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Change in porosity is the major determinant of the variation of cortical bone elasticity at the millimeter scale in aged women

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1 **Change in porosity is the major determinant of the variation of** 2 **cortical bone elasticity at the millimeter scale in aged women**

3 4 5 **Abstract**

6
7 At the mesoscale (i.e. over a few millimeters), cortical bone can be described as two-phase
8 composite material consisting of pores and a dense mineralized matrix. The cortical
9 porosity is known to influence the mesoscopic elasticity. Our objective was to determine
10 whether the variations of porosity are sufficient to predict the variations of bone
11 mesoscopic anisotropic elasticity or if change in bone matrix elasticity is an important
12 factor to consider. We measured 21 cortical bone specimens prepared from the mid-
13 diaphysis of 10 women donors (aged from 66 to 98 years). A 50-MHz scanning acoustic
14 microscope (SAM) was used to evaluate the bone matrix elasticity (reflected in impedance
15 values) and porosity. Porosity evaluation with SAM was validated against Synchrotron
16 Radiation μ CT measurements. A standard contact ultrasonic method was applied to
17 determine the mesoscopic elastic coefficients. Only matrix impedance in the direction of
18 the bone axis correlated to mesoscale elasticity (adjusted $R^2 = [0.16 - 0.25]$, $p < 0.05$). The
19 mesoscopic elasticity was found to be highly correlated to the cortical porosity (adj- $R^2 =$
20 $[0.72 - 0.84]$, $p < 10^{-5}$). Multivariate analysis including both matrix impedance and porosity
21 did not provide a better statistical model of mesoscopic elasticity variations. Our results
22 indicate that, for the elderly population, the elastic properties of the mineralized matrix do
23 not undergo large variations among different samples, as reflected in the low coefficients of
24 variation of matrix impedance (less than 6%). This work suggests that change in the
25 intracortical porosity accounts for most of the variations of mesoscopic elasticity, at least
26 when the analyzed porosity range is large (3-27% in this study). The trend in the variation
27 of mesoscale elasticity with porosity is consistent with the predictions of a
28 micromechanical model consisting of an anisotropic matrix pervaded by cylindrical pores.
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32

33 **Keywords:**

- 34
- 35 - anisotropic elasticity
- 36 - cortical porosity
- 37 - mechanical model
- 38 - scanning acoustic microscopy
- 39 - ultrasound
- 40
- 41
- 42
- 43

1 **Introduction**

2 Bones of different individuals not only have different sizes and shapes, but also different
3 material properties. These characteristics entirely determine the elastic response of a bone
4 to a given mechanical loading. The elastic properties of cortical bone tissue, which has a
5 hierarchical organization, must be described in a multiscale framework: the structure and
6 mechanical properties at one hierarchical level determine the properties of the subsequent
7 one. The *mesoscale* designates the intermediate scale between the microscale (lamellar
8 structures) and the macroscale (organ level). More precisely, the characteristic size of a
9 mesoscopic volume will be larger than 1.5 mm [1] and smaller than the thickness of the
10 cortical shell. The mesoscale elastic properties are of first interest because they depend on
11 tissue properties at all small-scale hierarchical levels and they have a direct influence on the
12 macroscopic mechanical response of bones. The observed intra-individual [2] and inter-
13 individual [3,4] variations of mesoscale elasticity are footprints of the remodeling process
14 and the structure-function adaptation mechanisms of bone. This calls for a clear
15 understanding of the variables that govern bone mesoscopic elasticity variations.

16 At the mesoscale, bone can be described as a two-phase composite material: a dense
17 mineralized matrix and a soft phase, hereinafter referred to as vascular porosity [5], which
18 consists of Haversian canals and resorption cavities containing fluids and soft tissues. The
19 porosity has been established to be an important determinant of the bone mesoscopic elastic
20 properties [6,7,8]. On the other hand, one would expect that variations of the mineralized
21 matrix properties strongly affect the mesoscopic elasticity because the matrix occupies
22 about 85% of the cortical bone volume. However, the actual influence of matrix properties
23 variations on mesoscale elasticity is still a matter of debate in the literature. Changes in

1 matrix mineralization have been shown to be correlated with the mesoscopic mechanical
2 properties variations when the data were combined from eighteen species [9], but not when
3 only human data were considered [10]. Rho et al. [7] found that the matrix elasticity
4 (probed with nanoindentation) was significantly correlated to the mesoscopic axial Young's
5 modulus. Since both vascular porosity and matrix properties determine mesoscale elasticity,
6 it is not possible to draw general conclusions unless both porosity and matrix properties are
7 measured on the same samples. To our knowledge, only Rho et al. [7] investigated to what
8 extent the changes in porosity and matrix elasticity contribute to the variations of the
9 mesoscopic elasticity. They found a significant correlation of both variables with the
10 mesoscopic elasticity variations. Unfortunately, the elastic properties and the porosity were
11 assessed on different specimens and along the bone axis direction only. Human cortical
12 bone possesses anisotropic elastic properties which are often approximated by transversely
13 isotropic or orthotropic properties both at the microscale [11] and mesoscale [3,12]. The
14 preferential orientation of the pores and the mineralized fibrils are such that the
15 relationships between matrix properties, porosity and mesoscale elasticity may be
16 significantly different in the axial, radial and tangential directions of bone.

17 The objective of this work was to assess the relative contributions of vascular porosity and
18 mineralized matrix elasticity to the mesoscopic elasticity variations in mature human
19 cortical bone. To this purpose, experiments were designed following two requirements,
20 which constitute the originality of the work. First, the bone matrix elasticity (reflected in
21 acoustical impedance values) and porosity, as well as the mesoscopic elasticity, were
22 measured on the same samples. Second, elasticity measurements at both the micro and the
23 mesoscale were performed in three orthogonal directions. Finally, the experimental results

1 were compared with the predictions of a micromechanical model to question the
2 assumption that cortical bone can be modeled as a homogeneous transversally isotropic
3 matrix pervaded by cylindrical pores.

