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

Mohammad J. Mirzaali^{a,1}, J. Jakob Schwiedrzik^{a,b,1}, Suwanwadee Thaiwichai^a, James P. Best^b, Johann Michler^b, Philippe K. Zysset^{a,2}, Uwe Wolfram^{*,a,c,2}

^a*Institute for Surgical Technology and Biomechanics, University of Bern, Switzerland*

^b*EMPA, Swiss Federal Laboratories for Materials Science and Technology, Laboratory of Mechanics of Materials and Nanostructures, Thun, Switzerland*

^c*School of Engineering and Physical Science, Institute for Mechanical, Process and Energy Engineering, Heriot-Watt University, Edinburgh, United Kingdom*

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 ap-

proximately 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 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.

Key words: cortical bone, strength, microindentation, Raman spectroscopy, microstructure, scale effect, ageing, reference point indentation

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

*Corresponding Author: u.wolfram@hw.ac.uk, Heriot-Watt University, School of Engineering and Physical Science, Institute for Mechanical, Process and Energy Engineering, James Nasmyth Building, Room JN 2.33 Edinburgh, EH14 4AS, UK

¹Both authors share first authorship.

²Both authors share last authorship.

amongst patients. As a remarkable proportion of physiological loading in the ageing hip is carried by the cortical shell (Holzer et al., 2009), it is important to investigate its role in the fracture resistance and strength of whole bones (Schaffler and Burr, 1984; Treece et al., 2010; Zebaze et al., 2010). 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 (Seeman and Delmas, 2006; Cooper et al., 2007). 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. %) (Rho et al., 1998). Collagen molecules self-assemble into fibrils that are reinforced periodically by mineral platelets (Weiner and Wagner, 1998; Fratzl and Weinkamer, 2007). Empty space is filled with water and non-collagenous proteins of which osteocalcin binds strongly to minerals while it complexes and links to collagen via osteopontin (Poundarik et al., 2012) to help to mineralise the collagen fibril. The mineralised collagen fibrils impregnated by extrafibrillar mineral particles (Currey, 1969; Lees et al., 1990; Currey, 2002) combine into fibril arrays, which form lamellae in a rotated plywood pattern (Giraud-Guille, 1988; Weiner et al., 1997; Varga et al., 2013; Reznikov et al., 2014). Osteocytes and their processes inhabit the lacuno-canalicular network making up for about 1 % of bone porosity (Martin, 1984).

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 (Fratzl and Weinkamer, 2007). It features a macroporosity of around 5-15 %, which is mainly oriented along the osteon direction (Wachter et al., 2001a; Zioupos et al., 2008). 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 (Burr et al., 1988), which is 1 to 5 μm in thickness (Currey, 2002; Burr et al., 1988). Its exact nature and mechanical role has been widely debated (Schaffler et al., 1987; Yeni and Norman, 2000; Skedros et al., 2005; Najafi et al., 2007; Ebacher and Wang, 2009; Montalbano and Feng, 2011; Nobakhti et al., 2014).

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 (Bain et al., 1990), classical histology (Bernhard et al., 2013), and scanning electron microscopy (Boyde and Jones, 1996). Morphological parameters like porosity and surface density have been identified using light-microscopic methods and quantitative stereology (Parfitt, 1984) 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 (μCT) and related morphological characterisation techniques (Hildebrand et al., 1999; Patsch et al., 2013).

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 (Robertson and Smith, 2000; Schaffler and Burr, 1988; McCalden et al., 1993; Rho et al., 1995; Sevostianov and Kachanov, 2000; Zioupos, 2001; Dong and Guo, 2004; Budyn et al., 2012). It has been shown that elastic modulus, strength, and energy absorption decrease with increasing porosity or with the osteons' area fraction (McCalden et al., 1993; Zioupos, 2001; Robertson and Smith, 2000). A change in mineral content due to ageing (Currey, 2002) affects the elastic, post-yield and ultimate properties of cortical bone (Martin et al., 1985; Martin, 1993; Courtney et al., 1996; Zioupos and Currey, 1998; Leng et al., 2009). 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 (Schaffler et al., 1990) and the post-yield behaviour of bone (Zhang et al.,

2013; Nalla et al., 2003). 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 (Schwiedrzik et al., 2014). 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 (1992); Swadener and Pharr (2001) 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 (Lewis and Nyman, 2008; Zysset, 2009). While most of the studies have concentrated more on elastic properties of bone (Zysset et al., 1999; Hoffer et al., 2005; Franzoso and Zysset, 2009; Reisinger et al., 2011), yield properties have also been extracted from microindentation data based on inverse methods (Tai et al., 2006; Mullins et al., 2009; Carnelli et al., 2010). Microindentation has been used in the past as part of validation strategies involving elastic micro-finite element models of trabecular bone (Wolfram et al., 2010c) or mineralised tendon (Spiesz, 2011), micromechanical homogenisation schemes describing the scale-dependency of the elastic properties of bone (Reisinger et al., 2011), and has also been compared to micro-tensile tests in bovine cortical bone (Hengsberger et al., 2003; Hoc et al., 2006). 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

89 microindentation techniques.

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

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

96 **2. Material and Methods**

97 **2.1. Whole Bone Level**

98 **Proximal femurs of 19 male and 20 female donors** with a median age of
99 77 (46 to 99) years. Donors had no known bone-related disease and passed away
100 due to natural causes. Lifestyle or medication history of the donors was unknown.
101 Investigations (cf. results) did not show any severe outliers or differences in the bone
102 samples investigated. The age of female donors varied from 46 to 99, of male donors
103 from 59 to 91 years. The specimens were provided by the Department of Applied
104 Anatomy of the Medical University of Vienna. Ethics approval (175/2011) based on
105 informed consent of the donors has been obtained. The specimens were frozen at
106 -20°C immediately after explantation.

107 **2.2. Macroscopic Tissue Level**

108 **Macromechanical specimens were prepared** from mid-diaphyseal sections
109 of approximately 120 mm length that were cut approximately 100 mm below the
110 cusp of the lesser trochanter (Figure 1). Each of the femoral sections was divided
111 into two approximately 50 mm long pieces by a central cut perpendicular to the shaft
112 using a band saw under constant water irrigation (Exakt Apparatebau, Norderstedt,
113 Germany). The proximal piece was divided and three neighbouring specimens from
114 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 μ CT (μ CT40, Scanco) at a spatial resolution of 8 μ m (Figure 1). Samples were submerged in 0.9 % saline solution during scanning and refrozen afterwards. μ CT images were manually cropped in ImageJ (National Institutes of Health, USA) and aligned to their main axis (TransformJ package (Meijering et al., 2001)). 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 (1978) 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 $2 \times 2 \times 6.5$ mm³ size using medtool 3.6 (Dr. Pahr Ingenieure e.U., Austria). Bone volume fraction (ρ) was defined as bone volume (sum of binarised grey levels) normalised by the volume of the whole sample cube. Fabric tensors (Harrigan and Mann, 1984) 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

143 of the specimens.

144 **Histology analysis** was performed on calcified bone infiltrated with polymethyl
145 methacrylate (PMMA). A 2 to 3 mm section was cut from each sample using the
146 band saw (Figure 1). The section was located between the gripping zone and the
147 shoulder of the gauge length. This volume was predominantly loaded elastically
148 during testing. The sections were embedded and prepared for histological analysis.
149 We used a modified von Kossa staining to highlight the cement interface and osteons.
150 The histological preparation and the staining procedure are described in more detail
151 in Appendix B.

152 Stained samples were visualised under an optical microscope (Leica DM750, Ger-
153 many) at 5-fold magnification. Three images were taken per specimen, two before
154 and one after the staining. Illumination and pixel size ($1.3 \mu\text{m}$) for each image were
155 kept constant.

156 Transmitted and reflected light images of every specimen before the staining
157 were registered to the transmitted light image of the stained sample. Image reg-
158 istration was performed in ImageJ based on an automatic sub-pixel registration
159 algorithm (Thévenaz et al., 1998). A constant region of interest (ROI) of $1800 \times$
160 1200 pixels (3.65 mm^2) was considered. The ROI was cropped from the centre of the
161 raw images in ImageJ. An overlay image of each stained and unstained sample was
162 created to check the segmentation method and to reduce artefacts due to gluing or
163 the milling process (Appendix B, Figure 8).

164 Cement lines in the stained images were manually segmented in Amira 5.6.0 (Zuse
165 Institute Berlin, Germany). The segmented images were skeletonised (DtfSkele-
166 tonization, MeVisLab, Germany). The pixels in the skeletonised binary image were
167 summed up and cement line density ρ_{CL} was defined as ratio of total number of
168 pixels of cement line l_{CL} to total number of ECM pixels in the image. To obtain
169 the number of osteons n_{osteon} per sample, the RGB image of the stained sample was
170 segmented based on a fixed threshold of (190, 190, 190) in RGB space. Secondary os-

teons were selected using a connected component analysis (MATLAB, Natick, MA). 8-connected neighbourhood connectivity was used in the algorithm and components larger than 500 pixels were considered as Haversian canals and each detected Haversian canal represented an osteon. The average osteon diameter d_{osteon} (in mm) can be obtained as $d_{\text{osteon}} = l_{CL}/n_{\text{osteon}}\pi$ approximating the osteonal cross-section with a circle.

For macroscopic mechanical testing, specimens were re-hydrated in Hank's buffered saline solution (HBSS) for 12 h at 4°C and kept in HBSS at room temperature for at least 2 h to reach thermal equilibrium. During testing, the specimens were wrapped in tissue soaked in HBSS and kept continuously hydrated.

Tests were performed in tension, compression and torsion using a biaxial servo-hydraulic testing system (MTS 858 Mini-Bionix, USA) with an axial and torsional capacity of 15 kN and 150 Nm. The specimens were loaded in displacement control along the cylindrical main axis. Axial and shear strains were measured using a custom made bi-axial extensometer (Epsilon Tech 3550HT, USA). Quasi-static tests were performed at a strain rate of $\approx 2.5 \cdot 10^{-4} \text{ s}^{-1}$ (displacement rate of $5 \cdot 10^{-3} \text{ mm s}^{-1}$) in uni-axial tension and uni-axial compression and a strain rate of $\approx 3 \cdot 10^{-4} \text{ s}^{-1}$ (angular rate of $0.25^\circ \text{ s}^{-1}$) in torsion (Mirzaali et al., 2015). The specimens were preconditioned with three load cycles up to 0.2 % strain in tension, compression and 0.3 % in torsion and subsequently monotonically loaded to failure.

All tests were performed at approximately 23°C and 44 % relative humidity. Data was acquired at a sampling rate of 100 Hz with a test duration of about 3 minutes. Time, displacement, angle of rotation ϕ , force F , torque T , axial strain ε and shear strain γ were recorded simultaneously.

Stress σ in compression and tension was calculated as F/A_0 . Engineering strain $\varepsilon = \Delta l \cdot l^{-1}$ in tension and compression was directly captured by the axial channel of the extensometer. Shear stress τ for a cylindrical sample with radius r was calculated

198 according to Nadai (1950) as

$$\tau = \frac{1}{2\pi r^3} \left(\phi \frac{dT}{d\phi} + 3T \right). \quad (1)$$

199 Yield stress and yield strain was determined from the monotonic stress-strain
200 curves using a 0.05 % and a 0.2 % offset criterion. We chose the 0.05 % criterion as
201 onset of yield and the 0.2 % criterion as an upper bound that allows to compare our
202 results to the literature. The initial elastic modulus (E_0) was determined using a
203 moving regression with an 0.2 % window width to identify the steepest section of the
204 loading curve. Ultimate stress and strain were measured as the maximum stress and
205 corresponding strain. Ultimate strain energy W_{ult} was calculated as the area under
206 the stress-strain curve until reaching the ultimate stress.

207 Due to the limited range of the extensometer, it was not possible to continue
208 the test to the ultimate fracture point for most of the torsional specimens. There-
209 fore, W_{ult} and γ_u could not be determined for this case. Ultimate shear stress was
210 determined as the asymptotic post-yield stress since torsion shows very little strain
211 hardening (Figure 1).

