Journal of the Mechanical Behavior of Biomedical Materials 84 (2018) 217–224

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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

Modal analysis of nanoindentation data, confirming that reduced bone turnover may cause increased tissue mineralization/elasticity

Maria-Ioana Pastrama[a,b], Romane Blanchard[c], John G. Clement[d], Peter Pivonka[c,e], Christian Hellmich[a,*]

[a] Institute for Mechanics of Materials and Structures, TU Wien - Vienna University of Technology, Vienna, Austria
[b] KU Leuven, Department of Movement Sciences, Human Movement Biomechanics Research Group, Tervuursevest 101, 3001 Leuven, Belgium
[c] St Vincent's Department of Surgery, University of Melbourne, Melbourne, Australia
[d] Melbourne Dental School, University of Melbourne, Melbourne, Australia
[e] School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, Brisbane, Australia

ARTICLE INFO

Keywords:
Extracellular bone matrix
Modal analysis of nanoindentation data
Undamaged elastic modulus
Resorption modeling

ABSTRACT

It is widely believed that the activities of bone cells at the tissue scale not only govern the size of the vascular pore spaces (and hence, the amount of bone tissue available for actually carrying the loads), but also the characteristics of the extracellular bone matrix itself. In this context, increased mechanical stimulation (in mediolateral regions of human femora, as compared to anteroposterior regions) may lead to increased bone turnover, lower bone matrix mineralization, and therefore lower tissue modulus. On the other hand, resorption-only processes (in endosteal versus periosteal regions) may have the opposite effect. A modal analysis of nanoindentation data obtained on femurs from the Melbourne Femur Research Collection (MFRC) indeed confirms that bone is stiffer in endosteal regions compared to periosteal regions ($\overline{E}_{endost}$ = 29.34 ± 0.75 GPa > $\overline{E}_{periost}$ = 24.67 ± 1.63 GPa), most likely due to the aging-related increase in resorption modeling on endosteal surfaces resulting in trabecularization of cortical bone. The results also show that bone is stiffer along the anteroposterior direction compared the mediolateral direction ($\overline{E}_{anteropost}$ = 28.89 ± 1.08 GPa > $\overline{E}_{mediolat}$ = 26.03 ± 2.31 GPa), the former being aligned with the neutral bending axis of the femur and, thus, undergoing more resorption modeling and consequently being more mineralized.

1. Introduction

As bones are the predominant load carriers in the vertebrate animal kingdom, their mechanical properties (in particular elasticity and strength) have always been of great interest for biomedicine and the scientific community at large. However, bone exhibits a hierarchical organization (Lakes, 1993; Katz et al., 1984; Weiner and Wagner, 1998), and mechanical properties involving quantities of the dimension "force per area" need to be assigned to a specific observation scale, on which these forces and areas are recorded. As regards elasticity and strength, two such observation scales are of particular interest for the biomedical field:

- the macroscopic scale with a material volume measuring some hundreds of micrometers to milimeters cubed, where cortical and trabecular bone are distinguished. At this length scale bone exhibits pore spaces with characteristic sizes of tens to hundreds of micrometers, populated by biological cells (Buckwalter et al., 1995); and
- the extracellular bone tissue, with material volumes at the tens of micrometers-scale, consisting of a nanocomposite made of hydroxyapatite, collagen, and water with non-collageneous organics (Lees, 1987).

It is widely believed that the activities of bone cells at the higher one of these two levels not only govern the size of these pore spaces (and hence, the amount of bone tissue available for actually carrying the loads), but also the characteristics of the extracellular bone matrix itself. In this context, several mechanisms have been proposed, and the present paper focusses on the following research question: *Does excessive bone resorption cause extracellular tissue elasticity increase?*

The relevance of this question is inferred from the following deliberations reported in literature:

- Within a certain physiological load window (Frost, 1964, 1992; Rubin and Lanyon, 1985; Turner, 1998; Hsieh et al., 2001; Rubin et al., 2001), mechanical stimulation is known to increase the rate of

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

https://doi.org/10.1016/j.jmbbm.2018.05.014
Received 7 September 2017; Received in revised form 20 January 2018; Accepted 9 May 2018

