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# Cortical bone viscoelastic damping assessed with resonant ultrasound spectroscopy reflects porosity and mineral content

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## Abstract

Viscoelasticity is an essential property of bone related to fragility, which is altered in aging and bone disease. Bone viscoelastic behavior is attributed to several mechanisms involving collagen and mineral properties, porosities, and bone hierarchical tissue organization. We aimed to assess the relationships between cortical bone viscoelastic damping measured with Resonant Ultrasound Spectroscopy (RUS), microstructural and compositional characteristics. We measured 52 bone specimens from the femur of 26 elderly human donors. RUS provided a shear damping coefficient at a frequency of the order of 150 kHz. The characteristics of the structure of the vascular pore network and tissue mineral density were measured using synchrotron radiation high-resolution computed tomography (SR- $\mu$ CT). Fourier transformed infrared microspectroscopy (FTIRM) was used to quantify mineral-to-organic phase ratio, mineral maturity, crystallinity, and collagen maturity. Cross-links were quantified from biochemistry. Viscoelastic damping was found to increase with vascular porosity ( $r = 0.68$ ), to decrease with the degree of mineralization of the extravascular matrix ( $r = -0.68$ ), and was marginally affected by collagen. We built a multilinear model suggesting that when porosity is controlled, the variation of mineral content explains a small additional part of the variability of damping. The work supports the consideration of viscoelasticity measurement as a potential biomarker of fragility and provides a documentation of bone viscoelastic behavior and its determinants in a frequency range rarely investigated.

*Keywords:* Cortical bone, Damping, Quality factor, Resonant ultrasound spectroscopy, Porosity, Mineral content

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## 1. Introduction

Viscoelasticity is an essential property of biological tissues [1]. For example, hard biological tissues, such as bone, exhibit creep and stress relaxation, rate dependent response to a dynamic loading, phase lag between stress and strain during oscillatory loading, and damping of elastic waves [2]. Bone viscoelastic behavior is attributed to several mechanisms [3] involving all scales from the nanoscale to the mesoscale, i.e. the scale of a few millimeters in cortical bone [4]. The collagen of the extracellular matrix is itself viscoelastic. The movement of fluids within the pores, heat flow under mechanical loading between heterogeneous regions such as osteons and lamellae, are some of the other mechanisms associated to the viscoelastic behavior.

Viscoelasticity of bone has been investigated by many authors motivated by the relationship between damping mechanisms and mechanical behavior beyond the elastic limit and related to bone fragility. Firstly, collagen was shown to have a profound effect on bone fragility, because changes in collagen content, or changes to collagen cross-linking, reduce the energy required to cause failure [5]. Secondly, bone strength [6, 7] and toughness [8] are rate dependent suggesting a role for viscous mechanisms. Thirdly, there is a close relationship between microdamage and viscoelasticity as loading cortical bone past the yield stress changes its viscoelastic properties [9]. Finally, the viscoelastic dissipation of energy at micron and sub-micron scales is related to crack initiation and propagation [10, 11]. Bone material quality and fragility are multifaceted phenomena involving several scales and they cannot be fully captured from a single mechanical measurement. This has led several authors to suggest that the measurement of viscoelasticity could bring unique information related to fragility associated with bone disease [12–14] and tissue alteration during aging [15]. Yet, viscoelastic data is scarce in comparison to published data on elastic properties. In particular, the relationships between viscoelasticity, extravascular matrix composition, and microstructure are poorly documented.

Viscoelasticity has been assessed with a variety of techniques such as microscale measurement of creep with nanoindentation [15–17], mesoscale measurement of an oscillatory response in torsion (up to 50 kHz) [18], or 3-point bending with dynamic mechanical analyzers (in the range 1-20 Hz) [19]. In material science, another popular approach is to use resonant ultrasound spectroscopy (RUS), a technique to measure the anisotropic stiffness and viscoelastic damping assessed from the width of a resonance of a freely vibrating specimen [20–23]. In a viscoelastic material such as bone, the resonant peaks corresponding to the different eigenmodes of the measured specimen tend to overlap. It follows that RUS is essentially practicable to measure bone damping associated with the first eigenmode which is associated to a shear modulus [23, 24]. A typical cortical bone specimen for RUS measurements is a cuboid with longest dimension around 5 mm, having its first eigenfrequency around 150 kHz.