212 2.3. Microscopic Tissue Level

213 **Specimens for microindentation** were prepared from a 2 to 3 mm section cut
214 from the posterior end of the most anterior tetragonal specimen extracted from each
215 donor using the band saw (Figure 1). Subsequently, the section was divided so that
216 an axial and a transverse cross-section was accessible. Sections were air-dried for
217 more than 48 h and embedded in but not infiltrated by PMMA (Technovit 4006SE,
218 Heraeus, Germany) (Wolfram et al., 2010c; Schwiedrzik et al., 2014). After curing
219 at an elevated pressure of 2 bar for 30 minutes, they were ultra-milled (Polycut E,
220 Reichert-Jung, Germany) to obtain flat and parallel surfaces. Prior to indentation,
221 samples were re-hydrated for at least 2 h in HBSS (Wolfram et al., 2010c) and stayed
222 submerged in HBSS during testing. Indentations were performed with an Ultra

223 Nano Hardness Tester (UNHT, CSM Instruments, Switzerland) equipped with long
224 shaft reference and Berkovich diamond indenter tips and a custom made liquid cell.
225 Osteonal and interstitial regions were indented on the axial cross sections. On the
226 transverse cross sections, indentations were placed close to Haversian channels, but
227 no clear visual distinction between osteonal and interstitial tissue could be made.

228 **For microindentation testing**, a trapezoidal protocol in load control up to
229 a maximum depth of 1 μm with a loading rate of 100 mN/min, holding time at
230 maximum force of 30 s (Wolfram et al., 2010c), and an unloading rate of 400 mN/min
231 was chosen in order to minimise the effects of creep on the measurement (Schwiedrzik
232 et al., 2014). Ten indentations in ten regions were performed per sample. On the
233 axial cross sections five indentations per region were placed in osteonal tissue and
234 five in interstitial tissue. On the transverse cross sections ten random indentations
235 were placed per region. Plane strain modulus E^* , indentation hardness H_{IT} , elastic
236 work W_{elast} , and total work W_{tot} were extracted using standard methods (Oliver and
237 Pharr, 1992; Reisinger et al., 2011; Wolfram et al., 2010c). Plane strain modulus is
238 recovered from the experimentally measured reduced modulus E_r (Oliver and Pharr,
239 1992) by

$$E^* = \left(\frac{1}{E_r} - \frac{1 - \nu_i^2}{E_i} \right)^{-1} \quad (2)$$

240 for known isotropic constants E_i and ν_i of the diamond indenter tip. Indentation
241 hardness is given by the ratio of maximum load P_{max} to projected contact area at
242 maximum depth A_c

$$H_{IT} = \frac{P_{max}}{A_c}. \quad (3)$$

243 The total, elastic, and plastic work are defined between 0, maximum depth h_m , and

244 residual depth h_p as:

$$W_{tot} = \int_0^{h_m} Pdh, \quad W_{elast} = \int_{h_m}^{h_p} Pdh \quad W_{plast} = W_{tot} - W_{elast}. \quad (4)$$

245 **To perform Raman spectroscopy**, a layer of approximately 50 μm was milled
 246 away to remove the indentations and possible influences of the submersion in HBSS
 247 on the surface chemistry (Figure 1). Ten Raman spectra were acquired in dry con-
 248 dition on each axial sample using an upright Raman microscope (Nova Spectra,
 249 ND-MDT, Russia). A continuous laser with an excitation wavelength of 633 nm
 250 and a power of 0.7 mW was focused on the sample surface using a 100 $\times$ objective
 251 with a numerical aperture of 0.95. Spectra were recorded with 2 acquisitions of 3 s
 252 exposure time each and a spectral resolution of 2.7 cm^{-1} . To account for autofluo-
 253 rescence, the Raman spectra were baseline corrected following James et al. (2012)
 254 using SpecTools³.

255 Following Akkus et al. (2004), Yerramshetty et al. (2006), Morris and Mandair
 256 (2011), Gamsjaeger et al. (2011), and Gamsjaeger et al. (2014) (and references
 257 therein), we analysed the ratio of the integrated areas of the $\nu_1\text{PO}_4$ (900 – 1000 cm^{-1})
 258 to the amide I (1620 – 1720 cm^{-1}) band to express the mineral/matrix ratio ρ_{MM}
 259 (Figure 1). The inverse of the full width half maximum (FWHM) of the $\nu_1\text{PO}_4$ peak
 260 was analysed to approximate maturity/crystallinity of the apatite crystals.

261 Peaks were isolated according to the given bands. Data was processed without
 262 smoothing. For each amide I peak in each of the ten spectra a Gaussian function
 263 was fitted directly using a least square scheme (`scipy.optimize.leastsq`). In case of the
 264 $\nu_1\text{PO}_4$ peak a two step procedure was employed. First a Cauchy-Lorentz function was
 265 fitted directly to the extracted peak and peak centre and half width half maximum
 266 was determined. Then, a Gaussian function was fit to the data ranging from peak
 267 centre minus half width half maximum to the end of the extracted band in order

³<http://spectools.sourceforge.net/> last visited May 5th, 2015.

268 to account for small overlapping bands at lower Raman shifts for the $\nu_1\text{PO}_4$ peak
269 (Appendix C, Figure 9).

270 From the final fitted Gaussian functions we identified the peak centre, intensity,
271 and FWHM as $2\sqrt{2\ln(2)}S$ with the standard deviation S . The integrals under this
272 function were computed using a numerical integration scheme (scipy.integrate.quad)
273 in Scipy (Jones et al., 2001). After analysing the peaks possible outliers were re-
274 moved. Subsequently, the data was averaged and treated as in a repeated measures
275 analysis to reduce the variance in a sample with a limited number of specimens (Craw-
276 ley, 2005). This means that the sample mean was regarded as representative and
277 subsequently used in the regression analyses.

278 2.4. Statistical Analyses

279 **Descriptive statistics and statistical testing** was performed using Gnu R (R
280 Development Core Team, 2008). A significance level of $p < 0.05$ was used. Normal-
281 ity was checked using quantile-quantile plots and post-hoc Shapiro-Wilk normality
282 tests (Shapiro and Wilk, 1965). In group wise comparisons, e. g. macroscopic gen-
283 der differences, t-tests were chosen where normal distributions were found, otherwise
284 Mann-Whitney tests were performed to check for significant differences. To analyse
285 the indentation results, analysis of variance (ANOVA) was performed where age,
286 gender, and sample direction were factors. R's linear least square function was used
287 to fit models between macro- and micromechanical, microstructural, and composi-
288 tional properties in log-space. Due to an unequal number of samples per donor, we
289 used a mixed model (Pinheiro et al., 2015) to find the correlation between age and
290 morphological properties. A data point was considered as outlier if it was 1.5 times
291 the inter-quartile range smaller than the 25 % or larger than the 75 % quartile.

292 **We used power law relationships** (5) to describe the relation between mi-
293 cromechanical X_{micro} , microstructural (ρ and ρ_{CL}), and macroscopic mechanical
294 properties X_{macro} . X_{micro} represents the donor mean from a repeated measure anal-
295 ysis and $\bar{X}_{micro}$ the population average of each property. X_0 , a , b , c , and d are fitting

parameters and the model is given as

$$X_{macro} = X_0 \left(\frac{X_{micro}}{\bar{X}_{micro}} \right)^a \rho^b (1 - \rho_{CL})^c \left(\frac{\rho_{MM}}{\bar{\rho}_{MM}} \right)^d. \quad (5)$$

In this case, X_0 represents the properties of a fictitious solid with average micromechanical properties and chemical composition, no porosity and no cement lines. The mechanical properties measured at the macroscale consisted of elastic modulus E_0 , strength σ_u , yield stress $\sigma_{0.05}$, yield strain $\varepsilon_{0.05}$, and ultimate work W_{ult} . Their corresponding micromechanical counterparts were plane strain modulus E^* , indentation hardness H_{IT} , ratio of hardness to modulus H_{IT}/E^* , and total work W_{tot} . Microstructural properties were bone volume fraction ρ measured by μ CT and the cement line density ρ_{CL} calculated from the stained histology images. The mineralisation ρ_{MM} with its population average $\bar{\rho}_{MM}$ measured with Raman spectroscopy was chosen to represent the chemical tissue composition.

To perform the fit we exploited log-space conversion of the power law. Significant parameters were identified by removing insignificant parameters from the linear model in log space in a backward, step-wise manner. After each step, the parameter with the highest p-value was removed until only significant slopes and the intercept remained. Subsequently, overall significance of the model in log-space was checked.

3. Results

Raman analyses show that mineral content and crystallinity were not significantly different between males and females ($p > 0.05$) so that the data was pooled. Neither mineralisation ρ_{MM} nor crystallinity correlated with donor age (Figure 2). Mineralisation was found to be 3.44 ± 0.39 and crystallinity 0.06 ± 0.0012 cm (mean $\pm$ standard deviation).

μ CT and histological analysis showed that the morphological parameters were not dependent on gender ($p > 0.05$). In addition the age distributions of the male

and female donors were not significantly different ($p > 0.05$). Therefore, the data was pooled (Table 1).

A significant positive trend was observed between donor age and porosity ($R^2 = 0.16$, $p < 0.05$) as well as cement line density ($R^2 = 0.06$, $p < 0.05$). No significant correlation with age was observed for osteon diameter and number of osteons ($p > 0.05$) (Figure 3).

The microindentation tests yielded micromechanical properties in axial and transverse direction independent of donor age ($p > 0.05$) except for H_{IT} in interstitial bone (axial direction), in which a weak trend with age was observed (Figure 4). Direction was a significant factor in the ANOVA ($p < 0.05$) for all measurements except H_{IT} confirming the anisotropy of cortical bone on the ultra-structural level (Franzoso and Zysset, 2009; Reisinger et al., 2011; Schwiedrzik et al., 2014; Schwiedrzik and Zysset, 2015). In axial direction, significant differences of approximately 10 % were found between interstitial and osteonal bone ($p < 0.05$), which is in line with earlier measurements reporting increased properties for the more mineralised interstitial tissue (Zysset et al., 1999). In the transverse direction, weakly significant differences of approximately 3.33 %, 14.31 %, and 3.97 % were found between male and female donors for indentation hardness H_{IT} , the ratio of hardness to modulus H_{IT}/E^* and the ratio of plastic to total work W_{plast}/W_{tot} respectively ($p < 0.05$). This was not the case in axial direction. Population mean $\pm$ standard deviation of the properties are reported in Table 2.

Standard deviation of all micromechanical variables was independent of age. Ex-

Table 1: Mean $\pm$ standard deviation of morphological parameters of the tested cohort. No significant differences were found between properties of male and female donors, therefore the data was pooled.

$1 - \rho$	ρ_{CL}	d_{osteon}	n_{osteon}	DA
$6.00 \pm 3.97 \%$	$1.2 \pm 0.1 \%$	$0.19 \pm 0.02 \text{ mm}$	$14.51 \pm 2.27 \text{ mm}^{-2}$	1.92 ± 0.077

Table 2: Population mean $\pm$ standard deviation of mechanical properties measured by microindentation in axial and transverse directions are shown. Microindentation properties were independent of gender except for H_{IT} , H_{IT}/E^* , and W_{plast}/W_{tot} measured by microindentation in the transverse direction.

Orientation and Tissue Type		Plane Strain Modulus	Hardness	Hardness/Modulus	Elastic Work	Total Work
		E^* in GPa	H_{IT} in MPa	$\frac{H_{IT}}{E^*}$ in %	W_{elast} in pJ	W_{tot} in pJ
axial	Osteonal	18.09 ± 1.73	408 ± 69	2.2 ± 0.2	1014 ± 454	4198 ± 1866
	Interstitial	20.07 ± 1.19	503 ± 56	2.5 ± 0.2	1212 ± 496	4836 ± 1972
	pooled	19.08 ± 1.78	455 ± 78	2.4 ± 0.2	1113 ± 483	4517 ± 1935
transverse	female	12.60 ± 2.00	367 ± 91	2.9 ± 0.3	709 ± 218	2176 ± 641
	male	13.68 ± 1.72	428 ± 75	3.1 ± 0.2	885 ± 358	2611 ± 1026
	pooled	13.10 ± 1.94	395 ± 89	3.0 ± 0.3	790 ± 302	2377 ± 859

cept for H_{IT} in osteonal bone where an average 14 % difference was found the standard deviation was independent of gender.