4

5 **Material and methods**

6 *Bone sample preparation*

7 Fresh bone specimens were prepared from a collection of ten left femurs of female cadavers
8 (mean donor age 81 years, range 66-98 years). Femurs were removed during multi-organ
9 collection and stored at -20°C . Ethical approval for the collection of samples was granted
10 by the Human Ethics Committee of the Centre du don des Corps at the University Paris
11 Descartes (Paris, France). The tissue donors or their legal guardians provided informed
12 written consent to give their tissue for investigation, in accord with legal clauses stated in
13 the French Code of Public Health. A cross-section of thickness approximately 7 mm was
14 cut in the mid-diaphysis of each femur. In order to maximize the variability of bone
15 properties, parallelepiped-shaped samples were harvested from different anatomical
16 quadrants (lateral, medial, posterior) of each cross-section. No sample was extracted in
17 areas where the cortical thickness was less than 4 mm. This led to a set of twenty-one
18 samples (nominally $5 \times 5 \times 7 \text{ mm}^3$): three samples from two of the femurs, two samples
19 from seven other femurs and one sample from the remaining femur. The samples faces
20 were oriented according to the radial (axis 1), circumferential (axis 2), and axial (axis 3)
21 directions defined by the anatomic shape of the femoral diaphysis [2]. The samples were
22 defatted for 12 hours in a chemical bath of diethylether and methanol (1:1). The parallelism
23 of the opposite faces was controlled with a $50 \mu\text{m}$ admitted error. The six faces of each

1 sample were polished with a hard synthetic cloth using 3 μm polycrystalline diamond
2 abrasive particles followed by a 0.05 μm aluminum oxide suspension (Metadi Supreme and
3 Masterprep, Buehler® GmbH, Düsseldorf, Germany). After preparation, the samples were
4 stored in gauze soaked in saline solution at 4°C for no more than 48 hours prior to
5 measurements.

6

7 *Assessment of mesoscale elasticity*

8 Mesoscale elasticity was determined using a well-established method based on the
9 measurements of ultrasonic bulk wave velocities and sample apparent mass density. The
10 method, which has been extensively described elsewhere [2,12], is the only existing method
11 which provides measurements of the shear and longitudinal elastic properties in the
12 different directions of a same bone material volume. In contrast, mechanical methods
13 (traction, torsion, three-point bending, etc.) usually require to prepare one sample for the
14 measurement of each property. Given the ultrasonic bulk wave velocities v and apparent
15 density ρ , the diagonal terms C_{ii} of the mesoscopic elastic tensor are calculated from:

$$\begin{aligned} C_{ii} &= \rho \cdot v_{ii}^2 \quad (i = 1,2,3) \\ C_{44} &= \rho \cdot v_{23}^2 = \rho \cdot v_{32}^2 \\ C_{55} &= \rho \cdot v_{13}^2 = \rho \cdot v_{31}^2 \\ C_{66} &= \rho \cdot v_{12}^2 = \rho \cdot v_{21}^2 \end{aligned} \quad (1)$$

17 where C_{11} , C_{22} , and C_{33} are the so called longitudinal elastic coefficients which represent
18 the stiffness in a traction-compression mode, and C_{44} , C_{55} , C_{66} are the shear coefficients.
19 Velocity v_{ji} denotes the velocity of a bulk wave propagating in direction i with particles
20 motion in the j -direction. For longitudinal waves, $i = j$, and for shear waves, $i \neq j$. Samples

1 were measured undrained in ambient conditions. The apparent mass density of each sample
2 was assessed by dividing its mass by its volume; geometrical dimensions were measured
3 with a digital caliper (accuracy: ± 0.02 mm) and mass with a laboratory scale (accuracy: \pm
4 0.1 mg). The ultrasonic (US) wave velocities were evaluated using a pulse transmission
5 method with a pair of frequency matched transducers in contact with the sample surface.
6 Longitudinal waves and shear waves were measured using 2.25 MHz and 1 MHz
7 transducers (respectively, V105RM and V152RM, Panametrics, Inc., Waltham, MA). Since
8 the longitudinal and shear wave velocities in bone are significantly different (~ 3700 m/s
9 and 1700 m/s, respectively), the use of different frequencies for these two propagation
10 modes allowed obtaining a similar wavelength, of the order of 1.7 mm. Hence, the resulting
11 wavelength, which defines the probing scale, guaranteed to retrieve the bone mesoscopic
12 elasticity (i.e. at a scale much larger than the vascular pores). The received signal was
13 acquired using an oscilloscope (TDS 2012, Tektronix Inc., Beaverton, OR) and post-
14 processed with a custom MatLab program (The Mathworks Inc., Natick, MA). The time
15 delay, Δt , for wave transmission through the specimen was obtained as the difference
16 between the arrival time of the US pulse with the sample inserted and the arrival time of a
17 reference signal (transducers in contact for the longitudinal waves, Plexiglas plate inserted
18 between the transducers for the shear waves). Each longitudinal coefficient was calculated
19 after averaging the velocities measured in ten successive acquisitions with intermediate
20 repositioning; each shear coefficient was obtained after averaging the two shear wave
21 velocities from which it could be calculated (equation (1)).
22 The accuracy of the elastic coefficients evaluation was determined from measurements on a
23 homogeneous calibrated pure polycrystalline (99.95%) copper plate (Goodfellow SARL,

1 Lille, France) and was found to be 2.1% and 0.9% for the longitudinal and shear elastic
2 coefficients, respectively. Measurement errors were assessed by repeating longitudinal and
3 shear waves velocity measurements on two human bone specimens for five consecutive
4 days with intermediate repositioning. The reproducibility was 3.2% and 4.7% for the
5 mesoscopic longitudinal and shear elastic coefficients, respectively. Finally, our
6 measurements were verified to be bulk wave velocities and not bar wave velocities [3]. For
7 this, longitudinal wave velocities were measured in eight artificial composite bone samples
8 (Sawbones, Pacific Research Laboratory Inc, Vashon WA) of dimensions $10 \times 20 \times d \text{ mm}^3$
9 (thickness \times cross-sectional dimension), the lateral dimension d varying from 2 to 10 mm.
10 The same velocity was measured for all the Sawbone samples ($2907 \pm 11 \text{ m/s}$). The value
11 corresponds to the tabulated bulk velocity for this material (2890 m/s). Thus, the velocities
12 measured in this study, even for the smallest samples, were proved to be bulk wave
13 velocities and equation (1) can be applied to derive the elastic coefficients.