The purpose of the present work was to assess the relationships between cortical bone viscoelastic damping measured with RUS, tissue composition, porosity, and microstructure. We measured a collection of bone specimens from elderly human donors with RUS, providing a quality factor (equivalent to  $\tan \delta$  in torsion experiments), associated to a shear modulus, at a frequency of the order of 150 kHz. Vascular porosity and the degree of mineralization of bone were obtained from synchrotron radiation high-resolution computed tomography (SR- $\mu$ CT), cross-links were quantified from biochemistry, Fourier transformed infrared spectroscopy

38 (FTIR) was used to quantify mineral-to-organic phase ratio, mineral maturity, crystallinity, and collagen ma-  
39 turity. Note that the relationship between elastic properties and these variables was previously reported in  
40 [25].

41 The work provides a documentation of bone viscoelastic behavior and its determinants in a frequency  
42 range rarely investigated, complementing existing data. Comparison with the viscoelastic behavior at other  
43 frequencies may provide insight into the mechanics of bone viscoelasticity. While our results do not reflect the  
44 viscoelastic behavior of bone at the frequencies of physiological loading, they are of practical interest for some  
45 ultrasonic applications around  $10^5$  Hz such as bone ultrasonic drilling, ultrasonic stimulation of bone healing  
46 and bone health assessment with guided waves.

## 47 2. Materials and methods

### 48 2.1. Specimens

49 We have used a collection of specimens from a previous study [25]. The preparation of specimens is briefly  
50 recalled here. Cortical bone specimens were harvested from the left femur of 26 human cadavers. The femurs  
51 were provided by the Département Universitaire d'Anatomie Rockefeller (Lyon, France) through the French  
52 program on voluntary corpse donation to science. Among the donors, 14 were females and 12 were males  
53 (50 – 95 years old,  $77.3 \pm 11.5$ , mean $\pm$ SD). As shown in Figure 1, in each of the lateral and medial anatomical  
54 quadrants, adjacent specimens (# 1, # 2 and # 3) were prepared along the axial direction, to be measured  
55 by several techniques described below. All specimens were frozen and stored at  $-20^\circ\text{C}$  between tests. They  
56 were then slowly thawed and immersed in 0.9% NaCl saline before testing to ensure full hydration [26]. The  
57 nominal size of the specimens #1 used for RUS was  $3 \times 4 \times 5\text{mm}^3$  in radial ( $x_1$ ), circumferential ( $x_2$ ) and axial  
58 ( $x_3$ ) directions, respectively. These specific dimensions were chosen so as to maximize the size of the specimens  
59 while complying with the technical requirements of RUS [22]. Figure 2 is the three-dimensional rendering of  
60 the SR- $\mu$ CT image of a bone specimen. Specimens #1 used in RUS were kept hydrated prior to experiment  
61 and all RUS measurements were made on a fully hydrated specimen. After a first set of RUS measurements,  
62 specimens #1 were defatted following a protocol which prevents the risk of infections and allows the specimen  
63 conservation at room temperature [26].

### 64 2.2. RUS experiment

65 Setup and signal processing methods dedicated to the RUS measurement of attenuative materials, exten-  
66 sively described elsewhere [22, 27], were used in this study. Briefly, the specimen #1 was mounted on opposite  
67 corners between two shear wave transducers (V154RM, Panametrics, Waltham, MA, USA). The frequency  
68 response (vibration spectrum) was recorded using a vectorial network analyzer (Bode 100, Omicron Electron-  
69 ics GmbH, Klaus, Austria) and a broadband charge amplifier (HQA-15M-10T, Femto Messtechnik GmbH,  
70 Berlin, Germany). The frequency band of analysis was 100-700 kHz, including the first resonant frequency  
71 of the specimen. Six successive spectra were recorded for each specimen, with intermediate rotation (without  
72 unmounting) of the specimen by a small angle between each measurement to vary the relative amplitudes of