Population average plane strain moduli in axial and transverse directions were used to fit a transversely isotropic tissue stiffness tensor (Franzoso and Zysset, 2009) (Figure 10).

In the macroscopic mechanical tests, 13 specimens were excluded based on the outlier criterion or extensometer slippage during testing. Finally, 96 successful tests were included in this study from which 32 samples were tested in tension, compression, and torsion, respectively (Figure 1).

Mean $\pm$ standard deviation of the identified macromechanical properties in tension, compression, and torsion are shown in Table 3. Elastic modulus in tension and compression were not significantly different ($p = 0.08$) and therefore pooled. Yield stress, yield strain, strength, and ultimate strain were significantly different in ten-

355 sion and compression ($p < 0.05$). Ultimate work in compression and work to fracture
in tension were not significantly different ($p > 0.05$).

Table 3: Descriptive statistics of the monotonic macroscopic mechanical tests in uniaxial tension, uniaxial compression, and torsion are reported.

Monotonic Tests	Variable and Unit		Load Case		
			Tension	Compression	Torsion
Modulus	E_0	GPa	18.16 ± 1.88	18.97 ± 1.84	-
Shear Modulus	μ_0	GPa	-	-	6.07 ± 0.57
Yield Stress 0.05 %	$\sigma_{0.05}$	MPa	71.56 ± 10.19	115.06 ± 16.36	-
Shear Yield Stress 0.05 %	$\tau_{0.05}$	MPa	-	-	31.62 ± 4.00
Yield Strain 0.05 %	$\varepsilon_{0.05}$	%	0.45 ± 0.05	0.66 ± 0.09	-
Shear Yield Strain 0.05 %	$\gamma_{0.05}$	%	-	-	0.57 ± 0.03
Yield Stress 0.2 %	$\sigma_{0.2}$	MPa	84.52 ± 10.47	147.89 ± 16.36	-
Shear Yield Stress 0.2 %	$\tau_{0.2}$	MPa	-	-	40.95 ± 5.16
Yield Strain 0.2 %	$\varepsilon_{0.2}$	%	0.67 ± 0.04	0.98 ± 0.09	-
Shear Yield Strain 0.2 %	$\gamma_{0.2}$	%	-	-	0.87 ± 0.04
Ultimate Stress	σ_u	MPa	92.95 ± 10.07	153.59 ± 21.63	-
Shear Ultimate Stress	τ_u	MPa	-	-	46.31 ± 5.82
Ultimate Strain	ε_u	%	1.9 ± 0.6	1.3 ± 0.3	-
Ultimate Work	W_{ult}	$\frac{\text{kJ}}{\text{m}^3}$	1.49 ± 0.55	1.27 ± 0.64	-

356
357 Elastic modulus in tension and compression as well as yield stress in compression
358 were significantly correlated with donor age. Yield stress in tension and torsion,
359 yield strain as well as ultimate work in tension and compression where independent
360 of donor age Figure 5.

361 Elastic modulus, yield stress and strength in tension, compression, and torsion as
 362 well as the ultimate work in compression were significantly correlated to porosity ($p <$
 363 0.05). No dependency of mechanical properties in tension, compression, and torsion
 364 on osteon diameter, osteon number, or cement line density could be identified. No
 365 significant correlation between yield strain and porosity in tension and compression
 366 and ultimate work in tension was observed (Figure 6).

367 **Correlating the results of the microindentation tests with the Raman**
 368 **results** yields no significant relationship. E^* , H_{IT} , H_{IT}/E^* , or W_{plast}/W_{tot} (Figure 4)
 369 are neither dependent on donor mean ρ_{MM} nor on mineral maturity/crystallinity
 370 (Figure 2).

371 **Correlating the results of the macroscopic tests with micromechanical,**
 372 **microstructural, and chemical properties** yields a significant ($p < 0.05$) depen-
 373 dence of macroscopic stiffness and strength on ρ (Table 4). For yield strain in tension
 374 and compression only the intercept X_0 was significant ($p < 0.05$). For ultimate work
 375 in tension only the intercept was significant while in case of compression the intercept
 376 and ρ were significant ($p < 0.05$).

377 4. Discussion

378 **Specimens of 39 donors** in an age range of 46 – 99 years were tested in a site
 379 matched manner at the micro- and the macroscale by means of microindentation and
 380 uniaxial tension, compression, and torsion testing. Specimen specific morphological
 381 parameters were measured using μ CT and histology. The chemical composition was
 382 investigated by Raman spectroscopy. Statistical analyses between mechanical prop-
 383 erties at two length scales, morphological, and chemical properties were performed
 384 with the aim to better understand the structure-mechanical property relationships
 385 and scale effects in bone.

386 **Raman spectroscopy** showed that the relative mineral content ρ_{MM} as well as
 387 the crystallinity/maturity did not vary with donor age or gender. Thus, the results

Table 4: The measured micromechanical properties X_{micro} are combined with microstructural features in a power law (5) to predict the macroscopic mechanical properties X_{macro} . Columns left of the vertical line show load cases, macro- and microscopic counterparts, and used units. To the right of the vertical line, the model parameters X_0 , a , b , and c are only shown if they were significant. The coefficient of variation (CV) is reported as the standard error of the estimate divided by the mean of the prediction.

Load Case	X_{macro}	X_{micro}	Unit	X_0	a	b	c	d	CV
UT, UC	E_0	E^*	GPa	19.32	–	0.68	–	–	0.10
UT	$\sigma_{0.05}$	H_{IT}	MPa	79.03	–	1.77	–	–	0.13
UT	$\varepsilon_{0.05}$	$\frac{H_{IT}}{E^*}$	–	0.00465	–	–	–	–	0.11
UT	σ_u	H_{IT}	MPa	100.76	–	1.40	–	–	0.09
UT	W_{ult}	W_{tot}	kJm^{-3}	1.37	–	–	–	–	0.41
UC	$\sigma_{0.05}$	H_{IT}	MPa	125.70	–	1.48	–	–	0.12
UC	$\varepsilon_{0.05}$	$\frac{H_{IT}}{E^*}$	–	0.00688	–	–	–	–	0.13
UC	σ_u	H_{IT}	MPa	180.46	–	2.59	–	–	0.07
UC	W_{ult}	W_{tot}	kJm^{-3}	1.67	–	5.34	–	–	0.37
TR	μ_0	E^*	GPa	6.71	–	1.74	–	–	0.07
TR	$\tau_{0.05}$	H_{IT}	MPa	36.66	–	2.58	–	–	0.09
TR	$\gamma_{0.05}$	$\frac{H_{IT}}{E^*}$	–	0.00596	–	0.76	–	–	0.05
TR	τ_u	H_{IT}	MPa	53.19	–	2.42	–	–	0.09

388 describe a well established ECM of an elderly cohort. This confirms results obtained
389 for ECM of paediatric donors (Gamsjaeger et al., 2014) where no dependence on
390 donor age could be found. With respect to the relative mineral content, the inde-
391 pendence of age is furthermore in line with findings of Boivin and Meunier (2002).

392 However, Gamsjaeger et al. (2014) also investigated newly forming bone where a sig-
 393 nificant dependence of all investigated parameters on tissue age, i. e. tissue maturity,
 394 was found. This may indicate that chemical composition as measured by Raman
 395 spectroscopy reaches a stable condition that is maintained throughout life. It was
 396 found that Raman spectra change with tissue age and that these changes can be
 397 related to decreasing mechanical competence. The phosphate bands relative to ma-
 398 trix collagen and crystallinity increased with tissue age in rats ranging in age from
 399 3 to 24 months (Akkus et al., 2004). Hence, forming bone shows differences in the
 400 chemical composition that are not present in elderly ECM.

401 Our results for crystallinity were comparable to results obtained by Kim et al.
 402 (2014) and only slightly higher than those reported by Yerramshetty and Akkus
 403 (2008). Our mineralisation ρ_{MM} was comparable to the average mineralisation re-
 404 ported by McCreadie et al. (2006). Kim et al. (2014) identified reduced tissue stiffness
 405 and mineralisation in an osteoporotic cohort that was significantly older than a non-
 406 osteoporotic control cohort (totally 11 donors). This is in contrast with our results
 407 where ageing seems not to affect microscale mechanical tissue properties or mineral
 408 content of the ECM. However, Kim et al. (2014) drove the indentations to 0.5 mN
 409 which is at least an order of magnitude lower than our loads, rendering a proper
 410 comparison difficult. The low indentation load may have lead to spurious surface
 411 artefacts that could have biased the results. The indentation modulus was slightly
 412 higher than ours which may be due to the alcohol dehydration and embedding of
 413 the specimens. The mineral to matrix ratio was not comparable to our data since
 414 $v_1\text{PO}_4$ was divided by the CH_2 band in their study. We decided against analysing
 415 CH_2 since it appeared as a right headed distribution while amide I better resembled
 416 a Gaussian. Finally, the age dependence was weak ($p = 0.053$), so that the used
 417 cohort may have been too small to conclude on an age dependence. Nevertheless the
 418 authors found no dependency of crystallinity on age which is in line with our results.
 419 Yerramshetty et al. (2006) identified age dependent mineral to matrix ratios in an

420 elderly male cohort of 16 donors. The variation around their mean mineral to matrix
421 ratio was less than $\pm 10\%$. The independence of crystallinity on age was in line with
422 our results.

423 Gamsjaeger et al. (2011) point out that determining the ratio $v_1\text{PO}_4/\text{amide I}$
424 is orientation dependent which seems not to be the case for the integrated area
425 of $v_2\text{PO}_4/\text{amide III}$. However, we only used samples in axial direction which have
426 been well aligned and averaged over 10 measurements. With this we exclude a
427 directional impact coming solely from the specimen direction. A further impact
428 of the direction within the axial samples on the measurements should have been
429 averaged out by the number of tests per sample. Furthermore, if the orientation of
430 the collagen network would have been age dependent it should have been observable
431 in E^* as well. Clearly this measure is anisotropic as axial and transverse specimens
432 are significantly different. Since there is no dependence we conclude that possible
433 directional effects are negligible. We averaged over interstitial and osteonal bone
434 and neglect, therefore, the difference in tissue age. For a composition analysis of the
435 specimens under investigation as a whole, this should be sufficient.

436 **From the morphological analysis**, microstructural properties such as porosity
437 $(1 - \rho)$, cement line density (ρ_{CL}) , number of osteons (n_{osteon}) and osteon diameter
438 (d_{osteon}) were calculated and analysed as a function of age and gender. The morpho-
439 logical parameters were found to be independent of gender which is in accordance
440 with results of Thomas et al. (2005); Britz et al. (2009) if compared for the same
441 age range as for the cohort in this study. A significant correlation between age and
442 porosity $(p = 0.04)$ was found confirming the results of McCalden et al. (1993);
443 Zioupos (2001); Malo et al. (2013). The significance, however, is lost if the female
444 point at 46 years is removed (Figure 3). This is due to the relatively low number of
445 donors younger than 60 years. The age of the 46 years old donor, however, did not
446 violate the outlier criterion so that it was not excluded from the analysis. Never-
447 theless, the tested age range is considered relevant since musculoskeletal diseases are

most prevalent in the elderly. The porosity, however, was lower as for instance in McCalden et al. (1993) where female donors showed porosity values of almost 30 % in specimens from a similar location in the femur.

