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Estimation of local anisotropy of plexiform bone: Comparison between depth sensing micro-indentation and Reference Point Indentation

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ABSTRACT

The recently developed Reference Point Indentation (RPI) allows the measurements of bone properties at the tissue level *in vivo*. The goal of this study was to compare the local anisotropic behaviour of bovine plexiform bone measured with depth sensing micro-indentation tests and with RPI. Fifteen plexiform bone specimens were extracted from a bovine femur and polished down to 0.05 μm alumina paste for indentations along the axial, radial and circumferential directions ($N=5$ per group). Twenty-four micro-indentations (2.5 μm in depth, 10% of them were excluded for testing problems) and four RPI-indentations (~50 μm in depth) were performed on each sample. The local indentation modulus E_{ind} was found to be highest for the axial direction (24.3 ± 2.5 GPa) compared to the one for the circumferential indentations (19% less stiff) and for the radial direction (30% less stiff). RPI measurements were also found to be dependent on indentation direction ($p<0.001$) with the exception of the Indentation Distance Increase (IDI) ($p=0.173$). In particular, the unloading slope US1 followed similar trends compared to the E_{ind}: 0.47 ± 0.03 N/μm for axial, 11% lower for circumferential and 17% lower for radial. Significant correlations were found between US1 and E_{ind} ($p=0.001$; $R^2=0.58$), while no significant relationship was found between IDI and any of the micro-indentation measurements ($p>0.157$). In conclusion some of the RPI measurements can provide information about local anisotropy but IDI cannot. Moreover, there is a linear relationship between most local mechanical properties measured with RPI and with micro-indentations, but IDI does not correlate with any micro-indentation measurements.

1. Introduction

Bone is a complex hierarchical material with mechanical properties that depend on the investigated dimensional scale, from the cell up to the body levels. Bone structural units (BSU) are formed of packages of bone lamellae that organize in macrostructures such as trabeculae and osteons. Due to the distribution of their BSUs, both cortical and trabecular bone have exhibited anisotropic mechanical properties at the tissue level as shown by micro-indentation (Dall'Ara et al., 2013; Franzoso and Zysset, 2009; Reisinger et al., 2011; Roy et al., 1999; Wolfram et al., 2010) and ultrasound (Turner et al., 1995, 1999); and at the macro-level as shown by mechanical testing (Li et al., 2013; Odgaard, 1997; Ohman et al., 2007; Rincon-Kohli and Zysset, 2009). The long bones of quickly growing large animals reveal one further sub-classification into laminar and plexiform tissue, formed of parallel-fibred bone and lamellar bone around macroporosities. Previous studies have shown that the structure of plexiform bone has an orthotropic mechanical behaviour macroscopically (Katz et al., 1984; Macione et al., 2010). However, not much is known about its mechanical anisotropy at the BSU level. The better understanding of the anisotropic properties of this structure at the micro-level is important in order to understand why it appears only in large quickly growing animals and not in more active species such as humans or small mammals.

The need to understand how bone properties relate and translate from the micro-architectural (BSU) to the macro-architectural level has become more acute with arrival of the Reference Point Indentation (RPI) method. This novel indentation technique allows for the study of bone quality *in vivo* (Hansma et al., 2008, 2009), something that until now could be done only on extracted bone biopsies. A few studies have reported that some of the RPI parameters, in particular the Indentation Distance Increase IDI (Diez-Perez et al., 2010; Guerri-