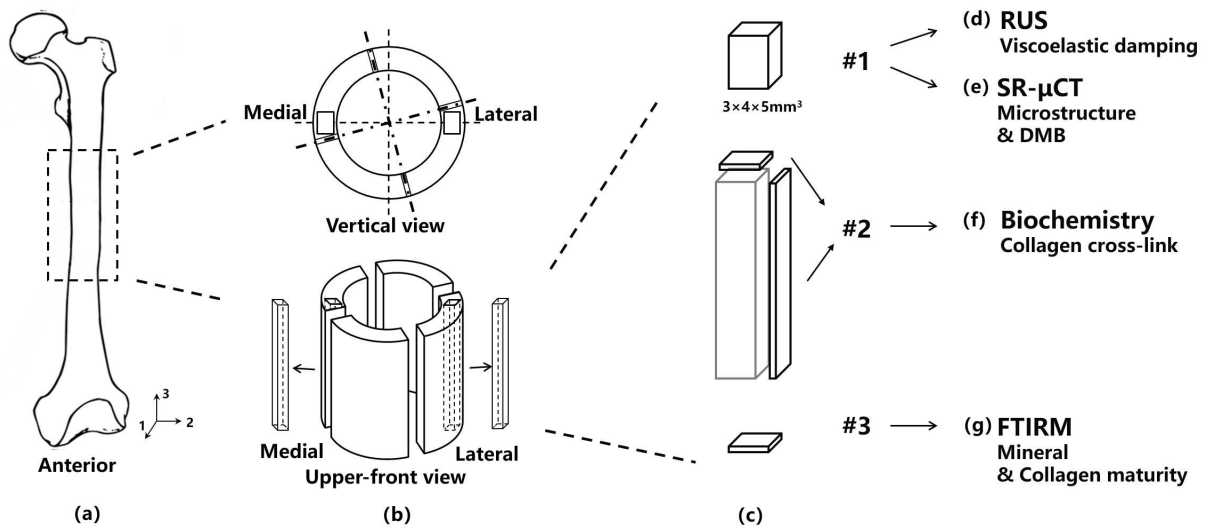


Figure 1: Specimen preparation procedure. (a) A cross-section of femoral bone at the mid-diaphysis was extracted. (b) The vertical and upper-front view of the cross section, which was then cut into 4 pieces (lateral, medial, anterior and posterior). Two of these pieces (lateral and medial) were then used. (c) 3 rectangular parallelepiped shaped specimens (set #1, #2 and #3) were prepared along the axial direction at both the lateral and medial quadrants. (d) RUS measurements for bone viscoelastic damping. (e) SR- $\mu$ CT scanning for Bone microstructural parameters and DMB. (f) Bone residues close-by #2 after cutting for #2 went to biochemistry experiments for the collagen and cross-links. The data experiments carried on #2 were not shown in this work. (g) FTIRM tests for bone compositional information.

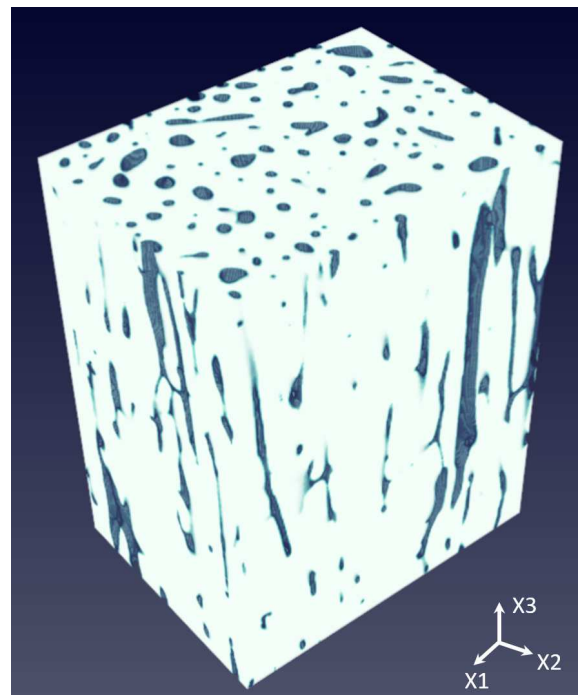


Figure 2: Three-dimensional rendering of the SR- $\mu$ CT image of a bone specimen of approximate dimensions  $3 \times 4 \times 5 \text{ mm}^3$ .

73 the excited resonant modes in order to maximize the number of detectable resonant frequencies (Fig. 3). A  
 74 selected portion of each complex spectrum can be fitted by a sum of  $M$  Lorentzian line-shapes (each describing

75 the behavior of a one degree-of-freedom mechanical resonator) :

$$L(f) = \sum_{k=1}^M \frac{a_k}{(f_k^2 - f^2) + i(f_k f / Q_k)}, \quad (1)$$

76 with  $f$  the frequency,  $a_k$  the complex amplitudes,  $f_k$  the resonant frequencies and  $Q_k$  the quality factors. The  
 77 quality factor is related to the width of the resonant peak as  $Q_k \sim f_k / \Delta f$ , where  $\Delta f$  is the -3db bandwidth  
 78 (half-power bandwidth).