It has been shown in the past that osteon size and number of osteons were significantly influenced by ageing leading to more and smaller osteons in older bones (Ortner, 1975; Evans, 1976; Burr, 1992; Havill, 2004). However, no significant correlation was found between age and osteon diameter or osteon number in the elderly cohort studied in this work ($p > 0.05$). Again, this could be due to the the relatively low number of donors younger than 60 years. When comparing our results for n_{osteon} to the results of Kerley (1965) who presented a correlation over an age range of 0 to 100 years, we observe a distribution with a plateau between 60 and 100 years which is similar to our cohort. In addition, variation in microstructural properties of mature bone have been shown to be independent of age (Ortner, 1975; Burr, 1992). The cement line density was found to change as a function of age which is in line with conclusions in Zimmermann et al. (2011).

The misalignment between the direction of the largest fabric eigenvalue and the cylindrical main axis of the tested macroscopic samples was less than 6° . In addition, the small variation of the degree of anisotropy (Table 1) indicates a homogeneous orientation distribution of porosity. Thus, the specimens can be considered properly aligned and suitable for the macroscopic tests. Also, the representation of porosity by a scalar instead of a tensor is justified in the case of an uniaxial test.

Microindentation properties were found to be independent of age ($p > 0.05$) except for indentation hardness H_{IT} of interstitial tissue indented in axial direction. The independence of age corresponds well to the reports of (Zysset et al., 1999; Hoffer et al., 2000; Wolfram et al., 2010a,b). In contrast to an earlier study on trabecular bone (Wolfram et al., 2010a), the plastic to total work ratio did not change as a function of age. However, age groups were compared in the earlier study, which could lead to spurious significance depending on the chosen age cutoff.

476 The indentation direction was found to be a significant parameter ($p < 0.05$) for all
 477 measurements except H_{IT} confirming the anisotropy of bone on the lamellar level
 478 reported in several studies in the past (Franzoso and Zysset, 2009; Wolfram et al.,
 479 2010a; Reisinger et al., 2011; Schwiedrzik et al., 2014; Schwiedrzik and Zysset, 2015).
 480 The lack of anisotropy in hardness arises from the multiaxial stress state under the
 481 indenter obscuring the anisotropy in yield properties and is in line with a study
 482 on indentation in anisotropic single crystals (Vlassak and Nix, 1994). In the axial
 483 direction, significant differences were found between interstitial and osteonal bone
 484 ($p < 0.05$). This is in line with earlier studies, which reported an increased hardness
 485 in interstitial tissue and attributed this difference to a higher degree of mineralisation
 486 due to an increased tissue age (Zysset et al., 1999; Schwiedrzik and Zysset, 2015).

487 In the transverse direction, small but significant differences were found between
 488 male and female donors for indentation hardness H_{IT} , hardness to modulus ratio
 489 H_{IT}/E^* and plastic to total work ratio W_{plast}/W_{tot} ($p < 0.05$). This has not been
 490 reported in the past and it may be attributed to a different number of interstitial
 491 sites in both groups. Unlike axial indentations, transverse indentations are prone to
 492 sampling errors between osteonal and interstitial tissue as both tissue types cannot
 493 be clearly distinguished. An additional reason could be swelling of the surface in
 494 transverse direction which is known to lead to artefacts (Spiesz et al., 2012). It
 495 was shown that the viscous properties of bone may be gender dependent (Wu et al.,
 496 2012). However, the reported dependence was as weak as the one found in the
 497 present study. Finally, the differences observed here are smaller than 15 % with
 498 mutually overlapping standard deviation ranges (Table 2). Together with the gender
 499 independence of the axial osteonal and interstitial properties, that were obtained
 500 under more controlled conditions, we conclude that the observed gender dependence
 501 in transverse direction is at least questionable if not absent.

502 The indentation data of this study could confirm trends seen in earlier studies
 503 on cortical bone (Rho et al., 1997; Zysset et al., 1999; Rho et al., 2002) with a

considerably larger number of donors and in two anatomical directions under fully hydrated conditions. Bone properties at the lamellar level were shown to lie within a reasonably constant band with significant differences between younger and older tissue (osteonal vs. interstitial), but donor age did not play a significant role. This means that there is no change in bone quality measurable by microindentation with age, although Zioupos and Currey (1998) and Zimmermann et al. (2011) have shown deterioration of post-elastic and fracture mechanics properties by ageing using different methods. Therefore, macroscopic strength deterioration must be caused by effects other than ECM deterioration, e.g. increased crack or interface densities or a change in porosity.

Microscopic investigations at the tissue level have shown that mineral heterogeneity has only a minor effect on the mechanical behaviour of bone (Roschger et al., 2008; Gross et al., 2012; Spiesz et al., 2013). Furthermore, only a weak positive correlation between hardness (Boivin et al., 2008) as well as stiffness (Raum et al., 2006) and tissue mineralisation was found. Our results do not support such a relation as no indentation property was dependent on mineral content ρ_{MM} or crystallinity. This is in contrast with the results of Kim et al. (2014) where a weakly significant correlation between indentation modulus and the mineral to matrix ratio ρ_{MM} was found. However, the points discussed above also apply for the mineral content. On the other hand the indentations and the Raman analysis was not locally matched. It would be interesting to know if such a local one-to-one study yields a correlation. In fact, a confocal optic with a finite focus diameter of $0.8\text{ }\mu\text{m}$ was used. With a layer thickness of $78.3\text{ }\mu\text{m}$ a cylindrical interaction volume of $39\text{ }\mu\text{m}^3$ can be approximated. The actual interaction volume should be closer to a conic section. This interaction volume is smaller than the tested indentation volume which can be approximated as a semi-sphere with a diameter of $10\text{ }\mu\text{m}$ giving an interaction volume of approximately $261\text{ }\mu\text{m}^3$. Several Raman measurements per donor were averaged as in case of the indentation results. Therefore, the correlation between both methods should

532 be possible. The relatively small variation of micromechanical properties measured
 533 by microindentation with donor age in the course of this study further support the
 534 small impact of heterogeneous mineralisation. Finally, our Raman data shows that
 535 the mineralisation has a similar small variation. This means micromechanical prop-
 536 erties and mineral content are rather constant so that no correlation between the
 537 two can be found.

538 **Macromechanical properties** of human cortical bone such as elastic modulus,
 539 yield stress and yield strain, ultimate stress and strain for tension and compression
 540 as well as for torsion are well comparable to the literature (Carter et al., 1981;
 541 Pattin et al., 1996; Kotha and Guzelsu, 2003; Zioupos and Currey, 1998; Reilly and
 542 Burstein, 1975; Jepsen et al., 1999). Elastic modulus (E_0), yield stress ($\sigma_{0.05}$), yield
 543 strain ($\varepsilon_{0.05}$), strength (σ_u), and ultimate work W_{ult} were independent of gender.

544 Elastic modulus, strength and yield stress were significantly correlated to poros-
 545 ity, while yield strain in tension and compression were not. This is in line with earlier
 546 findings (Evans, 1976; Schaffler and Burr, 1988; Currey, 1988; Wachter et al., 2002;
 547 Bayraktar et al., 2004; Doblar et al., 2004; Hayes and Carter, 1976; Wachter et al.,
 548 2001b). However, the dependence on donor age was not as strong as expected (Mc-
 549 Calden et al., 1993; Zioupos and Currey, 1998; Currey, 2002). This may be due to
 550 the elderly donor cohort and ultimately attributed to the weak tendency of porosity
 551 with age in the investigated material.

552 Interestingly, the ratio between yield strain in tension and compression was 0.68
 553 for both the 0.05 % and the 0.2 % criterion. This was close to 0.66 found numerically
 554 on the tissue level for trabecular bone (Gross, 2014). In contrast to the tensile
 555 or compressive case, there was a significant correlation between yield strain and
 556 porosity in torsion. This may be caused by the interaction of the loading condition
 557 and the sample geometry. A higher porosity implies more sectioned osteons at the
 558 circumferential surface of the sample. This leads to the presence of weak interfaces
 559 and preexisting surface cracks in the most stressed area, which might lead to a

560 decrease of apparent yield strains compared to a pristine sample.

561 Strength in compression decreases much faster with porosity than in tension or
562 torsion. When comparing this to the data for yield stress, where this is not the case
563 (Figure 6), this indicates a potential artefact of the experimental conditions. For
564 both tension and torsion, the sample is aligned by the applied load. For compressive
565 loading, the system tends to become unstable after reaching yield. The appearance
566 of microcracks is accompanied by the localisation of the deformation in a pore driven
567 plastic hinge and a change from a homogeneous stress state over the cross-section
568 into a state of plastic buckling, which can lead to a decreased strength. This process
569 may be amplified by a higher porosity, as it can act as a stress concentrator and
570 decreases the lateral support of locally buckling regions.

571 We did not find correlations between any macromechanical properties and os-
572 teon number or diameter. This was unexpected since a correlation between osteon
573 diameter and work-to-fracture from a Chevron notch experiment (Moyle and Bow-
574 den, 1984) suggests such a dependence. Although morphology and composition of
575 the cement interface (Burr et al., 1988; Schaffler et al., 1987; Skedros et al., 2005)
576 and its possible mechanical role in fracture of osteonal bone as a local interface and
577 crack arrestor or stress concentrator (Nobakhti et al., 2014; Yeni and Norman, 2000;
578 Burr et al., 1988; Ebacher and Wang, 2009) has been widely debated, we did not
579 find a significant correlation between cement line density and macroscopic mechan-
580 ical properties either. This inconsistency maybe explained by the different kind of
581 testing utilised. The role of this interface has been investigated in fracture mechan-
582 ics experiments where notches are exploited to start macrocracks that are strongly
583 influenced by the cement interfaces (Nalla et al., 2003). Such a notch was miss-
584 ing in our experiment and failure started by development of many microcracks in
585 different regions of the sample that coalesced into macrocracks at a later stage of
586 the experiment only. This explains the lack of correlation of pre-failure properties
587 with interface density and indicates that the cement interface is important for crack

propagation and not initiation.

To correlate micromechanical, microstructural, chemical, and macroscopic mechanical properties we chose a multiplicative power law relationship based on the assumption that the chosen variables are independent. ρ represents the bone volume fraction, which has been shown to correlate with bone mechanical properties in the past. ρ_{CL} represents the fraction of weak interfaces, where microcracks can be both started and stopped during loading (Schaffler et al., 1987; Burr et al., 1988; Currey, 2002; Ebacher and Wang, 2009). ρ_{MM} represents the relative mineralisation of the ECM. Including micromechanical properties in the analysis is a natural choice aiming at predicting macroscopic mechanical properties. Thus, a combination of these variables in a power law offers the chance to span two scales and discriminate dominant features determining macroscopic mechanical properties. This follows earlier efforts to do so for the stiffness of cortical bone (Schaffler and Burr, 1988; Currey, 1988; Dong and Guo, 2004).

Neither the micromechanical properties, nor the cement line density, nor the mineralisation ρ_{MM} were significant parameters in the model for any mechanical property leaving only the intercept X_0 and the bone volume fraction ρ . Zysset et al. (1999) found a significant difference of E^* and H_{IT} between the femoral diaphysis and femoral neck. A reason could be a different lamellar setup or different remodelling rates in these two sites. Whether porosity and intercept are the only significant parameters in correlations of micromechanical, microstructural and macromechanical properties in other sites such as the neck needs to be shown by an own experiment which is difficult to perform due to the thin cortical shell.

Fitting the elastic modulus in tension and compression resulted in an intercept of 19.32 GPa. This is similar to the mean apparent modulus in axial direction E_{33} of 19.87 GPa obtained from fitting a transversely isotropic stiffness tensor to the indentation data (Appendix D, Figure 10). Similarly, the shear stiffness intercept of 6.71 GPa was found to be only slightly higher than the mean tissue shear modulus of

5.73 GPa identified from the stiffness tensor (Figure 10). Yerramshetty and Akkus (2008) associated mineral crystallinity and macroscopic stiffness of cortical bone and found a significant relation. This was not the case for our data. The results of the fatigue tests performed by Yerramshetty and Akkus (2008) may have been biased by preexisting microcracks since a scale effect exists in bone (Schwiedrzik et al., 2014).

