Assessment of osteonal and interstitial bone quality using a novel custom-made microscope-indenter assembly

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INTRODUCTION: Prediction of bone fracture goes beyond bone mineral density measurement. The quality of bone matrix is instrumental in providing fracture resistance to bone. Thus assessment of bone quality is critical to understanding bone fracture. Reference point indentation (RPI) purports to measure bone matrix quality in vivo and has been shown to separate fracture and non-fracture groups with over-lapping BMD [1]. Here, we compare the material properties of osteonal and adjacent interstitial bone using RPI and evaluate its ability to distinguish bone with different tissue age. It is known that interstitial bone is more mineralized and contains post-translationally modified collagen. Consequently, it is likely to accumulate damage as linear microcracks while osteons are predisposed to accumulating diffuse damage [2, 3] a bone toughening mechanism. The accumulation of diffuse damage has been associated with higher fracture toughness [3].

METHODS: We developed a novel, custom-made micro-indentation set-up (Fig. 1). Our set-up includes an Olympus CKX41 inverted microscope with a mercury light source and standard color filters. This enables us to view 100 µm thick bone sections and distinguish various morphological features like osteonal and interstitial bone at magnifications of 100X-400X. Directly above the microscope stage, is affixed a BioDent (Active Life Scientific) RPI indenter. The assembly allows us to accurately locate osteonal bone and corresponding adjacent interstitial bone and perform multiple indentation measurements on them.

Fig. 1) Microscope-Indenter Assembly

Toluidine blue stained human tibial cross-sections (100um) were obtained from 11 human donors (ages 19-97yrs). For each specimen, an osteon with a distinct and sufficiently large adjacent interstitial region were located. The osteon and corresponding interstitial regions were indented with a maximum load of 3 N and a frequency of 2 Hz for 5 cycles (10 indentations). A maximum applied indentation load of 3N was chosen so as to limit the extent of cracking around the point of indentation. Care was taken to ensure that the osteonal and interstitial indentions were sufficiently apart so that matrix damage as a result of the primary indentation did not influence the secondary indentation.

RESULTS: Osteonal bone (blue) shows increased values of total indentation (Fig. 2a) as seen in total indentation distance (TID, p<0.05) and indentation distance increase (IDI, p<0.01). Osteonal bone also exhibits greater extent of creep as visible in creep indentation distance (CID, p<0.05) and average creep indentation distance (Avg. CID, p<0.01) (Fig. 2b).

DISCUSSION: Continuous bone remodeling results in the formation of new osteons that grow larger and mineralize with age. Old bone is visible as packets of interstitial bone – bone located in the vicinity of osteons. Nano-indentation studies show that interstitial bone has 15% greater indentation modulus than osteonal bone [4]. This has been attributed lower osteonal mineralization in comparison to interstitial bone. Osteons also have a less mature organic matrix (determined by the extent of cross-linking in collagen) that may be subject to greater deformation. Our results show that indentation depth (as measured by TID and IDI) is greater in osteonal bone. This implies that osteons allow the indenter probe to penetrate deeper into the matrix. Diffuse damage formation is associated with ductility [5]. Thus greater indentation depths in osteonal bone may stem from the material softening due to the diffuse damage accumulation.

We show that osteonal bone also undergoes greater creep-deformation. This is visible in the higher creep indentation distance (CID) and avg. CID values. We believe that the damage due to cyclic indentation loading seen in osteons mainly accumulates in the organic matrix through creep-related mechanisms. Creep correlates with fracture toughness [6] suggesting that osteonal bone may be tougher than interstitial bone. Lower indentation depths in interstitial bone indicate that the extent of material softening is lower than in osteons. The absence of softening can cause stress concentrations and microcrack propagation in the matrix. Indeed, a number of studies report microcracks at the osteonal-interstitial interface [2, 7]. Interstitial bone also undergoes lower creep deformation that could result from higher mineralization and an impaired organic matrix containing post-translational modifications [8].

SIGNIFICANCE: Using a novel custom-made microscope-micro-indentation assembly, we show that osteonal bone undergoes greater deformation. We attribute this to creep-related mechanisms and diffuse damage formation which serves to limit the growth of micro-cracks in bone and prevent bone fracture.


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