79 Upon combining the six spectra and fitting with Eq. (1), between 20 to 30 resonant frequencies  $f_k$  were  
 80 extracted for each specimen [22]. Frequencies  $f_k$ , which are nearly equal to the eigenfrequencies of the freely  
 81 vibrating specimen, were then used to determine the coefficients  $C_{ij}$  of the stiffness tensor [20, 27]. These  
 82 were obtained in a previous work presented in [25]. We used Voigt notation for the stiffness tensor and we  
 83 assumed that bone is a transversely isotropic material (plane 1-2 is the plane of isotropy). As a consequence,  
 84  $C_{11} = C_{22}$ ,  $C_{13} = C_{23}$ ,  $C_{44} = C_{55}$ .

85 For the purpose of the present study, we specifically processed the first resonant peak (around 150 kHz  
 86 in Fig. 3) to assess shear mode damping. As explained in section 2.3, the shear damping coefficient  $Q_{44}^{-1}$   
 87 (associated to elastic coefficient  $C_{44}$ ) can be obtained from the quality factor of the first resonant mode  $Q_1$   
 88 (defined in Eq. (1)). The quality factor  $Q_1$  in each spectrum was obtained as follows :

- 89 • A portion of the spectrum (bandwidth) was manually selected, containing only the first peak  $f_1$  (Case 1,  
 90 Fig. 4 left) or the two first peaks (Case 2, Fig. 4 right). Precisely, Case 1 corresponds to specimens with  
 91 a well-isolated first resonant peak. For other specimens with first two relatively close resonant peaks  
 92 (Case 2), we selected a portion of the spectrum with the first two peaks to account for the potential  
 93 influence of the second resonance on the Lorentzian lineshape of the first peak. The distance between  
 94 the two first peaks depends on the exact dimensions of the specimen and its elastic properties. Among  
 95 the 52 specimens, 10 were in Case 1 and 42 were in Case 2. The effect on frequency and Quality factor  
 96 determination of the bandwidth selection method is discussed further in Appendix A.
- 97 • To determine  $Q_1$ , Eq. (1) was fitted to the spectrum assuming  $M = 1$  (Case 1) or  $M = 2$  (Case 2) using  
 98 a time domain estimation method based on a linear predictive filter (black dash line in Fig 5), followed  
 99 by a frequency domain nonlinear optimization (black solid line) [22, 28].

100 Specimens were measured with RUS before defatting, after defatting, and finally after X-ray irradiation  
 101 during SR- $\mu$ CT.

### 102 2.3. Calculation of material damping

103 In this section, we present how the measured quality factor  $Q_1$  was used to calculate shear damping  
 104 coefficient  $Q_{44}^{-1}$  following [23, 24]. Bone viscoelasticity was modeled by introducing the complex modulus:

$$C_{ij}^* = C_{ij} + iC'_{ij} = C_{ij}(1 + iQ_{ij}^{-1}), \quad (2)$$

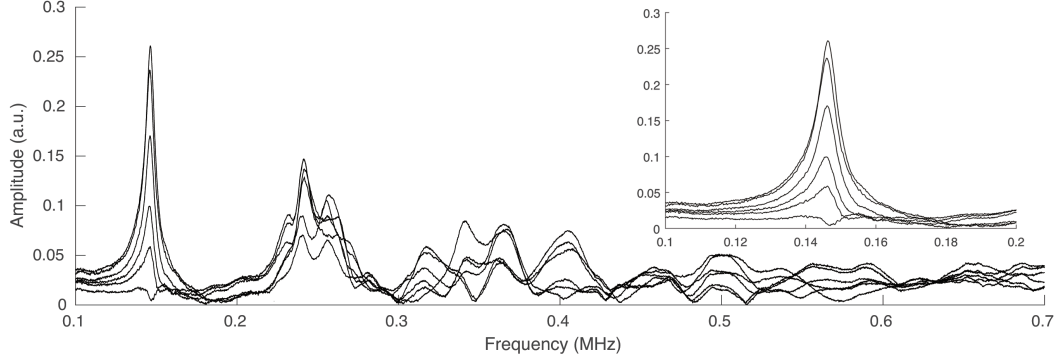


Figure 3: Typical set of spectra measured for a cortical bone specimen. The relative amplitudes of the resonant peaks vary as the specimen is rotated. The width of the first peak provided a measurement of damping. The inset figure is a zoom of in the frequency range of 100 to 200 kHz.