14

15 *50-MHz Scanning Acoustic Microscopy*

16 A custom scanning acoustic microscope (SAM), operating with a spherically focused 50-
17 MHz transducer (V605, Valpey Fisher, Hopkinton, USA), was used to probe the acoustic
18 impedance normal to the samples surfaces according to the measurement procedure
19 extensively detailed in previous studies [13,14]. The acoustic impedance (Z), which is
20 modeled as the square root of the product of the local mass density and elastic coefficient in
21 the beam direction, has been shown to be a surrogate measurement of the bone matrix
22 elasticity at the microscale [15]. This is also reflected by the good agreement between the
23 impedance and the Young's modulus of bone matrix as obtained from site-matched SAM

1 and nanoindentation measurements in bone [16,17] (although some discrepancies appear
2 presumably due to the assumptions made on the Poisson ratio). Calibrated impedance maps
3 were obtained with a lateral resolution of 30 μm for all six faces of each sample. The Z-
4 maps were segmented allowing the separation of vascular porosity and bone matrix as
5 previously reported [18].

6 The acoustic impedance of the matrix was determined from the segmented maps for each
7 face of the twenty-one samples. Note that the small pores (Volkmann's canal, osteocyte
8 lacunae) could not be resolved so that they contributed to the probed bulk matrix properties.

9 Matrix impedance in the probing direction, denoted \hat{Z}_i ($i=1,2,3$), was defined as the average
10 of the impedance values of the matrix pixels in two opposite faces of normal n_i ($i=1,2,3$).

11 The reproducibility of the assessment of \hat{Z} , obtained after imaging the face of the same
12 bone four times on different days, was found to be 1.4%.

13 The 2D cross-sectional porosity was calculated from the segmented Z-maps in the 1-2
14 plane, i.e. perpendicular to the bone axis (Fig. 1), as the ratio of the pores area to the total
15 bone surface. Porosity is usually assumed to vary only slightly across sample thickness.

16 This assumption is reasonably met with the typical sample thickness of 7 mm, given that
17 (1) the Haversian canals are roughly aligned with the bone axis and (2) the osteon length is
18 4 mm on average in human femoral mid-diaphysis [19]. However in our experience, large
19 resorption cavities visible on a cross-sectional surface can introduce a significant bias in the
20 estimation of volumetric porosity from surface porosity. To overcome this limitation, we
21 estimated the volumetric porosity of each sample (denoted Por) as the average value of the
22 cross-sectional porosities assessed on the two opposite faces in the 1-2 planes.

1

2 *Synchrotron Radiation Microtomography (SR- μ CT)*

3 To comfort our assumption that Por is a good surrogate for the volumetric porosity, a
4 subset of specimens was imaged using 3D SR- μ CT. SR- μ CT measurements were
5 performed on the imaging beamline ID19 at the ESRF (European Synchrotron Radiation
6 Facility, Grenoble, France). The beam energy was tuned to 27 keV by using a (Si111)
7 double crystal monochromator. A full set of 2D radiographic images was recorded using a
8 CDD detector (FReLoN camera; ESRF Detector group) by rotating the sample in 1999
9 steps within a 360° range of rotation in about 35 minutes. We selected a pixel size of $5.4\mu\text{m}$
10 on the detector providing a 3D reconstructed image volume with a measured spatial
11 resolution of about $10\mu\text{m}$. Due to time limitations at the ESRF facilities, only ten of the
12 twenty-one samples were imaged. After the 3D tomographic reconstruction and the
13 conversion of the linear attenuation coefficients to degree of mineralization values
14 expressed in g/cm^3 of hydroxyapatite (HA) crystals [20], the 3D-porosity was derived from
15 the segmented SR μ CT images, following a fixed threshold set to $0.7\text{ g HA}/\text{cm}^3$.

16

17 *Micromechanical model*

18 Micromechanical models are useful as a means of testing how changes of the bone
19 microscale properties affect its mesoscopic behavior. The modeled behavior depends in
20 particular on hypothesized organizational patterns and elastic symmetry of the model
21 material phases. In this work, a model of cortical bone mesoscopic elasticity based on
22 asymptotic homogenization (AH) was used (source code available online [21]). This
23 micromechanical method was chosen for its stability, even at high porosities. The theory

1 was described in details in the case of matrix isotropy in Parnell and Grimal [22]. The
 2 model hypothesizes that cortical bone can be regarded as a homogeneous transversely
 3 isotropic (TI) matrix pervaded by cylindrical pores, which are periodically distributed
 4 within the matrix material, specifically on a hexagonal lattice (Fig. 1). Here, the plane
 5 normal to the pores (1-2 plane) is the plane of isotropy for the matrix. The representation
 6 leads to transversely isotropic elasticity at the mesoscale (isotropy in the 1-2 plane), which
 7 is a reasonable approximation in human femoral mid-diaphysis [2,12]. Given an elastic
 8 tensor c^m describing the matrix elasticity, an elastic tensor c^p describing the elasticity of
 9 the material within the pores, and the volume fraction of pores, a homogenized elastic
 10 tensor C^* at the mesoscale is calculated. The elastic tensor of the bone matrix was identical
 11 for all samples. Its coefficients were determined by minimizing the L2-norm of the relative
 12 error between the experimental (C) and homogenized (C^*) mesoscopic elasticity values
 13 over the twenty-one samples. Hence c^m is the tensor which minimizes the objective
 14 function defined as:

$$15 \quad H_0(c^m) = \sqrt{\sum_{k=1}^{21} \sum_{i=1}^6 \left(\frac{C_{ii;k} - C_{ii;k}^*(c^m, c^p, Por_k)}{C_{ii;k}} \right)^2} \quad (2)$$

16 where Por_k refers to the estimate of porosity of sample k assessed from impedance maps,
 17 and $C_{ii;k}$ and $C_{ii;k}^*$ to its experimental and homogenized elastic coefficients. Since the
 18 samples were kept moist during the measurements, the material in pores (undrained) was
 19 assumed to behave like bulk water, that is, bulk modulus and Poisson ratio were set to 2.3
 20 GPa [23] and 0.4999 (quasi-incompressible), respectively, from which the terms of c^p can
 21 be calculated.

