al., 1997; Turner et al., 1999; Rho and Pharr, 1999; Zysset et al., 1999; Fan et al., 2002; Hengsberger et al., 2002; Ebenstein and Pruitt, 2006), exhibit strong stress and strain gradients. Namely, the latter impede direct determination of continuum mechanical properties like stiffness or strength, which are, by definition (Salencon, 2001; Zaoui, 2002), related to homogeneous stress or strain states imposed onto a representative volume element of solid matter. In order to overcome this problematic situation, an increasing number of researchers have more recently been investing into the Focused Ion Beam (FIB) milling technique, allowing them to carve out micropillars from a surrounding substrate. Some of them carved out horizontally oriented beams, which were then subjected to bending deformations (Jimenez-Palomar et al., 2012; Chan et al., 2009). Retrieving elastic properties from such, strictly speaking, structural tests inducing inhomogeneous stress and strain states requires the use of beam theories and respective inherent assumptions. In order to do without the latter, many researchers have produced vertically oriented pillars, and have compressed them by flat punches, so as to induce quasi-homogeneous stress states within the aforementioned micropillars (Barnoush et al., 2010; Battaile et al., 2012; Dietiker et al., 2011; Frick et al., 2007, 2008a,b; Kheradmand et al., 2013; Kirchlechner et al., 2011; Monnet and Pouchon, 2013; Nagoshi et al., 2013, 2014; Ng et al., 2011; Ng and Ngan, 2008; Raghavan et al., 2012; Schneider et al., 2009, 2011, 2013; Schwiedrzik et al., 2014; Stewart et al., 2012; Yang et al., 2009; Zhang et al., 2010). However, even if the problems of pillar tapering (a phenomenon which restricts stress and strain homogeneity to the pillar cross sections, while the stress magnitudes within the pillar decrease with increasing distance from the flat punch) are solved, see e.g. Kinashi et al. (2014), Kirchlechner et al. (2011), Mutoh et al. (2013), and Nagoshi et al. (2013, 2014) for the production of non-tapered pillars, then these tests still do not allow for direct determination of stiffness or strength values, since the machine-measured displacements of the quasi-rigid punch do not only relate to the deformations of the pillar itself, but also to those of the elastic half space below. The current contribution aims at meeting exactly this last challenge on the way to a truly continuum mechanical test at the single-micron scale, and to apply a correspondingly developed experimental protocol to bone. In more detail, the challenge lies in the production of non-tapered micropillars, and in detaching them from the original (deformable) substrate, so as to plant them onto a rigid substrate. To the very best knowledge of the authors, this has never been reported before – and the remainder of the paper is devoted to a concise description of the protocol, and a first batch of results obtained on miniature samples harvested from bovine cortical bone.

2. Materials and methods

2.1. Sample production

In order to arrive at the targeted non-tapered micropillars welded to a rigid substrate, the following sequence of processing steps was realized: first of all, a portion of an 18 month-old bovine femur was obtained from a local butcher [see Figs. 1(a) and 3(a)]. A hand saw was used to cut the aforementioned portion along the long bone axis, yielding smaller parts as seen in Figs. 1(b) and 3(b). Then, ten slices of approximately 1 mm thickness were cut, by means of a diamond saw (Isomet, Buehler, USA), from the aforementioned smaller parts. Thereby, the cutting direction was orthogonal to the long bone axis [see Figs. 1(b) and 3(b)]. Afterwards, the aforementioned slices [see Fig. 3(c)] were cut into again smaller pieces with dimensions of approximately $7 \times 2 \times 1$ mm [see Fig. 3(d)], and the latter were then kept frozen at $-20\,^{\circ}$C (Fölsch et al., 2011; Nazarian et al., 2009; Linde and Sørensen, 1993), in order to preserve their mechanical properties throughout the time lag between slice production and investigation of their single micron-scale elasticity and strength. For the latter purpose, the small pieces were, one after the other, unthawed and processed separately, in order to reduce the time of exposure to potentially harming environmental factors. FIB-milling is done under vacuum, and in order to prevent (i) possible vacuum pump-induced formation of drying cracks in the investigated specimens (Jimenez-Palomar et al., 2012), (ii) their negative charging under the SEMs electron beam (resulting in beam deflection, which may cause loss of resolution or astigmatism) (Bourne, 1972), as well as (iii) FIB-induced positive charging (which may lead to “surface melting” caused by incident Ga^{+} ions) (Nalla et al., 2005; Stokes et al., 2006; Drobne et al., 2007), each of the platy pieces described before, was coated by a 17 nm thick gold-palladium sheet (SC502-314B target, Quorum Technologies Ltd, East Sussex, United Kingdom) using the Q150T S High Resolution Sputter Coater (Quorum Technologies Ltd, East Sussex, United Kingdom).

The next major task was to mill, out of the aforementioned pieces, tiny micro-pillars in the Quanta 200 3D DualBeam (FEI, Hillsboro, Oregon, USA). The latter system is equipped with a vacuum chamber, an adjustable sample stage, a Focused Ion Beam (FIB), a Scanning Electron Microscope (SEM), and an in-built nano-manipulator (Kleindiek Nanotechnik GmbH, Reutlingen, Germany). The first Quanta-related processing step concerns its pin-mount sample holder to which both one of the tiny bone pieces and a silicon wafer (which would serve a s future “rigid” substrate for the micropillar samples) were glued, by means of a conductive silver adhesive (Plano GmbH,