In the case of strength and yield stress, the intercepts are larger than the mean values, as they reflect properties of a fictitious non-porous material. In case of compression, the intercept for strength is slightly higher than the value proposed by Carnelli et al. (2011) for ECM of bovine bone. As the properties reported by Carnelli et al. (2011) are inferred from back-calculation of microindentation data, they suffer from a potential lack of uniqueness and are not necessarily representative of the properties on the lamellar level. On the other hand, the intercept inferred from this study represents a poreless piece of material of macroscopic dimensions. This explains the large difference encountered when comparing it to the values obtained by micropillar compression experiments of ECM on the microscale (Schwiedrzik et al., 2014) demonstrating the presence of a considerable sample size effect in bone.

Interestingly, the ratio of indentation hardness H_{IT} to tissue yield stress σ_y^+ in tension is 5.76 and σ_y^- in compression 3.62. This ratio $H_{IT}/\sigma_y^\pm$ is called the constraint factor in the literature (Fischer-Cripps, 2002). It has been reported to be in the range of 1.5 for brittle materials like glasses (Fischer-Cripps, 2002), while for polycrystalline metals, which show pressure-independent yielding, it is a function of the plasticity index σ_y^-/E^* (Johnson, 1985; Fischer-Cripps, 2002). For a small plasticity index, the indentation is mostly plastic, which results in a constraint factor of 2.5 – 3, with 3 being an upper limit for materials following a von Mises criterion (Fischer-Cripps, 2002). However, for cohesive-frictional materials like bone (Tai et al., 2006) featuring a pressure-dependent yield point, the constraint factor H_{IT}/σ_y^- can reach values larger than 3 and depends on the friction angle as well as the elastic to total work ratio (Rodríguez et al., 2012). When using the constraint factor of 3.62 and

the elastic to total work ratio of 0.25 determined in this study for bone tested in the axial direction, it may be concluded that the friction angle of bone should be in the range of $30 - 36^\circ$. Earlier studies using a Mohr-Coulomb criterion reported a friction angle in the range of $12 - 18^\circ$ (Tai et al., 2006; Wang et al., 2008). The discrepancy of these results with the current study may be explained by the effect of specimen size on the yield and strength properties of bone, which has been shown experimentally recently using *in situ* micropillar compression (Schwiedrzik et al., 2014). This means that the constraint factor determined using hardness and yield stress determined on the microscale would be considerably lower leading to a smaller friction angle, which is consistent with Tai et al. (2006) and Wang et al. (2008).

It has to be noted that only 8 % of elastic modulus' variation in axial direction and 47 % of shear modulus' variation can be explained by porosity. Furthermore, porosity explains 24 %, 74 % and 49 % of the variation of strength in tension, compression and torsion, respectively. After accounting for porosity, coefficients of variation of between 5 and 13 % for stiffness, yield and ultimate stress and strain were found (Table 4), which is remarkably constant. However, in case of ultimate work in tension and compression coefficients of variation of 37 and 41 % were attained, respectively. This implies that macroscopic stiffness and strength were estimated with a relatively good approximation (maximally 13 % variation) with a measure of porosity for the whole population. This is not the case for mechanical work where the coefficients of variation remain high even after correction for porosity. This may be due to differences in the distribution of existing interfaces. According to Currey (2002) the variability in ultimate work is strongly influenced by the amount of cracks and the way and speed at which they travel through the tissue. The variability in the mechanical work reflects the load bearing capabilities up to failure including pre-existing submicroscopic defects. These defects are not detected during microindentation as the defects are located near interfaces, while indentations are performed in bulk material and the tested volume is very small.

When relating ultimate work and total indentation work, only X_0 was significant in tension, while in compression both X_0 and ρ were significant. Again, compression may lead to the localisation of the deformation in a plastic hinge which may lead to plastic buckling. This shifts the loading from a uniaxial compression to a bending state. This shift should be highly influenced by existing porosity and may explain the sensibility. The coefficient of variation for these models was 37 and 41 % which indicates a weak role of porosity in explaining the variation of these measures.

One could conclude that microindentation is mainly compressive and includes a large hydrostatic component. Therefore, it does not necessarily capture all relevant loading modes encountered in a macroscopic sample. However, the chosen correlations are a natural choice since it was shown that the ECM behaves in a plastic fashion (Schwiedrzik et al., 2014; Luczynski et al., 2015) and the variables should correlate unless the microstructure dominates the response. The poor correlations between the chosen variables indicate by means of elimination that macroscopic failure must be dominated by a structural feature. It was shown that the presence of defects could indeed explain the difference in material behaviour (Schwiedrzik et al., 2014). The authors proposed a model where the macroscale properties were given by a uniform distribution of defects and a normal distribution of the material properties at the ECM level. If the ECM properties vary slightly in this model, the overall macroscopic response is much less affected than due to the uniform distribution of the existing defects. Therefore, our results support the idea that pre-existing defects dominate failure and that sample specific microindentation experiments are not able to capture the individual failure point. At least one paper found a relation between macroscopic tensile toughness and preexisting microcracks (Norman et al., 1998). However, the relation was weak which could suggest that other factors are of importance that were not addressed in this study.

Nevertheless, recent developments in osteoporosis diagnostics exploit indentation strategies to discriminate individual patients at risk of fracture (Diez-Perez et al.,

2010). These indentations are an order of magnitude deeper than the ones performed in our study. The affected indentation volume features a semi-ellipsoid of approximately nine times the indentation depth under the tip and a radius of about seven times the indentation depth (Hengsberger et al., 2002). Therefore, these indentations create microcracks (Setters and Jasiuk, 2014) and are affected by lacuno-canalicular porosity, individual osteons, and cement lines which may explain the increased sensitivity of these measurements. However, based on the data reported in this study it may be concluded that microindentation experiments in the order of 1 μm indentation depth are not a good discriminator for conditions decreasing macroscopic bone strength in the elderly.

In conclusion, this exhaustive study consisting of multiple macro- and micromechanical tests, μCT , histomorphometry as well as Raman spectroscopy confirms that porosity is the major significant but often weak determinant of macroscopic tensile, compressive and torsional mechanical properties of human cortical bone. The compositional and indentation properties at the ECM level appear essentially independent of age and gender, but it should be emphasised that preexisting microdamage, bound water and collagen cross-links were not quantified in this study. The results suggest that another structural feature such as preexisting microdamage might play a decisive role in the post-yield and fracture behaviour of cortical bone.

The presented data can help to form a better understanding of the mechanisms through which and the length scale at which bone tissue is affected by ageing. This knowledge may be used for prediction of fracture risk in the elderly in the future. Furthermore, such knowledge may be helpful for the development of numerical tools to predict overall bone strength or the development of biomaterials mimicking the mechanical properties of bone tissue at several length scales.

A. Clinical Density Information

Dual energy X-ray absorptiometry (DEXA) data for each femur was obtained (Discovery QDR, Hologic Inc., USA) in an earlier study (Dall'Ara et al., 2013). Normal, osteopenic, and osteoporotic donors were identified based on the total femoral neck areal bone mineral density (aBMD) using cut-off values of 0.82 for a T-Score of -1 and 0.637 for a T-score of -2.5⁴. To investigate a possible impact of osteoporosis on endocortical porosity, we correlated the porosity measured by μ CT averaged for each donor with the BMD in the femoral neck.

aBMD of the femoral neck determined for each donor by DEXA was available and a significant correlation between aBMD and endocortical porosity was found ($R^2 = 0.18$, $p < 0.05$). However, the cohort contained osteoporotic bones with both very low and comparatively high cortical porosity (Figure 7).

Due to the increased surface to volume ratio and a higher remodelling rate (Eriksen et al., 1994), trabecular bone is more strongly affected by resorption and, thus, a faster bone loss would be expected compared to cortical bone (Brandi, 2009). Nevertheless, bone loss due to osteoporosis may lead to thinning of cortical tissue from the endosteal site. We produced specimens that were fully cortical and tried to avoid trabecularised tissue. This may be a further reason why osteoporotic donors with a low porosity were found. However, we also found normal donors with a high porosity in cortical bone. This may be explained by possible effects of personal lifestyle or general medical history of the donors, e.g. a lack of regular exercise, healthy nutrition, etc. Chemical composition as well as porosity was checked for each donor and did not indicate suspicious specimens.

Finally, an ANOVA between the microindentation properties and aBMD of the femoral neck revealed no significant dependence of E^* , H_{IT} , H_{IT}/E^* or W_{plast}/W_{tot}

⁴The analysis was performed according to <http://courses.washington.edu/bonephys/opbmd.html#tz> last visited March 27, 2015.

on the pathology. This means that osteopenia and osteoporosis do not affect the ECM micromechanical properties of cortical bone as measured by microindentation, at least in the femoral midshaft. Whether or not these properties are changed within the femoral neck cannot be deduced from the dataset of this study.

B. Histological Specimen Preparation

Bone samples were submerged in a 37 % formaldehyde fixation solution for 14 hours. Subsequently, they were rinsed 4 times with tap water for 60 minutes and dehydrated with an Ethanol series of 70 %, 80 %, 96 % and absolute Ethanol. During the process of dehydration, samples were placed on a shaker. Subsequently, samples were submerged in xylol under vacuum over night. The pieces were then transferred to a clean glass jar with the cutting side towards the bottom of the jar. Polymethyl-methacrylate (PMMA) was added to the jar and filled to approximately 20 mm above the sample. The solution consisted of 0.5 gr perkadox 16, 89.5 ml methyl methacrylate, and 10 ml dibutyl phthalate. The infiltration process took 1 day while the samples were placed under low vacuum at 4°C. During polymerisation, the samples were placed under a hood without further movement to avoid formation of bubbles. After polymerisation, the jars were transferred to an oven (UE400, Memmert GmbH + Co. KG, Germany) and cured at 60 °C for about 2 hours without a lid. At the end, samples were cooled down, the glasses were broken and embedded bone specimens were retrieved.

Bottom surfaces of the embedded undecalcified bones were flattened using an ultra-miller (Reichert-Jung Polycut E, Germany) and a transverse slice of approximately 1 mm thickness was cut using the band saw. The ultra-milled side of the slice was glued to a microscopy slide using cyanoacrylate. The other side of the slice was ultra-milled until a thickness between 70 to 130 µm was reached. On each carrier slide, three specimens were placed in a triangular shape. Milling was performed at a spindle speed of 750 rpm, a cutting speed of 1.5 mm/s, a feed of 100 µm in each

777 step, and 10 μm for the last two steps.

778 For the modified Von Kossa staining procedure we used the following protocol:

779 (i) etching with formic acid 0.25 % 10 min; (ii) 5 % silver nitrate solution under
780 bulb light 60 min; (iii) rinse in demineralised water 3x; (iv) 5 % Sodium carbonate-
781 formaldehyde 2 min; (v) rinse in running tap water 10 min; (vi) rinse in demineralised
782 water 1x; (vii) 1 % sodium thiosulfate 4 min; (viii) rinse in running tap water 5 min;
783 (ix) rinse in demineralised water 1x; (x) 1 % Nuclear Fast Red 5 min; (xi) rinse in
784 demineralised water 1x; (xii) rinse in 80 % Ethanol 1x; (xiii) rinse in 96 % Ethanol
785 1x; (xiv) rinse in absolute Ethanol 1x. An example on the results is given in Figure 8.

786 C. Raman Peak Evaluation

787 D. Tissue Stiffness Tensor

788 Conflict of interest statement

789 None.

790 Acknowledgements

791 The project was financially supported by the AO Foundation (AO S-12-13W).
792 The authors would like to thank Prof. Pretterklieber of the Department of Applied
793 Anatomy of the Medical University of Vienna for providing bone samples, Dr. Samantha
794 Chan and Ursula Maile for help with the histology, Mark Siegrist for help with
795 the μCT , Dr. Fabian Blank for providing access to the stereoscope microscope of the
796 ARTORG Center for Biomedical Engineering Research, Aurore Balmet for prepar-
797 ing the transverse indentation samples, Nadine Siegfried for help with the Raman
798 measurements, Alexander Bürki, Urs Rohrer, and the team of the ISTB workshop
799 for designing and machining parts needed for the experiments.