1

2 *Statistics*

3 The distribution normality and variance equality were confirmed using Shapiro-Wilk and
4 Bartlett's tests respectively. One-way analysis of variance (ANOVA) followed by post-hoc
5 comparisons using Tukey's HSD test were performed to evaluate the differences in the
6 different directions for the longitudinal and shear elastic coefficients and for the mean
7 acoustic impedance. Note that the influence of the anatomical quadrant on the elasticity was
8 not investigated due to the small number of samples (posterior (n=2), lateral (n=9), and
9 medial (n=10)). Adjusted R^2 (adj- R^2) from single linear and stepwise multiple regression
10 analyses were used to characterize the relative contributions of the vascular porosity (Por)
11 and bone matrix mean impedance in the different directions (\hat{Z}_i) to the mesoscopic elastic
12 coefficients (C_{ii}). After the determination of the optimal matrix properties c^m in the AH
13 model (equation 2), the agreement between the experimental and homogenized elastic
14 coefficients as obtained from the AH model was deduced from the linear regression
15 parameters (adj- R^2 and root mean square error (RMSE)). All statistical results were
16 considered significant for p-values less than 0.05. Statistics were made using the MatLaB
17 Statistics Toolbox (The Mathworks Inc., Natick, MA, USA) and JMP (SAS Institute Inc.,
18 Cary, NC).

19

20 **Results**

21 We evaluated the anisotropic elastic properties of the samples at two scales. At the
22 mesoscale, ANOVA showed that the samples exhibited a strong elastic anisotropy which

1 was reflected in the longitudinal elastic coefficients ($F = 98, p < 10^{-5}$) as well as in the shear
2 elastic coefficients ($F = 26, p < 10^{-5}$). Precisely, we observed (Tukey HSD) $C_{33} > C_{11}$ (not
3 different from C_{22}) and $C_{66} < C_{44}$ (not different from C_{55}). At the microscale, the bone
4 matrix also exhibited anisotropy ($F = 96, p < 10^{-5}$), which was reflected in a significant
5 higher impedance value along the bone axis compared to the two transverse directions
6 \hat{Z}_1 and \hat{Z}_2 , which did not significantly differ. The average values of the mesoscopic elastic
7 coefficients and the bone matrix mean impedance are summarized in Table 1. The p-values
8 of the Tukey tests are given in Figure 2.

9 The comparison, for a subset of ten samples, of the 3D-porosity obtained from the SR- μ CT
10 to the estimated porosity value (Por) allowed to validate the assessment of volumetric
11 porosity from the segmented impedance maps. Precisely, Por and the 3D-porosity were not
12 significantly different (paired t-test, $p = 0.48$) and were highly correlated as shown by the
13 linear regression results ($adjR^2 = 0.98, RMSE = 0.94\%$, slope not significantly different
14 from 1) (Fig. 3). Por was found to be (mean \pm sd) $13.5 \pm 6.8\%$, covering a wide range of
15 values [3–27%].

16 A weak but significant correlation was found between all C_{ii} , except C_{22} , and \hat{Z}_3 (bone axis
17 direction) ($adj-R^2 < 0.25, p = [0.01-0.04]$) (Table 2). No significant correlation was found
18 between the C_{ii} and the matrix impedance in the radial and circumferential directions
19 (\hat{Z}_1 and \hat{Z}_2). The mesoscopic elastic coefficients were well correlated to the porosity ($adj-$
20 $R^2 = [0.72 - 0.84], p < 10^{-5}$). The use of a stepwise regression analysis showed no
21 improvement of the correlation when adding the bone matrix impedance (\hat{Z}_i) to the
22 porosity to explain the mesoscopic elasticity variations between samples.

1 The transversely isotropic elastic tensor of the matrix (c^m) which allowed the best
2 agreement (in the sense of equation 2) between measured and modeled mesoscopic elastic
3 properties was found to be $c_{11}^m = c_{22}^m = 26.8$ GPa, $c_{33}^m = 35.1$ GPa, $c_{44}^m = c_{55}^m = 7.3$ GPa,
4 $c_{66}^m = 5.8$ GPa, $c_{13}^m = c_{23}^m = 15.3$ GPa, and thus $c_{12}^m = c_{11}^m - 2c_{66}^m = 15.2$ GPa.

5 The experimental mesoscopic elastic coefficients correlated well with the effective elastic
6 coefficients as computed from the AH model (adj- $R^2 = [0.78-0.82]$, $p < 10^{-5}$) (Fig. 4). The
7 precision of the model prediction was evaluated by means of the RMSE absolute and
8 relative values: $C_{11} = 1.0$ GPa (5.2 %), $C_{22} = 1.2$ GPa (6 %), $C_{33} = 1.7$ GPa (5.6 %), $C_{44} = 0.3$
9 GPa (5.5 %), $C_{55} = 0.4$ GPa (8.5 %), $C_{66} = 0.3$ GPa (7.6 %).

10

11 **Discussion**

12 To our knowledge, the current work is the first to provide, for the same set of samples,
13 measurements of the anisotropic elastic properties at two scales together with an evaluation
14 of the cortical porosity. A set of human femoral cortical bone data, obtained on twenty-one
15 samples from ten donors, was used to investigate the relative contributions of both the
16 matrix elasticity and the porosity to the bone mesoscopic elasticity.

17 The experimental data corroborated well with previous studies, be it in respect of the
18 mesoscopic elastic coefficients [2,3,12], the mean acoustic impedance of the bone matrix
19 [14,17], or the range of the intracortical porosity [24,25,26].

20 Impedance measurements suggested that the average elastic properties of the mineralized
21 matrix did not undergo large variations in the different samples (with coefficients of
22 variation of the \hat{Z}_i all inferior to 6%). The limited variations of bone matrix elasticity

1 reflected in Z might explain the lack of correlation between the mean acoustic impedance of
2 the matrix and the mesoscopic elastic coefficients. A literature review reveals that such
3 modest variations of the bone matrix properties have been observed in a number of studies.
4 Cross-sectional reports have shown that the mean degree of mineralization of bone does not
5 exhibit large variations between individuals, independently of age [10,27,28] and gender
6 [27]. A few studies have measured the matrix elasticity on several individuals at the same
7 cortical bone site (femoral diaphysis and neck [29], femoral diaphysis [7], radius [14]).
8 Similarly, they all reported small changes in the mean value of the matrix elasticity
9 (average of several measurements points on a surface of at least 1 mm) with coefficients of
10 variation ranging between 3 and 10 %. Hence, although the bone matrix elasticity is known
11 to display strong local heterogeneities (in particular between the osteonal and interstitial
12 tissues), its mean value over a few millimeters remains relatively constant in healthy
13 individuals. However, a selection of bone specimens in a population with known bone
14 pathologies could result in a wider variation of matrix material properties and lead to
15 different conclusions.