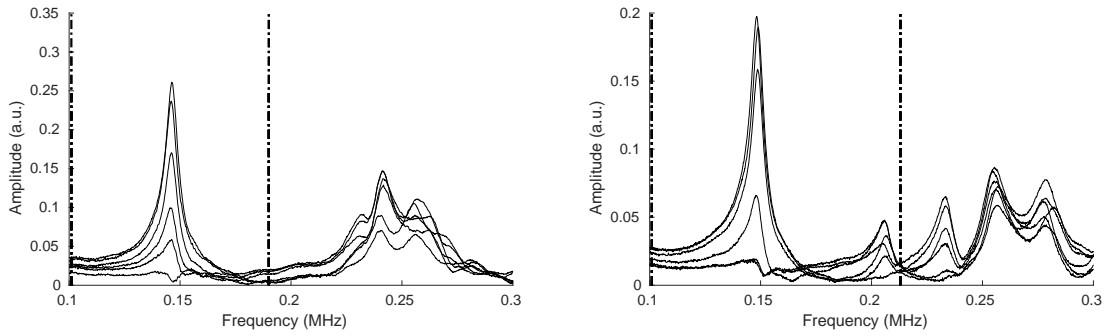


Figure 4: To calculate  $Q_1$ , a portion of the spectrum was selected between the two dash-dot lines. (left) Case 1: for specimens with a well-isolated first resonant peak, only the peak was selected. (right) Case 2: for other specimens, the first two peaks were selected together.

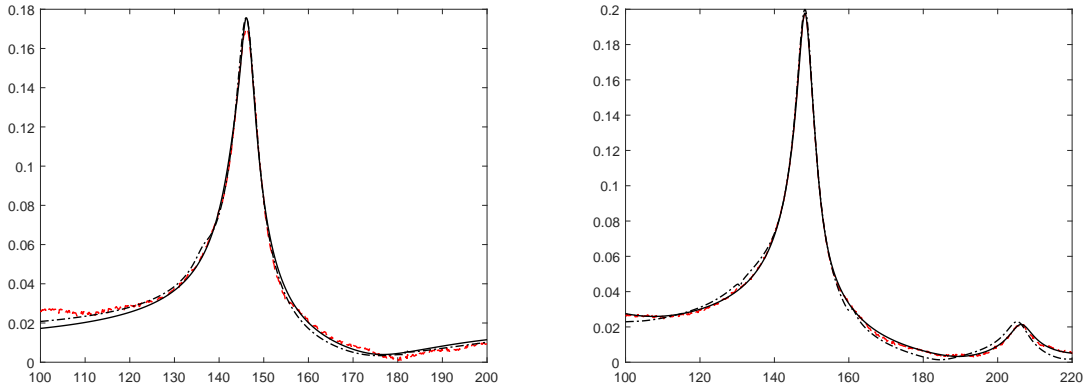


Figure 5: Illustration of the fit of the first peak with Eq. (1). Red dash lines show experimental frequency response of Case 1 (left) and Case 2 (right), frequency response reconstructed with a linear predictive filter (black dash line), and then followed by a frequency domain nonlinear optimization (black solid line).

105 where  $C_{ij}$  and  $C'_{ij}$  are the stiffness coefficient and loss modulus, respectively, and  $Q_{ij}^{-1} = \frac{C'_{ij}}{C_{ij}}$ . This definition  
 106 of the complex modulus holds for a dynamic loading at a given frequency, and  $C_{ij}$  and  $C'_{ij}$  are in general  
 107 functions of frequency. Note that  $Q_{ij}^{-1} \sim \tan \delta$ , where  $\tan \delta$ , another popular way of reporting damping, is  
 108 the phase shift between a harmonic loading and the mechanical response in a vibrational mechanical test [2].

109 In the present work, the complex modulus is evaluated using the first resonant peak centered at  $f_1$  of each  
 110 specimen.