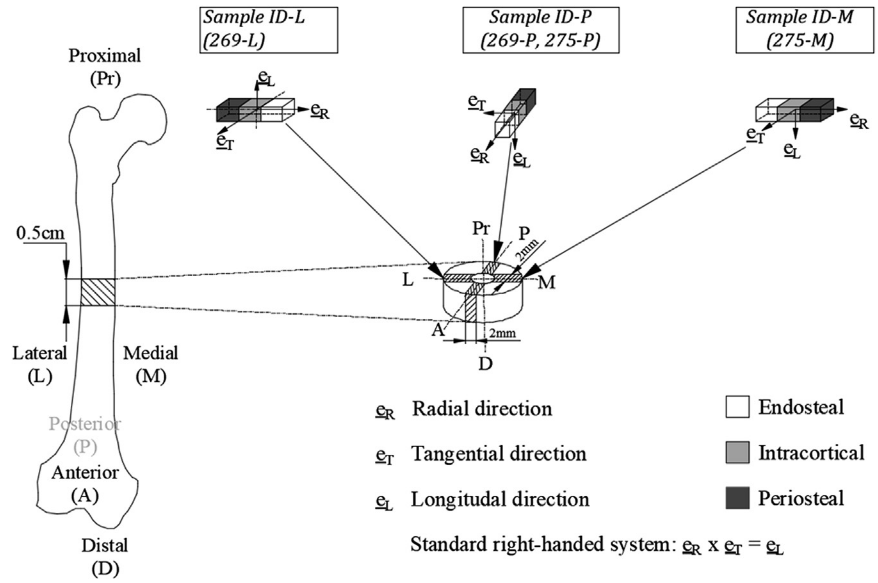

Fig. 1. Extraction scheme for match stick-like samples.

bone remodeling or turnover, i.e. the cellular processes leading to resorption of old extracellular bone tissue and subsequent formation of new tissue. On the other hand, bone turnover is associated to the mineralization degree at the tissue scale (Boivin and Meunier, 2002; Meunier and Boivin, 1997; Boivin et al., 2000), with higher turnover resulting in lower mineralization. The "complete" (or secondary) mineralization of the tissue would last up to years (Marotti et al., 1972; Bala et al., 2010), and would be hindered simply by tissue resorption before its full "maturation". Conversely, the fingerprint of low turnover would be highly mineralized tissue.

- Increased bone tissue mineralization causes an increase in the tissue elastic modulus, according to the micromechanical theory applied to, and experimentally validated for, extracellular bone tissue, as reported, among others, by Crolet et al. (1993), Hellmich et al. (2004), Fritsch and Hellmich (2007), Grimal et al. (2011).

The present paper aims at giving an answer to the aforementioned research question, by presenting a correspondingly designed nanoindentation study and its evaluation. In this context, it is important that the tested samples all consist of very similar tissue, so that any differences between tissue properties from one tested sample to another result only from the resorption activities as described above, with any other causes remaining highly improbable. A short literature review, see e.g. the Appendix of Hellmich et al. (2008), evidences that bone tissue within adult bony organs is, when spatially averaged over long bone cross sections, constant over space and time. This was independently shown by both microscopic and radiographic analyses revealing mineral density distributions (Boivin and Meunier, 2002; Akkus et al., 2003; Roschger et al., 2003; Bossy et al., 2004), and by nanoindentation studies (Hoffler et al., 2000a; Rho et al., 2002; Feng and Jasiuk, 2011; Wolfram et al., 2010).

Accordingly, the current study was performed on mid-shafts from adult female human femurs provided by the Melbourne Femur Research Collection (MFRC), discriminating regions of potentially higher and lower mineralization, and hence, higher and lower tissue elastic modulus:

- anteroposterior regions, being closely aligned with the neutral axis of the bending beam structure "human femur" (Feik et al., 2000; Thomas et al., 2005, 2006), therefore undergoing lower mechanical stimulation, lower turnover, and exhibiting higher mineralization and tissue elasticity) versus mediolateral regions (with higher mechanical stimulation, higher turnover, lower mineralization and lower tissue elasticity); and
- endosteal regions with significantly reduced formation activities indicated by trabecularization of cortical bone (Simmons et al., 1991; Cooper et al., 2007), and with expectedly higher mineralization and higher tissue modulus, versus periosteal regions (with expectedly lower mineralization and lower tissue modulus).

The correspondingly obtained nanoindentation data underwent a modal analysis as introduced by Furin et al. (2016), Kariem et al. (2015), in order to identify those indented half-spaces the stiffnesses of which were not affected by microcracks.

2. Materials and methods

2.1. Selection and preparation of femoral mid shaft samples

Two femoral mid-shaft sections were obtained from bone samples collected in 1990–1993 and 1998 by the Victorian Institute of Forensic Medicine as part of the Melbourne Femur Research Collection (MFRC). Ethical approval was given by the Office for Research Ethics and Integrity as part of a larger study, conducted by the researchers at Melbourne Dental School, where the collection is housed. The femurs, coded 269 and 275, originated from female donors without diseases directly affecting bone, and of ages 28 and 38 years, respectively. A diamond-impregnated metal disc cooled by a continuous water spray was used to cut an approximately 0.5 cm thick transverse segment from the mid-shaft of each of the investigated femurs, see Fig. 1. Thereafter, the segments were cleaned of adhering soft tissue, coarsely dehydrated in a series of graded alcohols under occasional ultrasonication and vacuum, and then refluxed for 7–14 days in a 50:50 heptane-isopropanol mixture. The bone sections were then transferred through two 12-h changes of 100% methanol, soaked in two one-hour long changes of xylene, both under occasional ultrasonication and vacuum, and embedded in poly-methyl-methacrylate (PMMA) according to a previously described procedure (Boyde et al., 1984).

2.2. Cutting of anatomical location-specific, match stick-like samples for nanoindentation

The Linea Aspera (LA), i.e. the rough longitudinal ridge on the posterior surface of each of the two femoral mid-shaft samples, was