16 Our results demonstrate that, for an elderly population, the change in porosity is the major
17 determinant of the variations of the anisotropic elastic coefficients at the mesoscale, at least
18 in the femoral mid-diaphysis. To our knowledge, only one study has experimentally
19 examined the impact of porosity variations on the elasticity of human femoral cortical bone
20 in several directions [8]. While they also found a strong dependence of the Young's moduli
21 and shear moduli on porosity ($R^2 = [0.66 - 0.72]$), they observed no significant correlation
22 between the elastic properties in the transverse direction and the porosity, in contrast to our
23 findings.

1 The fact that all the mesoscopic elastic coefficients have a dependency on the porosity is
2 supported by the theoretical results obtained with several models using different
3 homogenization approaches [1,30,31,32,33,34]. We compare the outcome of a
4 homogenization model to experimental data for known values of porosities associated to a
5 number of bone material volumes. As far as we know, only two previous studies confronted
6 experimental results with the predictions of a micromechanical model. However, the elastic
7 constants were not assessed on the same specimens [31], or the shear constants were
8 lacking [35]. In our study, because six elastic coefficients have been measured for each
9 sample, a large data set is available for the comparison. We found that modeling cortical
10 bone as a two-phase composite with a transversely isotropic matrix pervaded by cylindrical
11 pores provided a good estimate of the elasticity variations at the mesoscale, as shown by the
12 strong correlations (relative RMSE = [5.2 – 8.5 %]) between the experimental results and
13 the prediction of the micromechanical model. Note, however, that the homogenized
14 elasticity C^* is not strictly independent of the mesoscale experimental data C because the
15 matrix elasticity of the model (c^m) was determined such that the agreement between C and
16 C^* is optimum (equation 2).

17 It is noteworthy that the model was particularly efficient considering its ability to fit all
18 experimental mesoscopic elastic coefficients with a relatively good accuracy using a unique
19 elastic tensor for the matrix and the pores and a sample-dependent porosity. This was
20 despite the many idealizations of the model, in particular the elastic properties of the matrix
21 and the modeling of the pores. Universal, homogeneous, elastic properties were assigned to
22 the bone matrix. The choice of a unique matrix was supported by the small change in the

1 average elastic properties of the matrix, as testified by the matrix impedance data. We
2 verified that the optimized set of TI elastic properties assigned to the bone matrix (c^m)
3 were physically acceptable. In fact, once converted into engineering moduli ($E_T = 16.5$
4 GPa, $E_L = 24.0$ GPa, $G_T = 5.8$ GPa, $G_L = 7.3$ GPa), the matrix elastic properties were found
5 consistent with the nanoindentation values in human femoral bone available in literature
6 [36,37,38]. Moreover, the matrix elastic coefficients (c^m) used in our model compared well
7 with those derived from the experimental acoustic impedance mean values using the
8 conversion relationship between Z and c^m [15]. Precisely, the elastic coefficients of the
9 matrix as derived from the Z_i ($i=1,2,3$) ($c_{11}^{m\text{exp}} = 28.7 \pm 3.1$ GPa, $c_{22}^{m\text{exp}} = 28.5 \pm 2.3$ GPa and
10 $c_{33}^{m\text{exp}} = 40.7 \pm 3.3$ GPa) were in agreement with the elastic coefficients assigned in the
11 model ($c_{11}^m = c_{22}^m = 26.8$ GPa, $c_{33}^m = 35.1$ GPa). The vascular porosity was idealized as
12 infinite cylinders of circular cross-section aligned along the bone long axis. Hence, the
13 pores were modeled as continuous even though a discontinuous representation might seem
14 more realistic. However, we have found that, for aspect ratios (length of the pore / diameter
15 of the pore) larger than 5, modeling the pores as infinite cylinders yields a very good
16 approximation (less than 1% error) of discontinuous pores with typical aspect ratio of the
17 Haversian canal [39]. Although this representation has been commonly used for modeling
18 cortical bone [30,31,35], it does not take into account the variability of pores shapes, size,
19 and distribution. Considering the gradient of porosity from the endosteal to the periosteal
20 region [14,24] or the change in the pores size [26,32] may improve the predictions of the
21 bone effective elastic properties.

1 The remaining part of experimentally determined elasticity C which is not explained by the
2 model is due to experimental uncertainties and model assumptions. The latter comprise the
3 assumptions regarding the pores as mentioned above and the fact that some variability of
4 the matrix properties exists between different samples.

5 A first limitation of the study arises from the estimation of the sample porosity as the
6 average value of the cross-sectional porosities assessed on the two opposite transverse
7 faces. However, the validation of the porosity evaluation with 2D SAM on ten samples
8 against the vascular porosity as obtained from 3D SR μ CT data confirmed that Por is a good
9 proxy for the vascular porosity. A second limitation in the study is the fact that all donors
10 were elderly female donors (with a mean age superior to 80 years). Although the bone
11 matrix elasticity has been shown to be independent from age and gender [40], aging
12 strongly affects the range of porosity and could change the relative contributions of the
13 matrix elasticity and the porosity to the mesoscopic elasticity in younger individuals. Thus
14 the conclusions of this study hold true only for an aged population, which is most
15 commonly affected by osteoporosis and bone fragility. Finally, in spite of a limited sample
16 size ($n = 21$ from 10 subjects), the range of values covered by the porosity (from 3 to 27%)
17 was wide enough to provide conclusive results.