References

- Akkus, O., Adar, F., Schaffler, M. B., 2004. Age-related changes in physicochemical properties of mineral crystals are related to impaired mechanical function of cortical bone. *Bone* 34 (3), 443–453.
- Bain, S. D., Impeduglia, T. M., Rubin, C. T., 1990. Cement line staining in undecalcified thin sections of cortical bone. *Stain Technology* 65, 159 – 163.
- Bayraktar, H. H., Morgan, E. F., Niebur, G. L., Morris, G. E., Wong, E. K., Keaveny, T. M., 2004. Comparison of the elastic and yield properties of human femoral trabecular and cortical bone tissue. *Journal of Biomechanics* 37 (1), 27 – 35.
- Bernhard, A., Milovanovic, P., Zimmermann, E. A., Hahn, M., Djonic, D., Krause, M., Breer, S., Püschel, K., Djuric, M., Amling, M., Busse, B., 2013. Micro-morphological properties of osteons reveal changes in cortical bone stability during aging, osteoporosis, and bisphosphonate treatment in women. *Osteoporosis International*, 1–10.
- Boivin, G., Bala, Y., Doublier, A., Farlay, D., Ste-Marie, L. G., Meunier, P. J., Delmas, P. D., 2008. The role of mineralization and organic matrix in the microhardness of bone tissue from controls and osteoporotic patients. *Bone* 43 (3), 532–538.
- Boivin, G., Meunier, P. J., 2002. The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. *Calcified Tissue International* 70 (6), 503–511.
- Boyde, A., Jones, S. J., 1996. Scanning electron microscopy of bone: Enstrument, specimen, and issues. *Microscopy Research and Technique* 33 (2), 92–120.
- Brandi, M. L., 2009. Microarchitecture, the key to bone quality. *Rheumatology* 48 (suppl 4), iv3–iv8.
- Britz, H. M., Thomas, C. D. L., Clement, J. G., Cooper, D. M. L., 2009. The relation of femoral osteon geometry to age, sex, height and weight. *Bone* 45, 77 – 83.

- 827 Budyn, E., Jonvaux, J., Funfschilling, C., Hoc, T., 2012. Bovine cortical bone stiffness
828 and local strain are affected by mineralization and morphology. *Journal of Applied*
829 *Mechanics* 79, 011008–1 – 011008–12.
- 830 Burr, D. B., 1992. Estimated intracortical bone turnover in the femur of growing
831 macaques: Implications for their use as models in skeletal pathology. *Anatomical*
832 *Record* 232, 180 – 189.
- 833 Burr, D. B., Schaffler, M. B., Frederickson, R. G., 1988. Composition of the cement
834 line and its possible mechanical role as a local interface in human compact bone.
835 *Journal of Biomechanics* 21 (11), 939–945.
- 836 Carnelli, D., Gastaldi, D., Sassi, V., Contro, R., Ortiz, C., Vena, P., 2010. A finite
837 element model for direction-dependent mechanical response to nanoindentation of
838 cortical bone allowing for anisotropic post-yield behavior of the tissue. *Journal of*
839 *Biomechanical Engineering* 132 (8), 081008–1 – 081008–9.
- 840 Carnelli, D., Lucchini, R., Ponzoni, M., Contro, R., Vena, P., 2011. Nanoindentation
841 testing and finite element simulations of cortical bone allowing for anisotropic
842 elastic and inelastic mechanical response. *Journal of Biomechanics* 44 (10), 1852–
843 1858.
- 844 Carter, D. R., Caler, W. E., Spencler, D. M., Frankel, V. H., 1981. Fatigue behav-
845 ior of adult cortical bone: The Influence of mean strain and strain range. *Acta*
846 *Orthopaedica* 52 (1), 481 – 490.
- 847 Cooper, D. M., Thomas, C. D. L., Clement, J. G., Turinsky, A. L., Sensen, C. W.,
848 Hallgrímsson, B., 2007. Age-dependent change in the 3d structure of cortical poros-
849 ity at the human femoral midshaft. *Bone* 40, 957 – 965.
- 850 Courtney, A. C., Hayes, W. C., Gibson, L. J., 1996. Age-related differences in post-
851 yield damage in human cortical bone. Experiment and model. *Journal of Biome-*
852 *chanics* 29 (11), 1463 – 1471.
- 853 Crawley, M. J., 2005. *Statistics – An Introduction using R*. John Wiley and Sons,
854 Ltd.

- 855 Currey, J. D., 1969. The relationship between the stiffness and the mineral content
856 of bone. *Journal of Biomechanics* 2 (4), 477–480.
- 857 Currey, J. D., 1988. The effects of drying and re-wetting on some mechanical prop-
858 erties of cortical bone. *Journal of Biomechanics* 21 (5), 439–441.
- 859 Currey, J. D., 2002. *Bones: Structure and mechanics*. Princeton University Press.
- 860 Dall'Ara, E., Luisier, B., Schmidt, R., Pretterklieber, M., Kainberger, F., Zysset,
861 P. K., Pahr, D., 2013. Dxa predictions of human femoral mechanical properties
862 depend on the load configuration. *Medical Engineering and Physics* 35 (11), 1564–
863 1572.
- 864 Diez-Perez, A., Güerri, R., Nogues, X., Cáceres, E., Pena, M. J., Mellibovsky, L.,
865 Randall, C., Bridges, D., Weaver, J. C., Proctor, A., Brimer, D., Koester, K. J.,
866 Ritchie, R. O., Hansma, P. K., 2010. Microindentation for in vivo measurement
867 of bone tissue mechanical properties in humans. *Journal of Bone and Mineral*
868 *Research* 25 (8), 1877–1885.
- 869 Doblar, M., Garc, J. M., Gomez, M. J., 2004. Modelling bone tissue fracture and
870 healing: A review. *Engineering Fracture Mechanics* 71, 1809 – 1840.
- 871 Dong, X. N., Guo, X. E., 2004. The dependence of transversely isotropic elasticity
872 of human femoral cortical bone on porosity. *Journal of Biomechanics* 37, 1281 –
873 1287.
- 874 Ebacher, V., Wang, R., 2009. A unique microcracking process associated with the
875 inelastic deformation in haversian bone. *Advanced Functional Materials* 19 (1), 57
876 – 66.
- 877 Eriksen, E. F., Axelrod, D. W., Melsen, F., 1994. *Bone histomorphometry*. Raven
878 Press.
- 879 Evans, F. G., 1976. Mechanical properties and histology of cortical bone from younger
880 and older men. *Anatomical Record* 185, 1 – 12.
- 881 Fischer-Cripps, A. C., 2002. *Nanoindentation*. Springer-Verlag.

- 882 Franzoso, G., Zysset, P. K., 2009. Elastic anisotropy of human cortical bone sec-
883 ondary osteons measured by nanoindentation. *Journal of Biomechanical Engineer-*
884 *ing* 131, 021001–1 – 021001–11.
- 885 Fratzl, P., Weinkamer, R., 2007. Nature’s hierarchical materials. *Progress in Mate-*
886 *rials Science* 52 (8), 1263 – 1334.
- 887 Gamsjaeger, S., Buchinger, B., Zwettler, E., Recker, R., Black, D., Gasser, J. A.,
888 Eriksen, E. F., Klaushofer, K., Paschalis, E. P., 2011. Bone material properties
889 in actively bone-forming trabeculae in postmenopausal women with osteoporosis
890 after three years of treatment with once-yearly zoledronic acid. *Journal of Bone*
891 *and Mineral Research* 26 (1), 12–18.
- 892 Gamsjaeger, S., Hofstetter, B., Fratzl-Zelman, N., Roschger, P., Roschger, A., Fratzl,
893 P., Brozek, W., Masic, A., Misof, B. M., Glorieux, F. H., Klaushofer, K., Rauch, F.,
894 Paschalis, E. P., 2014. Pediatric reference raman data for material characteristics
895 of iliac trabecular bone. *Bone* 69, 89–97.
- 896 Giraud-Guille, M. M., 1988. Twisted plywood architecture of collagen fibrils in human
897 compact bone osteons. *Calcified Tissue International* 42 (3), 167–180.
- 898 Gross, T., 2014. Development and application of 3d ct image-based micro and macro
899 finite element models for human bones and orthopedic implant systems. Ph.D.
900 thesis, Vienna University of Technology.
- 901 Gross, T., Pahr, D. H., Peyrin, F., Zysset, P. K., 2012. Mineral heterogeneity has
902 a minor influence on the apparent elastic properties of human cancellous bone:
903 a sruct-based finite element study. *Computer Methods in Applied Mechanics and*
904 *Engineering* 15 (11), 1137 – 1144.
- 905 Harrigan, T. P., Mann, R. W., 1984. Characterisation of microstructural anisotropy
906 in orthotropic materials using a second rank tensor. *Journal of Materials Science*
907 19, 761 – 767.
- 908 Havill, L. M., 2004. Osteon remodeling dynamics in macaca mulatta: Normal varia-
909 tion with regard to age, sex, and skeletal maturity. *Calcified Tissue International*

- 910 74, 95 – 102.
- 911 Hayes, W. C., Carter, D. R., 1976. Postyield behavior of subchondral trabecular
912 bone. *Journal of Biomedical Materials Research Symposium* 10 (4), 537 – 544.
- 913 He, Q.-C., Curnier, A., 1995. A more fundamental approach to damaged elastic
914 stress-strain relations. *International Journal of Solids and Structures* 32 (10), 1433
915 – 1457.
- 916 Hengsberger, S., Enstroem, J., Peyrin, F., Zysset, P. K., 2003. How is the indentation
917 modulus of bone tissue related to its macroscopic elastic response? A validation
918 study. *Journal of Biomechanics* 36 (10), 1503 – 1509.
- 919 Hengsberger, S., Kulik, A., Zysset, P. K., 2002. Nanoindentation discriminates the
920 elastic properties of individual human bone lamellae under dry and physiological
921 conditions. *Bone* 30 (1), 178 – 184.
- 922 Hildebrand, T., Laib, A., Müller, R., Dequeker, J., Rüeggsegger, P., 1999. Direct three-
923 dimensional morphometric analysis of human cancellous bone: Microstructural
924 data from spine, femur, iliac crest and calcaneus. *Journal of Bone and Mineral
925 Research* 14 (7), 1167 – 1174.
- 926 Hoc, T., Henry, L., Verdier, M., Aubry, D., Sedel, L., Meunier, A., Apr 2006. Effect
927 of microstructure on the mechanical properties of haversian cortical bone. *Bone*
928 38 (4), 466–474.
- 929 Hoffer, C. E., Guo, X. E., Zysset, P. K., Goldstein, S. A., 2005. An application of
930 nanoindentation technique to measure bone tissue lamellae properties. *Journal of
931 Biomechanical Engineering* 127 (7), 1046 – 1053.
- 932 Hoffer, C. E., Moore, K. E., Kozloff, K., Zysset, P. K., Brown, M. B., Goldstein,
933 S. A., 2000. Heterogeneity of bone lamellar-level elastic moduli. *Bone* 26 (6), 603
934 – 609.
- 935 Holzer, G., von Skrbensky, G., Holzer, L. A., Pichl, W., 2009. Hip fractures and the
936 contribution of cortical versus trabecular bone to femoral neck strength. *Journal
937 of Bone and Mineral Research* 24 (3), 468 – 474.