111 In a RUS experiment, a series of resonant frequencies  $f_k$  and Quality factors  $Q_k$  can in principle be obtained  
 112 from the resonant spectrum. These are related to the complex modulus through [29–31] :

$$Q_k^{-1} = \sum_{i,j} \frac{2C_{ij}}{f_k} \frac{\partial f_k}{\partial C_{ij}} Q_{ij}^{-1}, \quad (3)$$

113 where

$$\sum_{i,j} \frac{2C_{ij}}{f_k} \frac{\partial f_k}{\partial C_{ij}} = 1. \quad (4)$$

114 The linear system of equations Eq. (3) between  $Q_{ij}^{-1}$  and  $Q_k^{-1}$  is derived with the assumption that  $Q_{ij}^{-1} \ll 1$ ,  
 115 which is in practice the case for cortical bone [23, 24]. The coefficients of the linear system are the relative  
 116 sensitivities of the eigenmode  $k$  to the loss moduli  $Q_{ij}$ .

117 When the stiffness coefficients  $C_{ij}$  are available, the system of equations (3) can be inverted to derive  
 118  $Q_{ij}^{-1}$  from the measured  $Q_k^{-1}$ . In practice, quality factors  $Q_k$  for  $k = 2, 3, \dots$  can hardly be estimated with  
 119 a sufficient precision in high damping materials such as bone because of strong resonant peak overlapping.  
 120 Furthermore, several relative sensitivity terms are small, so that the inversion is ill-conditioned and sensitive  
 121 to errors in the measured  $Q_k$ . Here, we take advantage of the fact that:

- 122 1. The first resonant peak is well-separated from the subsequent peaks (Fig. 4) for all specimens. This is a  
 123 result of the specific aspect ratio of the specimens which was selected so as to optimize the separation of  
 124 low frequency resonances. As a consequence,  $Q_1$  is estimated with a satisfactory precision ;
- 125 2. The terms

$$\frac{2C_{ij}}{f_1} \frac{\partial f_1}{\partial C_{ij}}$$

125 for  $ij \neq 44$  are relatively small.

126 Hence, the relationship between  $Q_1^{-1}$  and  $Q_{44}^{-1}$  simplifies to,

$$Q_1^{-1} \approx \frac{2C_{44}}{f_1} \frac{\partial f_1}{\partial C_{44}} Q_{44}^{-1}. \quad (5)$$

127 The quality of this approximation was checked by calculating the relative sensitivity  $\frac{2C_{44}}{f_1} \frac{\partial f_1}{\partial C_{44}}$  of the first  
 128 eigenmode to the shear coefficient  $C_{44}$  for the collection of specimens. We found that close to 90% of the value of  
 129  $Q_1^{-1}$  is determined by  $Q_{44}^{-1}$ , i.e.,  $\frac{2C_{44}}{f_1} \frac{\partial f_1}{\partial C_{44}} = 89.7\% \pm 2.0\%$  (mean $\pm$ SD). From a physical perspective, the first  
 130 eigenmode of the specimens is a pure shear mode involving mostly the shear modulus  $C_{44}$  and corresponding  
 131 damping  $Q_{44}^{-1}$  [20].

#### 132 2.4. Microstructural, mineral and collagen variables

133 In the present work, we use the same data set as [25] where the measurement protocols of the microstruc-  
 134 tural, collagen and mineral variables were extensively described. Briefly, (i) Fourier transform infrared mi-

135 crospectroscopy (FTIRM) was used to measure collagen maturity (CollMat), mineral-to-organic ratio (Mi-  
136 nOrga), mineral maturity (Minmat), carbonation, and crystallinity index (CryInd); (ii) biochemical measure-  
137 ments on hydrolyzates prepared from powdered demineralized bone residues provided the amount of enzymatic  
138 cross-links (DHLNL, HLNL, PYD and DPD), non-enzymatic cross-links (PEN), and the amount of collagen  
139 (Coll); (iii) SR- $\mu$ CT (pixel size 6.5  $\mu$ m) was used to determine vascular porosity (Ct.Po) and other microstruc-  
140 tural variables (PoS/PoV, PoN, PoDm, PoSp, PoPf, ConnD and SMI), and the degree of mineralization of  
141 bone (DMB). The definitions of these variables are collected in Table 1 grouped as collagen, mineral, and  
142 microstructural variables.