18 In summary, the findings of this paper demonstrate that, in aged women, the changes in
19 porosity prevail over those of matrix elasticity to drive the variations of the bone
20 mesoscopic elasticity. The impact of the porosity on the elasticity is all the more important
21 considering the increased intracortical porosity as a consequence of aging [10,26,41,42,43]
22 and disease, e.g. hyperparathyroidism, osteoporosis [44]. In particular, Zebaze et al. [43]
23 showed that 84% of the bone loss occurs after the age of 65 of which 68% would be

1 cortical bone manifested as an increase of cortical porosity. Moreover, the increase of
2 cortical porosity, pointed out as the dominant factor occurring in elderly individuals, is
3 known to reduce bone strength [45]. A simple mechanical model was proposed to interpret
4 the experimental data: the dependence on porosity of shear and longitudinal elastic
5 properties in the radial, circumferential, and axial directions of bone is correctly described
6 when idealizing bone as a two-phase material with a ‘universal’ (same for all bone samples)
7 transversely isotropic matrix pervaded by cylindrical pores.

8

9

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12 Based Assessment of Bone” (ULAB) and was supported by the ESRF Long Term Proposal
13 MD431.

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- 13

1 **Figures captions**

2 **Figure 1** (a) 3D reconstruction of a cortical bone volume from SR- μ CT data. The samples
3 faces are oriented according to the radial (1), circumferential (2), and axial (3) axes defined
4 by the anatomic shape of the femoral diaphysis. (b) Idealization of cortical bone as a
5 homogeneous anisotropic matrix pervaded by infinite cylindrical pores, which are
6 periodically distributed within the matrix material, specifically on a hexagonal lattice.

7

8 **Figure 2** Experimental results: (a) mesoscopic elastic coefficients (b) mean acoustic
9 impedance of the bone matrix. On each box the central mark is the median, the edges are
10 the 25th and 75th percentiles, the whiskers extend indicate the extreme values. The p -values
11 from the post hoc multiple comparison Tukey's HSD tests are also given.

12

13 **Figure 3** Validation of the assessment of volumetric porosity from the segmented
14 impedance maps on a subset of ten samples: the estimated value of the 'volumetric'
15 porosity (Por) is plotted against the 3D-porosity obtained from SR- μ CT.

16

17 **Figure 4** (a) Longitudinal and (b) shear mesoscopic elastic coefficients versus porosity:
18 results from experiments (■, o, ▲) and asymptotic homogenization model solid and dotted
19 lines). Note that all the homogenized elastic coefficients computed from the AH model are
20 obtained using a unique set of elastic constants for the bone matrix.

21

22

23

1 **Tables captions**

2 **Table 1** Experimental data (mean \pm std [range])

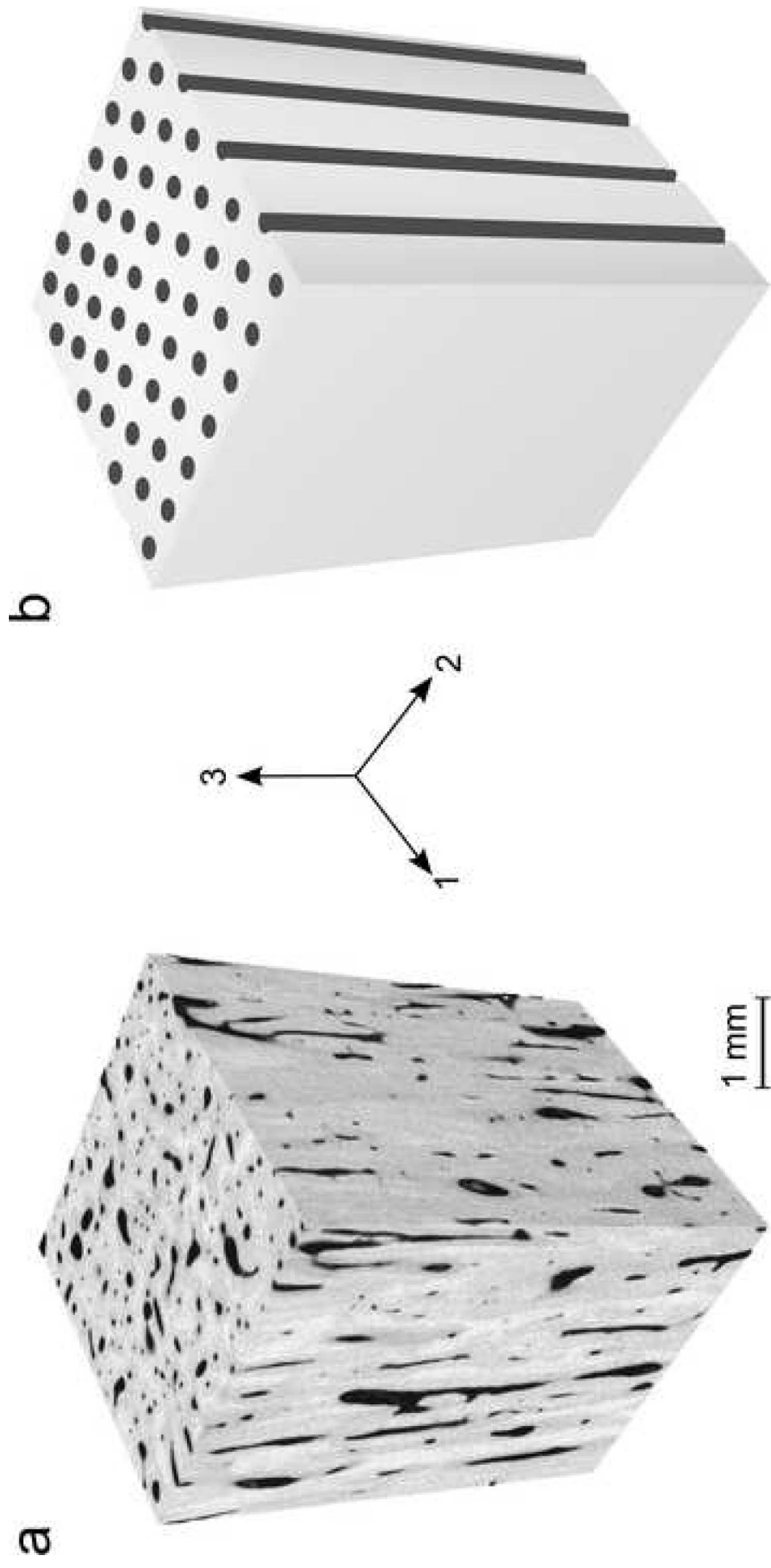
3

4 **Table 2** Multivariate analysis regression (adjusted R^2 and RMSE): relative contributions

5 of the vascular porosity (Por) and the matrix impedance (\hat{Z}_i) to the mesoscopic elastic

6 coefficients (C_{ij}).