- James, T. M., Schlösser, M., Lewis, R. J., Fischer, S., Bornschein, B., Telle, H. H.,
2012. Automated quantitative spectroscopic analysis combining background subtraction, cosmic-ray removal and peak fitting. *Applied Spectroscopy*.
- Jepsen, K. J., Davy, D. T., Krzypow, D. J., 1999. The role of the lamellar interface during torsional yielding of human cortical bone. *Journal of Biomechanics* 32 (3), 303 – 10.
- Johnson, K. L., 1985. *Contact mechanics*. Cambridge University Press, Cambridge.
- Jones, E., Oliphant, T., Peterson, P., et al., 2001. SciPy: Open source scientific tools for Python. [Online; accessed 2015-05-11].
- Kerley, E. R., 1965. The microscopic determination of age in human bone. *American Journal of Physical Anthropology* 23 (2), 149–163.
- Kim, G., Cole, J. H., Boskey, A. L., Baker, S. P., van der Meulen, M. C., 2014. Reduced tissue-level stiffness and mineralization in osteoporotic cancellous bone. *Calcified Tissue International* 95 (2), 125–131.
- Kotha, S. P., Guzelsu, N., 2003. Tensile damage and its effects on cortical bone. *Journal of Biomechanics* 36 (1), 1683 – 1689.
- Lees, S., Tao, N.-J., Lindsay, S. M., 1990. Studies of compact hard tissues and collagen by means of brillouin light scattering. *Connective Tissue Research* 24 (3-4), 187–205.
- Leng, H., Dong, X. N., Wang, X., 2009. Progressive post-yield behavior of human cortical bone in compression for middle-aged and elderly groups. *Journal of Biomechanics* 42, 491 – 497.
- Lewis, G., Nyman, J. S., 2008. The use of nanoindentation for characterizing the properties of mineralized hard tissues: State-of-the art review. *Journal of Biomedical Materials Research B* 288 – 303 (1), 87.
- Luczynski, K. W., Steiger-Thirsfeld, A., Bernardi, J., Eberhardsteiner, J., Hellmich, C., 2015. Extracellular bone matrix exhibits hardening elastoplasticity and more than double cortical strength: Evidence from homogeneous compression of non-

- 966 tapered single micron-sized pillars welded to a rigid substrate. *Journal of the Me-*
967 *chanical Behavior of Biomedical Materials*.
- 968 Malo, M., Rohrbach, D., Isaksson, H., Töyräs, J., Jurvelin, J., Tamminen, I., Kröger,
969 H., Raum, K., 2013. Longitudinal elastic properties and porosity of cortical bone
970 tissue vary with age in human proximal femur. *Bone* 53 (2), 451–458.
- 971 Martin, B., 1993. Aging and strength of bone as a structural material. *Calcified*
972 *Tissue International* 53 (1), S34–S40.
- 973 Martin, R., 1984. Porosity and specific surface of bone. *Critical Reviews in Biomed-*
974 *ical Engineering* 10 (3), 179–222.
- 975 Martin, R. B., Burr, D. B., Schaffler, M. B., 1985. Effects of age and sex on the
976 amount and distribution of mineral in eskimo tibiae. *American Journal of Physical*
977 *Anthropology* 67, 371–380.
- 978 McCalden, R. W., McGeough, J. A., Barker, M. B., Court-Brown, C. M., 1993. Age-
979 related changes in the tensile properties of cortical bone. The relative importance
980 of changes in porosity, mineralization, and microstructure. *Journal of Bone and*
981 *Joint Surgery American Edition* 75 (8), 1193 – 1205.
- 982 McCreddie, B. R., Morris, M. D., Chen, T.-C., Rao, D. S., Finney, W. F., Widjaja,
983 E., Goldstein, S. A., 2006. Bone tissue compositional differences in women with
984 and without osteoporotic fracture. *Bone* 39 (6), 1190–1195.
- 985 Meijering, E. H. W., Niessen, W. J., Viergever, M. A., 2001. Quantitative evalua-
986 tion of convolution-based methods for medical image interpolation. *Medical Image*
987 *Analysis* 5, 111 – 126.
- 988 Mirzaali, M., Bürki, A., Schwiedrzik, J. J., Zysset, P. K., Wolfram, U., 2015. Con-
989 tinuum damage interactions between tension and compression in osteonal bone.
990 *Journal of the Mechanical Behavior of Biomedical Materials* (49), 355 – 369.
- 991 Montalbano, T., Feng, G., 2011. Nanoindentation characterization of the cement
992 lines in ovine and bovine femurs. *Journal of Materials Science* 26, 1036 – 1041.
- 993 Morris, M. D., Mandair, G. S., 2011. Raman assessment of bone quality. *Clinical*

- Orthopaedics and Related Research 469 (8), 2160–2169.
- Moyle, D. D., Bowden, R. W., 1984. Fracture of human femoral bone. *Journal of Biomechanics* 17 (3), 203 – 213.
- Mullins, L. P., Bruzzi, M. S., McHugh, P. E., 2009. Calibration of a constitutive model for the post-yield behaviour of cortical bone. *Journal of the Mechanical Behavior of Biomedical Materials* 2 (5), 460–470.
- Nadai, A., 1950. Torsion of a round bar. The stress-strain curve in shear. McGraw-Hill Book Company, Inc. New York, Toronto and London, Ch. 21, pp. 347 – 352.
- Najafi, A. R., Arshi, A. R., Eslami, M. R., Fariborz, S., Moeinzadeh, M. H., 2007. Micromechanics fracture in osteonal cortical bone: A study of the interactions between microcrack propagation, microstructure and the material properties. *Journal of Biomechanics* 40 (12), 2788–2795.
- Nalla, R. K., Kinney, J. H., Ritchie, R. O., 2003. Mechanistic fracture criteria for the failure of human cortical bone. *Nature Materials* 2, 164 – 168.
- Nobakhti, S., Limbert, G., Thurner, J. J., 2014. Cement lines and interlamellar areas in compact bone as strain amplifiers—contributors to elasticity, fracture toughness and mechanotransduction. *Journal of the Mechanical Behavior of Biomedical Materials* 29, 235–251.
- Norman, T. L., Yeni, Y. N., Brown, C. U., Wang, Z., 1998. Influence of microdamage on fracture toughness of the human femur and tibia. *Bone* 23 (3), 303–306.
- Oliver, W., Pharr, G., 1992. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *Journal of Materials Science* 7 (6), 1564 – 1583.
- Ortner, D. J., 1975. Aging effects on osteon remodeling. *Calcified Tissue Research* 18, 26 – 36.
- Parfitt, A., 1984. Age-related structural changes in trabecular and cortical bone: Cellular mechanisms and biomechanical consequences. *Calcified Tissue International* 36, S123–S128, 10.1007/BF02406145.

- 1022 Patsch, J. M., Burghardt, A. J., Yap, S. P., Baum, T., Schwartz, A. V., Joseph, G. B.,
1023 Link, T. M., 2013. Increased cortical porosity in type 2 diabetic postmenopausal
1024 women with fragility fractures. *Journal of Bone and Mineral Research* 28 (2), 313–
1025 324.
- 1026 Pattin, C. A., Caler, W. E., Carter, D. R., 1996. Cyclic mechanical property degra-
1027 dation during fatigue loading of cortical bone. *Journal of Biomechanics* 29, 69 –
1028 79.
- 1029 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2015. nlme: Linear
1030 and nonlinear mixed effects models. R package version 3.1-120.
- 1031 Poundarik, A. A., Diab, T., Sroga, G. E. and Ural, m. A., Boskey, A. L., Gundberg,
1032 C. M., Vashishth, D., 2012. Dilatational band formation in bone. *Proceedings of*
1033 *the National Academy of Sciences* 109 (47), 19178 – 19183.
- 1034 R Development Core Team, 2008. R: A Language and Environment for Statistical
1035 Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-
1036 900051-07-0.
- 1037 Raum, K., Cleveland, R. O., Peyrin, F., Laugier, P., 2006. Derivation of elastic
1038 stiffness from site-matched mineral density and acoustic impedance maps. *Physics*
1039 *in Medicine and Biology* 51 (3), 747.
- 1040 Reilly, D. T., Burstein, A. H., 1975. The elastic and ultimate properties of compact
1041 bone tissue. *Journal of Biomechanics* 8 (6), 393 – 405.
- 1042 Reisinger, A. G., Pahr, D., K., Z. P., 2011. Principal stiffness orientation and degree
1043 of anisotropy of human osteons based on nanoindentation in three distinct planes.
1044 *Journal of the Mechanical Behavior of Biomedical Materials* 4 (8), 2113 – 27.
- 1045 Reznikov, N., Shahar, R., Weiner, S., 2014. Three-dimensional structure of human
1046 lamellar bone: The presence of two different materials and new insights into the
1047 hierarchical organization. *Bone* 59, 93–104.
- 1048 Rho, J. Y., Hobato, M. C., Ashman, R. B., 1995. Relations of mechanical properties
1049 to density and ct numbers in human bone. *Medical Engineering and Physics* 5,

- 1050 347 – 355.
- 1051 Rho, J. Y., Kuhn-Spearing, L., Zioupos, P., 1998. Mechanical properties and the
1052 hierarchical structure of bone. *Medical Engineering and Physics* 20 (2), 92 – 102.
- 1053 Rho, J.-Y., Tsui, T. Y., Pharr, G. M., 1997. Elastic properties of human cortical
1054 and trabecular lamellar bone measured by nanoindentation. *Biomaterials* 20 (18),
1055 1325 – 1330.
- 1056 Rho, J. Y., Zioupos, P., Currey, J., Pharr, G., 2002. Microstructural elasticity and
1057 regional heterogeneity in human femoral bone of various ages examined by nano-
1058 indentation. *Journal of Biomechanics* 35, 189 – 198.
- 1059 Ridler, T. W., Calvard, S., August 1978. Picture thresholding using an iterative
1060 selection method. *IEEE Transactions on Systems, Man, and Cybernetics SMC-*
1061 *8* (8), 630–632.
- 1062 Robertson, D. M., Smith, D. C., 2000. Compressive strength of mandibular bone as
1063 a function of microstructure of bone. *Journal of Biomechanics* 11, 455 – 471.
- 1064 Rodríguez, M., Molina-Aldareguía, J. M., González, C., LLorca, J., 2012. Determi-
1065 nation of the mechanical properties of amorphous materials through instrumented
1066 nanoindentation. *Acta Materialia* 60 (9), 3953–3964.
- 1067 Roschger, P., Paschalis, E. P., Fratzl, P., Klaushofer, K., 2008. Bone mineralization
1068 density distribution in health and disease. *Bone* 42, 456 – 466.
- 1069 Schaffler, M. B., Burr, D. B., 1984. Primate cortical bone microstructure: relation-
1070 ship to locomotion. *American Journal of Physical Anthropology* 65, 191–197.
- 1071 Schaffler, M. B., Burr, D. B., 1988. Stiffness of compact bone: Effect of porosity and
1072 density. *Journal of Biomechanics* 21, 13 – 16.
- 1073 Schaffler, M. B., Burr, D. B., G., F. R., 1987. Morphology of the osteonal cement
1074 line in human bone. *Anatomical Record* 217, 223–228.
- 1075 Schaffler, M. B., Radin, E. L., Burr, D. B., 1990. Long-term fatigue behavior of
1076 compact bone at low strain magnitude and rate. *Bone* 11 (5), 321 – 326.

