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# Mechanical properties of cortical bone and their relationships with age, gender, composition and microindentation properties in the elderly

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ABSTRACT

The growing incidence of skeletal fractures poses a significant challenge to ageing societies. Since a major part of physiological loading in the lower limbs is carried by cortical bone, it would be desirable to better understand the structure-mechanical property relationships and scale effects in this tissue. This study aimed at assessing whether microindentation properties combined with chemical and morphological information are usable to predict macroscopic elastic and strength properties in a donor- and site-matched manner.

Specimens for quasi-static macroscopic tests in tension, compression, and torsion and microindentation were prepared from a cohort of 19 male and 20 female donors (46 to 99 years). All tests were performed under fully hydrated conditions. The chemical composition of the extra-cellular matrix was investigated with Raman spectroscopy. The results of the micro-mechanical tests were combined with morphological and compositional properties using a power law relationship to predict the macro-mechanical results.

Microindentation properties were not gender dependent, remarkably constant over age, and showed an overall small variation with standard deviations of approximately 10 %. Similar results were obtained for chemical tissue composition. Macro-mechanical stiffness and strength were significantly related to porosity for all load cases ($p<0.05$). In case of macroscopic yield strain and work-to-failure this was only true in torsion and compression, respectively. The correlations of macro-mechanical with micro-mechanical, morphological, and chemical properties showed no significance for cement line density, mineralisation, or variations in the microindentation results and were dominated by porosity with a moderate explanatory power of predominately less than 50 %.

The results confirm that age, with minor exceptions gender, and small variations in average mineralisation have negligible effect on the tissue microindentation properties of human lamellar bone in the elderly. Furthermore, our findings suggest that microindentation experiments are suitable to predict macroscopic mechanical properties in the elderly only on average and not on a one to one basis. The presented data may help to form a better understanding of the mechanisms of ageing in bone tissue and of the length scale at which they are active. This may be used for future prediction of fracture risk in the elderly.



## 1. Introduction

As modern societies age, the growing incidence of fractures poses a significant challenge for health care systems all over the world. Hip fractures are among the most detrimental, as they lead to a loss of mobility and show an increased mortality amongst patients. As a remarkable proportion of physiological loading in the ageing hip is carried by the cortical shell [47], it is important to investigate its role in the fracture resistance and strength of whole bones [96,114,130]. Whole bone strength has been shown to depend on the tissue mineralisation measured by clinical densitometry, but also on the micromechanical properties of the hierarchical organisation of bone tissue [102,16]. It is therefore important to study its mechanical and morphological properties on several length scales to identify structure-mechanical property relationships.

Bone is a natural composite with a cell-seeded mineralised collagen matrix featuring a hierarchical structure. It is mainly composed of mineral (50-60 wt. %), collagen (30-40 wt. %) and water (10-20 wt. %) [89]. Collagen molecules self-assemble into fibrils that are reinforced periodically by mineral platelets [122,31]. Empty space is filled with water and non-collagenous proteins of which osteocalcin binds strongly to

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minerals while it complexes and links to collagen via osteopontin [82] to help to mineralise the collagen fibril. The mineralised collagen fibrils impregnated by extra-fibrillar mineral particles [19,55,21] combine into fibril arrays, which form lamellae in a rotated plywood pattern [34,121, 115,87]. Osteocytes and their processes inhabit the lacuno-canalicular network making up for about 1 % of bone porosity [61].

Cortical bone is a dense mineralised tissue that encloses trabecular bone in epiphyses and is also found in diaphyses of long bones. It consists mainly of concentric bone lamellae arranged around blood vessels forming osteons and interstitial areas [31]. It features a macroporosity of around 5-15 %, which is mainly oriented along the osteon direction [117,135]. Secondary osteons result from a continuous remodelling process that counteracts the development of fatigue damage. They are separated from the surrounding tissue by a cement interface [12], which is 1 to 5 μm in thickness [21,12]. Its exact nature and mechanical role has been widely debated [98,127,106,72,26,67,74].

Several methods have been used in the past to investigate the microstructure of cortical bone. Among them are staining of the cementous interfaces in calcified thin sections [2], classical histology [4], and scanning electron microscopy [7]. Morphological parameters like porosity and surface density have been identified using light-microscopic methods and quantitative stereology [78] on cross sections. The three-dimensional investigation of bone morphology has been helped greatly by the development of new technologies such as micro-computed tomography ( CT) and related morphological characterisation techniques [43,79].

The mechanical properties of cortical bone on the macroscale are closely related to its microstructure and composition. Their relation has been extensively studied in the past [93,97,63,88,104,133,25,10]. It has been shown that elastic modulus, strength, and energy absorption decrease with increasing porosity or with the osteons' area fraction [63,133,93]. A change in mineral content due to ageing [21] affects the elastic, post-yield and ultimate properties of cortical bone [62,60,17, 134,56]. Another factor that may affect the mechanical integrity of cortical bone is the accumulation of microcracks due to a reduced remodelling activity, which has been shown to affect both the fatigue [99] and the post-yield behaviour of bone [131,73]. Finally, it has been recently shown that bone exhibits a strong scale effect in its post-elastic properties. The behaviour on the microscale is characterised by an increased strength and ductility and an absence of damage [100,58]. A thorough study of the effect of tissue properties measured on the lamellar level combined with chemical and morphological parameters on the macroscopic mechanical behaviour of cortical bone has not been performed for a large cohort ($n \geq 35$) of human donors so far.