Figure1
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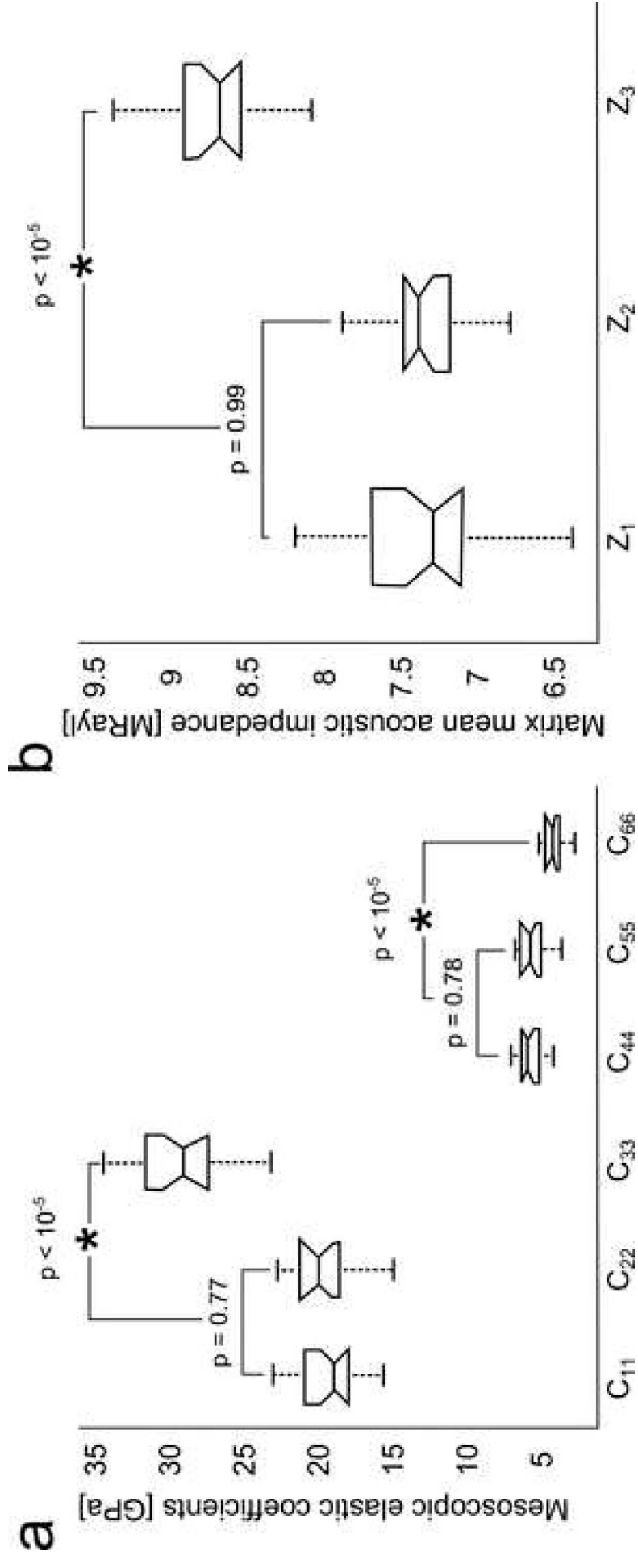
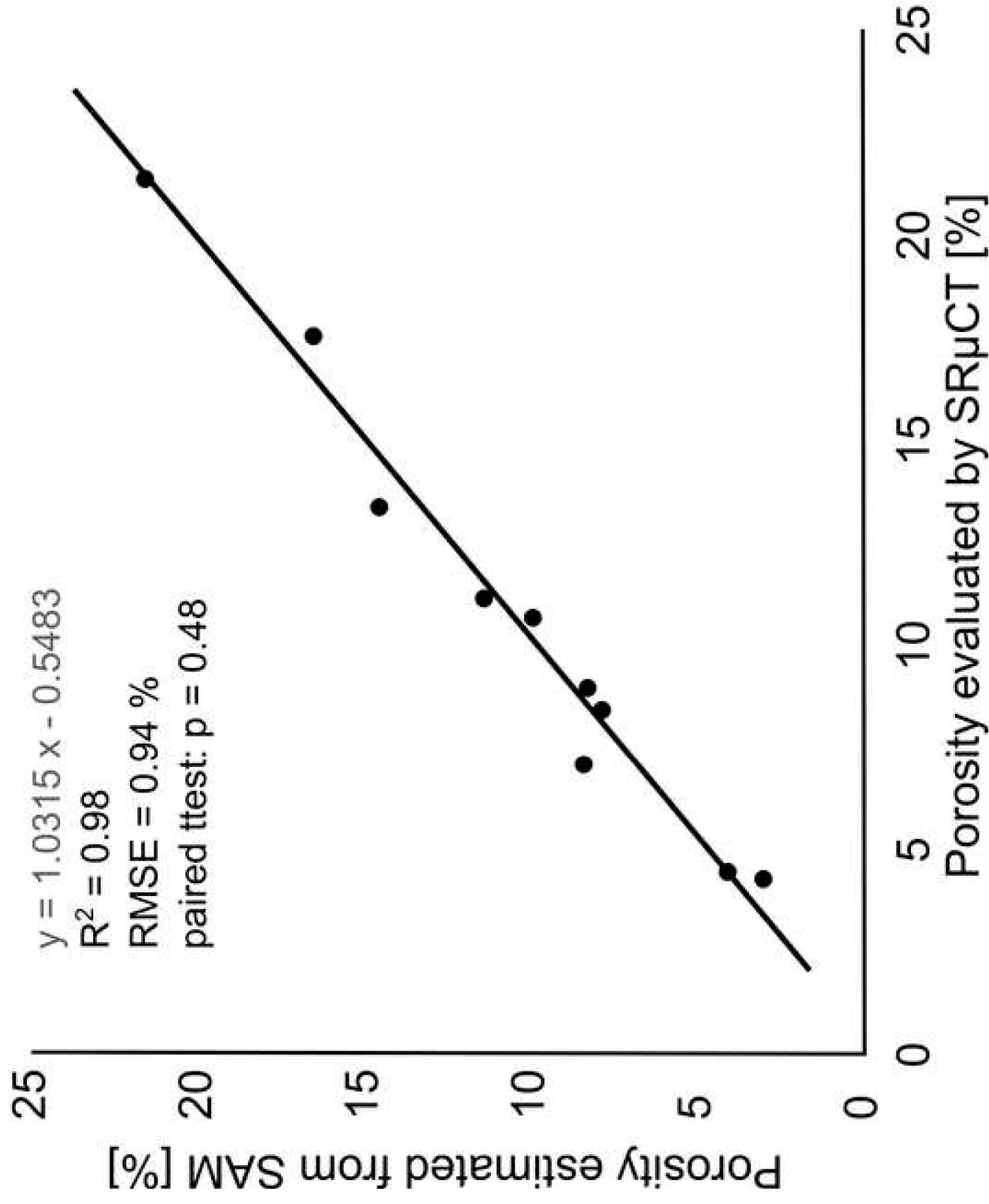