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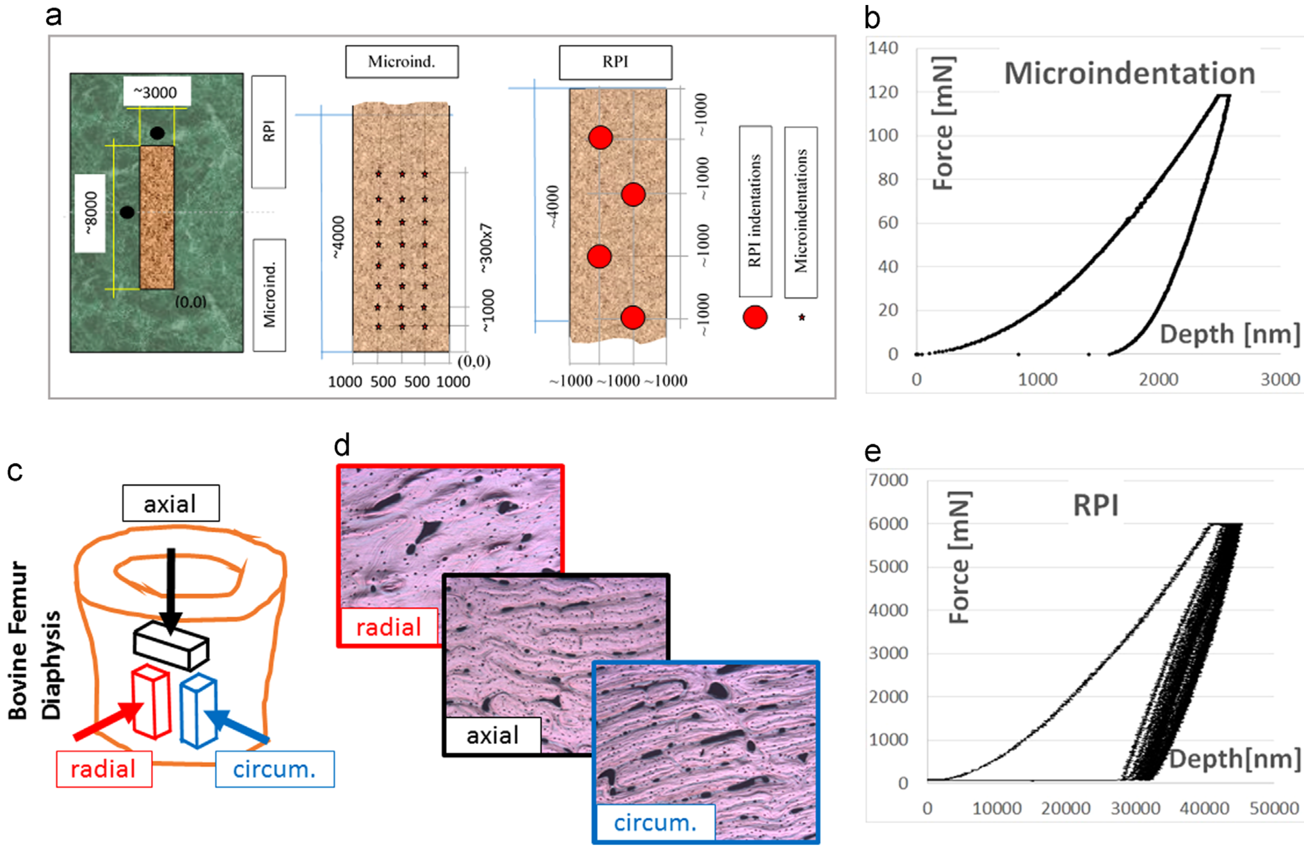

Fig. 1. Overview of the methods. Specimens were extracted from a bovine femur in order to perform indentations along axial, circumferential and radial directions (c). Only specimens with plexiform structure were analyzed (d). Specimens were embedded in PMMA and indented thirty times with micro-indentation test (a, b) and four times with Reference Point Indentation (RPI) test (a, e).

Fernandez et al., 2013), differ between patients who did or did not suffer of a bone fracture. However, it is not clear yet how the properties measured with this tool are related to the intrinsic mechanical properties of the tested bones at the tissue level and to its structural anisotropy. Very recently some methodological studies investigated the sensitivity of the RPI indentation properties to the selected testing procedure *in vitro* (Jenkins et al., 2015; Setters and Jasiuk, 2014). Due to the relatively large indentations (50–200 μm in depth) and the potential relationship between the local resistance to fracture and the IDI, some other studies have compared the RPI outputs with measurements performed at the macro-level such as bone toughness and reported mixed if not controversial results. In particular, IDI was not related to the whole mouse bone stress intensity fracture toughness (Carriero et al., 2014), was weakly correlated to ultimate stress ($R^2 \sim 0.25$) and toughness ($R^2 \sim 0.24$) of cortical bone specimens extracted from the human femur (Granke et al., 2014; Katsamenis et al., 2015) and was correlated ($R^2 \sim 0.50$) with work to failure for pooled data obtained from rat femur, rat vertebra and dog rib (Gallant et al., 2013). Moreover, IDI measurements performed along the longitudinal or transverse directions were found to be similar for the human femoral cortical bone (Granke et al., 2014) but significantly different for porcine cortical bone (Rasoulian et al., 2013). If the relationship between RPI measurements and macro-mechanical properties is not clear, little is known about the relationship between the RPI measurements method and the mechanical properties at the BSU. Surprisingly only two studies (Feng et al., 2012; Rasoulian et al., 2013) compared local lamellar elastic moduli measured with depth sensing nano-indentation (lamellar level, a few microns in size) and RPI measurements on porcine cortical bone, reporting that both techniques measure higher mechanical properties along the longitudinal direction compared to the transverse one, but it is not clear if indentations were performed on the same set of specimens and along which direction. On the basis of all these studies it appears that there are two crucial factors on which interpretation of the clinical RPI results will depend on in the future: the relationship of RPI measures to the underlying BSUs and the issue of directionality which will determine if and how the application of the RPI locally and *in vivo* will be interpreted as reflecting the properties of the bone at the higher scale.

In this study we propose to investigate the local anisotropic mechanical behaviour of plexiform bone at two dimensional levels with two indentation techniques on the same set of specimens: depth sensing micro-indentation (BSU level, penetration depth equal to 2.5 μm) and RPI (tissue level, penetration depth approximately 50 μm).

The hypotheses of this study were that:

- plexiform bone exhibits orthotropic mechanical behaviour at the BSU scale;
- RPI can measure differences in the mechanical properties of the plexiform bone if indented along the axial, radial and circumferential directions;
- there is a relationship between the local mechanical properties measured with RPI and with depth sensing micro-indentation.

2. Material and methods

One bovine femur was obtained from an 18 months old animal killed for alimentary purpose. The femur was stored in freezer at −20 C until it was dissected.