### 143 2.5. Data analysis

144 Normality of the variables was evaluated using the Shapiro-Wilk test. One-way analysis of variance  
145 (ANOVA) and Wilcoxon test (for the variables failing the normality test) were performed to evaluate the  
146 differences of the data sets from lateral and medial anatomical quadrants. As some variables were not nor-  
147 mally distributed, Spearman rank correlation coefficients between  $Q_{44}^{-1}$  and each of the mineral, collagen, and  
148 microstructural variables were calculated.

149 The variables significantly correlated with  $Q_{44}^{-1}$  were retained for stepwise multiple linear regression anal-  
150 yses. To highlight the relative importance of the explanatory variables in the model, all the variables except  
151  $Q_{44}^{-1}$  were normalized between  $-1$  and  $1$  using the equation,

$$\bar{x} = 2 \frac{x - \min x}{\max x - \min x} - 1, \quad (6)$$

152 where  $x$  is the variable to be normalized. The multiple linear regression analyses were firstly carried out in  
153 each group of variables (microstructure, mineral and collagen). Then, the most significant variable of each  
154 group was retained to find the optimal multiple linear regression model. Linear models were evaluated using  
155 the adjusted- $r^2$  (Adj- $r^2$ ) and root-mean-square-error ( $RMSE$ ).

156 Note that for some variables which showed significant differences between lateral and medial specimens,  
157 the multiple linear regression analyses were carried out both on the data sets of lateral and medial specimens  
158 separately and the corresponding model are reported. If these variables were not retained as explanatory  
159 variables in the regression model, analyses were run again pooling the data sets from lateral and medial  
160 specimens.

161 Data were considered statistically significant for  $p < 0.05$ . Statistical analyses were made using the Matlab  
162 2017a Statistics Toolbox (Mathworks Inc., Natick, MA, USA).

163 We present below the statistical results for  $Q_{44}^{-1}$  measured by RUS before defatting. We found that defatting  
164 or irradiation by X-ray for tomography imaging did not significantly modify  $Q_{44}^{-1}$  (see Appendix B).

Table 1: Microstructure, mineral and collagen variables and their definitions.

Variable	Unit	Definition	Modality
<b>Microstructure group</b>			
Ct.Po	%	pore volume fraction	SR- $\mu$ CT
PoS/PoV	$\text{mm}^{-1}$	pore surface to pore volume ratio	SR- $\mu$ CT
PoN	$\text{mm}^{-1}$	pore number per millimeter	SR- $\mu$ CT
PoDm	$\mu\text{m}$	average diameter of the pores	SR- $\mu$ CT
PoS	$\mu\text{m}$	average separation between pores	SR- $\mu$ CT
PoPf	$\text{mm}^{-1}$	pore pattern factor, lower PoPf indicates higher concavity, i.e., better-connected pore network	SR- $\mu$ CT
ConnD	$\text{mm}^{-3}$	connectivity density, a measure of the degree to which a pore is multiply connected	SR- $\mu$ CT
SMI	a.u.	structure model index, the relative prevalence of rods and plates in a 3D pore network	SR- $\mu$ CT
<b>Mineral group</b>			
DMB	$\text{g}/\text{cm}^3$	Degree of mineralization of bone	SR- $\mu$ CT
MinOrga	no unit	Mineral to organic ratio, the ratio of the $\nu_1\nu_3\text{PO}_4$ area ( $910 - 1184 \text{ cm}^{-1}$ ) over the Amide I area ( $1592 - 1730 \text{ cm}^{-1}$ )	FTIRM
MinMat	no unit	Mineral maturity, the ratio of the apatitic ( $\sim 1030 \text{ cm}^{-1}$ peak) over non apatitic ( $\sim 1110 \text{ cm}^{-1}$ peak)	FTIRM
Carbon	no unit	Carbonation, the ratio of the $\nu_2\text{CO}_3$ area ( $862 - 894 \text{ cm}^{-1}$ ) over the $\nu_1\nu_3\text{PO}_4$ area	FTIRM
CryInd	cm	Crystallinity index, the inverse of the full width FTIRM at half maximum of the $\sim 604 \text{ cm}^{-1}$ peak	FTIRM
<b>Collagen group</b>			
CollMat	no unit	Collagen maturity, $\sim 1660 \text{ cm}^{-1}$ peak over $\sim 1690 \text{ cm}^{-1}$ peak	FTIRM
DHLNL	mmol/mol collagen	Didhydroxylysionorleucine, immature enzymatic cross-links	Biochemistry
HLNL	mmol/mol collagen	Hydroxylysionorleucine, immature enzymatic cross-links	Biochemistry
PYD	mmol/mol collagen	Pyridinoline, mature enzymatic cross-links	Biochemistry
DPD	mmol/mol collagen	Deoxypyridinoline, mature enzymatic cross-links	Biochemistry
PEN	mmol/mol collagen	Pentosidine, non-enzymatic cross-links	Biochemistry
Coll	%	Collagen percentage by weight	Biochemistry