Microindentation is a mechanical testing technique allowing to assess local material properties at the lamellar level. A diamond tip with a known geometry, often a three sided pyramid, is pushed into a flat surface. Tip displacement and axial force are measured simultaneously. The pioneering work of Oliver and Pharr [76,110] allows to extract local elastic properties from the unloading part of the indentation curve. Indentation in bone up to 1 μm of depth aims at characterising the anisotropic mechanical properties on the lamellar level [57,136]. While most of the studies have concentrated more on elastic properties of bone [137,45,30,86], yield properties have also been extracted from microindentation data based on inverse methods [111,70,13]. Microindentation has been used in the past as part of validation strategies involving elastic micro-finite element models of trabecular bone [125] or mineralised tendon [107], micromechanical homogenisation schemes describing the scale-dependency of the elastic properties of bone [86], and has also been compared to micro-tensile tests in bovine cortical bone [41,44]. A rigorous correlation of microindentation and macroscopic mechanical data in both the elastic and post yield region for a large number of human donors would be desirable in order to better understand the structure-mechanical property relationships and scale effects in bone. Such a correlation would also shed light on the clinical usability of microindentation techniques.

Therefore, this study aims (i) to examine the dependence of extracellular matrix (ECM) composition and micro-indentation properties with respect to age and gender; (ii) to test whether ECM properties contribute to the prediction of macroscopic properties next to porosity and histomorphometric variables.

Fig. 1 provides an overview of the study to better illustrate the interconnection of experiments and length scales.

## 2. Material and Methods

### 2.1. Whole Bone Level

**Proximal femurs of 19 male and 20 female donors** with a median age of 77 (46 to 99) years were used in this study. Donors had no known bone-related disease and passed away due to natural causes. Lifestyle or medication history of the donors was unknown. Investigations (cf. results) did not show any severe outliers or differences in the used bone samples. The age of female donors varied from 46 to 99, of male donors from 59 to 91 years. The specimens were provided by the Department of Applied Anatomy of the Medical University of Vienna. Ethics approval (175/2011) based on informed consent of the donors has been obtained. The specimens were frozen at -20 °C immediately after explantation.

### 2.2. Macroscopic Tissue Level

**Macromechanical specimens were prepared** from mid-diaphyseal sections of approximately 120 mm length that were cut approximately 100 mm below the cusp of the lesser trochanter (Fig. 1). Each of the femoral sections was divided into two approximately 50 mm long pieces by a central cut perpendicular to the shaft using a band saw under constant water irrigation (Exakt Apparatebau, Norderstedt, Germany). The proximal piece was divided and three neighbouring specimens from the anterior-lateral region with a tetragonal cross-section of approximately 16 $mm^2$ were cut with the band saw. From these pieces, dumbbell-shaped bone specimens were prepared using a desktop lathe (Promac, Taiwan). Specimens were lubricated using ethylenglycol during lathing. All specimens were oriented along the long axis of the femur. The central sections of the specimens had a diameter of 3 mm and a gauge length of 6.5 mm, the total length of the specimens was 30 mm. The specimens were kept hydrated in 0.9 % saline solution throughout the whole process. After preparation, samples were stored frozen at -20°C. In total, 111 specimens (54 male, 57 female) were prepared of which 2 specimens broke before testing.

**For morphology analysis of macroscopic specimens** a 14.4 mm long central section of the dumbbell shaped specimens was scanned in a CT (μCT40, Scanco) at a spatial resolution of 8 μm (Fig. 1). Samples were submerged in 0.9 % saline solution during scanning and refrozen afterwards. CT images were manually cropped in ImageJ (National Institutes of Health, USA) and aligned to their main axis (TransformJ package [65]). Subsequently, all datasets were cropped to the gauge length of the specimens. Cross-sectional area $A_0$ and radius $r$ of each sample were calculated from the middle slice of the image stack and used to compute apparent stress from the force signal recorded during mechanical testing.

Cropped images were filtered using a Gauss filter with radius and standard deviation of 1 voxel. Then, they were segmented using the single level thresholding of Ridler and Calvard [92] with a global threshold equal to 34 % of the maximum grey value. Images were cleaned and unconnected bone regions and islands were removed.

Morphological analysis was performed on a hexahedron of $2 \times 2 \times 6.5$ $mm^3$ size using medtool 3.6 (Dr. Pahr Ingenieurs e.U., Austria). Bone volume fraction ($\rho$) was defined as bone volume (sum of binarised grey levels) normalised by the volume of the whole sample cube. Fabric tensors [37] and the degree of anisotropy ($DA$) as the largest eigenvalue divided by the smallest eigenvalue of the fabric tensor for the Haversian porosity were computed to judge the alignment of the specimens.

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