- 1077 Schwiedrzik, J., Raghavan, R., Bürki, A., LeNader, V., Wolfram, U., Michler, J., Zys-
1078 set, P. K., 2014. In situ micropillar compression of lamellar bone reveals superior
1079 strength and ductility but no damage. *Nature Materials* 13 (7), 740 – 747.
- 1080 Schwiedrzik, J. J., Zysset, P. K., 2015. Quantitative analysis of imprint shape and its
1081 relation to mechanical properties measured by microindentation in bone. *Journal*
1082 *of Biomechanics* 48, 210 – 216.
- 1083 Seeman, E., Delmas, P. D., 2006. Bone quality – the material and structural basis
1084 of bone strength and fragility. *New England Journal of Medicine* 354 (21), 2250 –
1085 2261.
- 1086 Setters, A., Jasiuk, I., 2014. Towards a standardized reference point indentation
1087 testing procedure. *Journal of the Mechanical Behavior of Biomedical Materials* 34,
1088 57 – 65.
- 1089 Sevostianov, I., Kachanov, M., 2000. Impact of the porous microstructure on the
1090 overall elastic properties of the osteonal cortical bone. *Journal of Biomechanics*
1091 33 (7), 881–888.
- 1092 Shapiro, S. S., Wilk, M. B., 1965. An analysis of variance test for normality (complete
1093 samples). *Biometrika* 52 (3-4), 591 – 611.
- 1094 Skedros, J. G., Holmes, J. L., Vajda, E. G., Bloebaum, R., 2005. Cement lines
1095 of secondary osteons in human bone are not mineral-deficient: New data in a
1096 historical perspective. *Anatomical Record A* 286A, 781 – 803.
- 1097 Spiesz, E., 2011. Experimental and computational micromechanics of mineralized
1098 tendon and bone. Ph.D. thesis, Ph. D. thesis, Vienna University of Technology.
- 1099 Spiesz, E. M., Reisinger, A. G., Kaminsky, W., Roschger, P., Pahr, D. H., Zysset,
1100 P. K., 2013. Computational and experimental methodology for site-matched inves-
1101 tigations of the influence of mineral mass fraction and collagen orientation on the
1102 axial indentation modulus of lamellar bone. *Journal of the Mechanical Behavior*
1103 *of Biomedical Materials* 28, 195–205.
- 1104 Spiesz, E. M., Roschger, P., Zysset, P. K., 2012. Elastic anisotropy of uniaxial miner-

- 1105 alized collagen fibers measured using two-directional indentation. effects of hydra-
1106 tion state and indentation depth. *Journal of the Mechanical Behavior of Biomedical*
1107 *Materials* 12, 20–28.
- 1108 Swadener, J. G., Pharr, G. M., 2001. Indentation of elastically anisotropic half-spaces
1109 by cones and parabolae of revolution. *Philosophical Magazine* 81, 447 – 466.
- 1110 Tai, K., Ulm, F.-J., Ortiz, C., 2006. Nanogranular origins of the strength of bone.
1111 *Nano Letters* 6 (11), 2520–2525.
- 1112 Thomas, C. D. L., Feik, S. A., Clement, J. G., 2005. Regional variation of intracortical
1113 porosity in the midshaft of the human femur: age and sex differences. *Journal of*
1114 *Anatomy* 206, 115 – 125.
- 1115 Thévenaz, P., Ruttimann, U. E., Unser, M., 1998. A pyramid approach to subpixel
1116 registration based on intensity. *IEEE Transactions on Image Processing* 7, 27 – 41.
- 1117 Treece, G. M., Gee, A. H., Mayhew, P. M., Poole, K. E., 2010. High resolution cortical
1118 bone thickness measurement from clinical ct data. *Medical Image Analysis* 14 (3),
1119 276 – 290.
- 1120 Varga, P., Pacureanu, A., Langer, M., Suhonen, H., Hesse, B., Grimal, Q., Cloetens,
1121 P., Raum, K., Peyrin, F., 2013. Investigation of the 3D orientation of mineral-
1122 ized collagen fibrils in human lamellar bone using synchrotron x-ray phase nano-
1123 tomography. *Acta Biomaterialia* 9, 8118 – 8127.
- 1124 Vlassak, J. J., Nix, W. D., 1994. Measuring the elastic properties of anisotropic
1125 materials by means of indentation experiments. *Journal of the Mechanics and*
1126 *Physics of Solids* 42 (8), 1223–1245.
- 1127 Wachter, N. J., Augat, P., Krischak, G. D., Mentzel, M., Kinzl, L., Claes, L., 2001a.
1128 Prediction of cortical bone porosity in vitro by microcomputed tomography. *Cal-*
1129 *cified Tissue International* 68, 38 – 42.
- 1130 Wachter, N. J., Augat, P., Krischak, G. D., R., S. M., Mentzel, M., Kinzl, L.,
1131 Claes, L., 2001b. Prediction of strength of cortical bone in vitro by microcomputed
1132 tomography. *Clinical Biomechanics* 16, 252 – 256.

- 1133 Wachter, N. J., Krischak, G. D., Mentzel, M., Sarkar, M. R., Ebinger, T., Kinzl, L.,
1134 Claes, L., Augat, P., 2002. Correlation of bone mineral density with strength and
1135 microstructural parameters of cortical bone in vitro. *Bone* 31, 90 – 95.
- 1136 Wang, X., Allen, M. R., Burr, D. B., Lavernia, E. J., Jeremić, B., Fyhrie, D. P., 2008.
1137 Identification of material parameters based on mohr–coulomb failure criterion for
1138 bisphosphonate treated canine vertebral cancellous bone. *Bone* 43 (4), 775–780.
- 1139 Weiner, S., Arad, T., Sabanay, I., Traub, W., 1997. Rotated plywood structure of
1140 primary lamellar bone in the rat: Orientations of the collagen fibril arrays. *Bone*
1141 20 (6), 6.
- 1142 Weiner, S., Wagner, H. D., 1998. The material bone: structure-mechanical function
1143 relations. *Annual Review of Materials Science* 28 (1), 271–298.
- 1144 Wolfram, U., Wilke, H.-J., Zysset, P. K., 2010a. Rehydration of vertebral trabecular
1145 bone: Influences on its anisotropy, its stiffness and the indentation work with a
1146 view to age, gender and vertebral level. *Bone* 46 (2), 348 – 354.
- 1147 Wolfram, U., Wilke, H.-J., Zysset, P. K., 2010b. Transverse isotropic elastic proper-
1148 ties of vertebral trabecular bone matrix measured using microindentation under
1149 dry conditions (effects of age, gender and vertebral level). *Journal of Mechanics in*
1150 *Medicine and Biology* 10 (1), 139 – 150.
- 1151 Wolfram, U., Wilke, H.-J., Zysset, P. K., 2010c. Valid μ finite element models of
1152 vertebral trabecular bone can be obtained using tissue properties measured with
1153 nanoindentation under wet conditions. *Journal of Biomechanics* 43 (9), 1731 –
1154 1737.
- 1155 Wu, Z., Ovaert, T. C., Niebur, G. L., 2012. Viscoelastic properties of human cor-
1156 tical bone tissue depend on gender and elastic modulus. *Journal of Orthopaedic*
1157 *Research* 30 (5), 693–699.
- 1158 Yeni, Y. N., Norman, T. L., 2000. Calculation of porosity and osteonal cement line
1159 effects on the effective fracture toughness of cortical bone in longitudinal crack
1160 growth. *Journal of Biomedical Materials Research* 51 (3), 504–509.

- 1161 Yerramshetty, J. S., Akkus, O., 2008. The associations between mineral crystallinity
1162 and the mechanical properties of human cortical bone. *Bone* 42 (3), 476–482.
- 1163 Yerramshetty, J. S., Lind, C., Akkus, O., 2006. The compositional and physicochem-
1164 ical homogeneity of male femoral cortex increases after the sixth decade. *Bone*
1165 39 (6), 1236–1243.
- 1166 Zebaze, R. M., Ghasem-Zadeh, A., Bohte, A., Iuliano-Burns, S., Mirams, M., Price,
1167 R. I., Mackie, E. J., Seeman, E., 2010. Intracortical remodelling and porosity in the
1168 distal radius and post-mortem femurs of women: A cross-sectional study. *Lancet*
1169 375 (9727), 1672 – 1673.
- 1170 Zhang, W., Tekalur, S. A., Baumann, M., McCabe, L. R., 2013. The effects of
1171 damage accumulation on the tensile strength and toughness of compact bovine
1172 bone. *Journal of Biomechanics* 46, 946 – 972.
- 1173 Zimmermann, E. A., Schaible, E., Bale, H., Barth, H. D., Tang, S. Y., Reichert, P.,
1174 Busse, B., Alliston, T., Ager III, J. W., Ritchie, R. O., 2011. Age-related changes
1175 in the plasticity and toughness of human cortical bone at multiple length scales.
1176 *Proceedings of the National Academy of Sciences* 108 (35), 14416 – 14421.
- 1177 Zioupos, P., 2001. Ageing human bone: Factors affecting its biomechanical properties
1178 and the role of collagen. *Journal of Biomaterials Applications* 15 (3), 187–229.
- 1179 Zioupos, P., Currey, J. D., 1998. Changes in the stiffness, strength, and toughness of
1180 human cortical bone with age. *Bone* 22 (1), 57 – 66.
- 1181 Zioupos, P., Richard, B. C., Hutchinson, J. R., 2008. Some basic relationships be-
1182 tween density values in cancellous and cortical bone. *Journal of Biomechanics* 41,
1183 1961 – 1968.
- 1184 Zysset, P. K., 2009. Indentation of bone tissue: A short review. *Osteoporosis Inter-*
1185 *national* 20 (6), 1049–1055.
- 1186 Zysset, P. K., Guo, E. X., Hoffer, E. C., Moore, K. E., Goldstein, S. A., 1999.
1187 Elastic modulus and hardness of cortical and trabecular bone lamellae measured
1188 by nanoindentation in the human femur. *Journal of Biomechanics* 32 (10), 1005 –

1189

1012.

ACCEPTED MANUSCRIPT

Figure 1: The study overview illustrates the combination of the different experimental techniques used on different length scales.

Figure 2: Raman analyses of the microindentation samples showed no significant dependence on donor age or gender for the relative amount of mineral and its maturity/crystallinity.

Figure 3: Porosity $1 - \rho$ (top left) and histological cement line density ρ_{CL} (top right) showed a significant correlation with age while osteon diameter d_{osteon} (bottom left) and the number of osteons n_{osteon} (bottom right) did not. Solid circles represent male, hollow circles female donors. As morphological properties are independent of gender, the regression analysis was performed on the pooled dataset.

Figure 4: Donor averaged indentation measurements of axial osteonal (dark green), axial interstitial (blue violet), and transverse samples (brown) as a function of age. Solid lines indicate a significant correlation.

Figure 5: Distribution of macromechanical properties as a function of donor age. Elastic modulus for uniaxial tension (UT) and uniaxial compression (UC) are pooled (black points). Loading modes are distinguished by colour: red for UT, blue for UC, and green for torsion (TR). No significant difference between male (solid circles) and female donors (hollow circles) was found.

Figure 6: Distribution of macromechanical properties as a function of porosity. Elastic modulus for uniaxial tension (UT) and uniaxial compression (UC) are pooled (black points). Loading modes are distinguished by colour: red for UT, blue for UC, and green for torsion (TR). No significant difference between male (solid circles) and female donors (hollow circles) was found.

Figure 7: Areal BMD in the proximal femur as measured by dual energy X-ray absorptiometry is negatively correlated to endocortical porosity from the diaphysis of the same femurs. Dashed vertical lines illustrate the cut-off values to identify normal (T-Score > -1), osteopenic (-1 > T-Score > -2.5), and osteoporotic (-2.5 > T-Score) donors.

Figure 8: Transverse cross section of cortical bone of a 96 year old female donor before (top row) and after staining (bottom row). The section has a thickness of 100 μm .

Figure 9: figure

The exemplary band fits shown for one spectrum of a 60 year old male donor illustrate how the Gaussian functions were fitted to evaluate the $\nu_1\text{PO}_4$ and the amide I band. Note that the left shoulders in the $\nu_1\text{PO}_4$ distribution are avoided by including only those points right of the full width half maximum in the fitting.

Figure 10: figure

Visualisation of the transverse isotropic (five independent constants) tissue stiffness tensor $\mathbb{S}$ for human cortical bone. The tensor was obtained by fitting the average axial and transverse plane strain moduli measured by indentation using the method of Franzoso and Zysset (2009). The shape of the ellipsoid represents the elongation modulus and the colour coding depicts the bulk modulus (He and Curnier, 1995; Wolfram et al., 2010a).

Statistical Analyses:

- microindentation tests vs. Raman data
- macroscopic mechanical tests vs. microindentation + μ CT + histology + Raman data

Key Findings:

- tissue microindentation properties independent of age, with minor exceptions gender, and mineralisation
- microindentation experiments predict macroscopic mechanical properties only on average and not on one to one basis

Highlights

multiple macro- and micromechanical tests, μ CT, histomorphometry, Raman spectroscopy

porosity major significant but often weak determinant of macroscopic mechanical properties

compositional and indentation properties at ECM level appear essentially independent of age and gender

microindentation predicts macroscopic mechanical properties only on average not on one to one basis