Figure3
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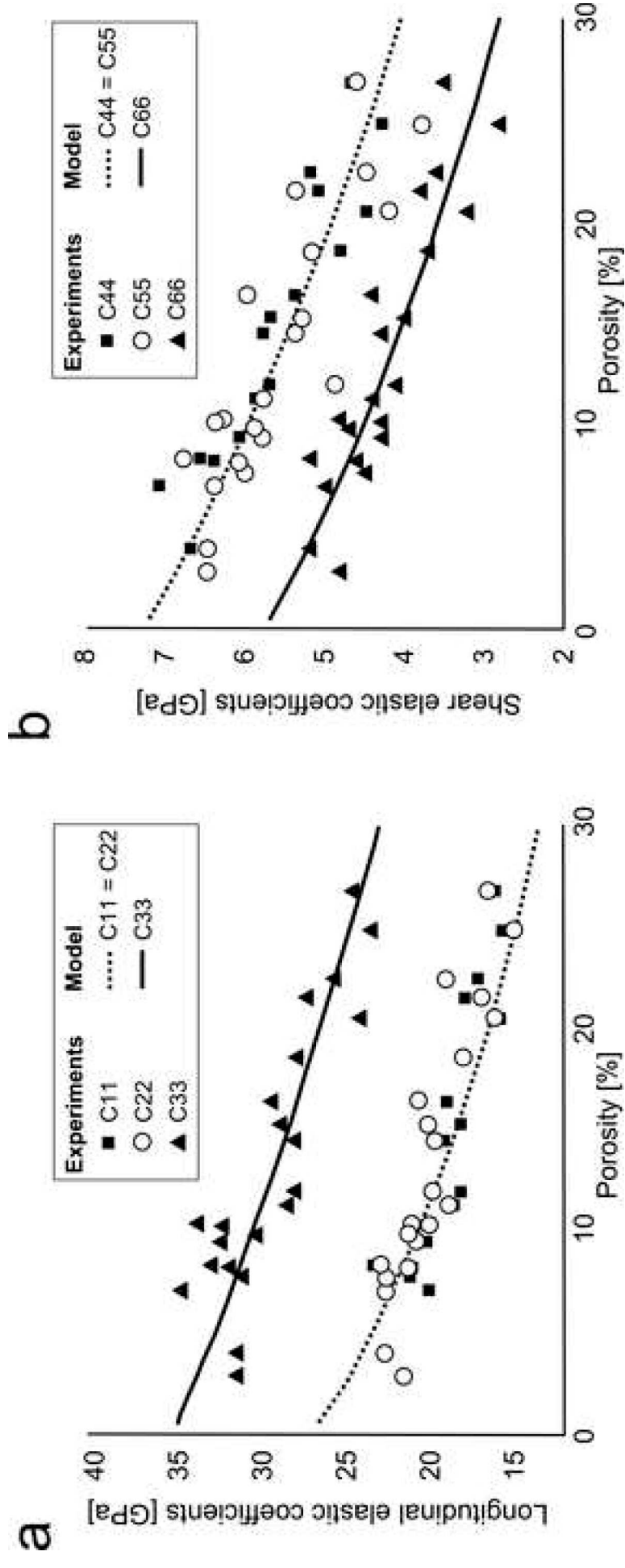


Table1

Mesoscopic elastic coefficients [GPa]					
C_{11}	C_{22}	C_{33}	C_{44}	C_{55}	C_{66}
19.3 ± 2.2	19.8 ± 2.2	29.2 ± 3.2	5.8 ± 0.8	5.6 ± 0.8	4.2 ± 0.6
[15.6 – 23.2]	[15.0 – 22.8]	[23.3 – 34.5]	[4.3 – 7.1]	[3.8 – 6.8]	[2.8 – 5.2]
Mean acoustic impedance of the bone matrix [MRayl]					
\hat{Z}_1		\hat{Z}_2		\hat{Z}_3	
7.4 ± 0.4		7.3 ± 0.3		8.7 ± 0.4	
[6.4 – 8.2]		[6.7 – 7.9]		[8.1 – 9.6]	
Vascular porosity [%]					
		13.5 ± 6.8			
		[2.9 – 26.9]			

Table2

Adjusted R ² RMSE [GPa]	C_{11}	C_{22}	C_{33}	C_{44}	C_{55}	C_{66}
\hat{Z}_1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
\hat{Z}_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
\hat{Z}_3	0.21 *	n.s.	0.26 *	0.22 *	0.26 *	0.16 *
	1.96		2.75	0.68	0.71	0.59
<i>Por</i>	0.79 **	0.76 **	0.74 **	0.84 **	0.72 **	0.78 **
	1.01	1.09	1.64	0.31	0.44	0.30
<i>Por, \hat{Z}_1, \hat{Z}_2, \hat{Z}_3</i>	0.79 **	0.76 **	0.74 **	0.84 **	0.72 **	0.78 **
	1.01	1.09	1.64	0.31	0.44	0.30

n.s.: not significant ($p > 0.05$); * $p < 0.05$; ** $p < 10^{-5}$