### 165 3. Results

#### 166 3.1. Descriptive statistics

167 Microstructural, mineral and collagen variables and  $Q_{44}^{-1}$  values have been obtained for 52 specimens. For  
168 each specimen measured by RUS, in average 5 values of  $Q_1$  were successfully retrieved from the 6 available  
169 spectra. The average of these values, which was used for the analyses, ranged from 23.93 to 35.31 ( $30.07 \pm$   
170  $2.02$ ). The frequency  $f_1$  of the first peak ranged from 115.4 to 160.3 kHz ( $146.1 \pm 8.4$  kHz).

171 Descriptive statistics of all variables are given in Table 2. Except for  $Q_{44}^{-1}$ , the data is the same as published  
 172 in [25] but is recalled here for the convenience of the reader.

Table 2: Descriptive statistics (Mean $\pm$ SD) of  $Q_{44}^{-1}$ , microstructure and compositional variables. \* Variables in which significant difference were found between the data from lateral and medial specimens.

$Q_{44}^{-1}$				
0.0373 $\pm$ 0.0031				
Microstructure variables				
Ct.Po (%)	PoS/PoV (mm <sup>-1</sup> )	PoN (mm <sup>-1</sup> )	PoDm ( $\mu$ m)	PoSp ( $\mu$ m)
7.47 $\pm$ 4.03	60.53 $\pm$ 18.29	0.80 $\pm$ 0.22	89.39 $\pm$ 31.98	320.22 $\pm$ 31.51
PoPf (mm <sup>-1</sup> )	ConnD* (mm <sup>-3</sup> )		SMI* (a.u.)	
	L	M	L	M
30.85 $\pm$ 8.75	10.32 $\pm$ 4.99	24.75 $\pm$ 5.84	3.23 $\pm$ 0.25	3.05 $\pm$ 0.22
Mineral variables				
DMB (g/cm <sup>3</sup> )	MinOrga* (n.u.)		MinMat* (n.u.)	
	L	M	L	M
1.02 $\pm$ 0.02	5.26 $\pm$ 0.30	5.55 $\pm$ 0.26	1.84 $\pm$ 0.10	1.72 $\pm$ 0.07
Carbon* (n.u.)		CryInd* (cm)		
L	M	L	M	
0.0071 $\pm$ 0.0003	0.0066 $\pm$ 0.0002	0.0384 $\pm$ 0.0011	0.0396 $\pm$ 0.0006	
Collagen variables				
CollMat* (n.u.)		DHLNL (mmol/mol collagen)	HLNL (mmol/mol collagen)	PYD (mmol/mol collagen)
L	M			
4.54 $\pm$ 0.37	4.33 $\pm$ 0.29	567.8 $\pm$ 195.6	260.4 $\pm$ 73.4	353.1 $\pm$ 44.5
DPD (mmol/mol collagen)	PEN (mmol/mol collagen)	Coll (mmol/mol collagen)		
103.1 $\pm$ 18.3	9.9 $\pm$ 2.7	13.5 $\pm$ 0.7		

### 173 3.2. Univariate correlation analysis

174 Spearman rank correlation coefficients ( $r$ ) between  $Q_{44}^{-1}$  and the other variables are summarized in Table 3.  
 175 For the variables displaying a significant difference between the lateral and medial quadrants, i.e., ConnD, SMI,  
 176 MinOrga, MinMat, Carbon, CryInd and CollMat,  $r$  was calculated for lateral and medial group separately.

177 Among the microstructure variables, Ct.Po, PoN, and PoDm were positively correlated with  $Q_{44}^{-1}$ ,  $r$  was  
 178 0.68, 0.51 and 0.68, respectively. Negative correlations were found between PoS/PoV, PoSp, PoPf and  $Q_{44}^{-1}$ ,  $r$   
 179 was -0.68, -0.44 and -0.61, respectively. Among the mineral variables, DMB was significantly correlated with  
 180  $Q_{44}^{-1}$  ( $r = -0.68$ ). Carbon from medial quadrant was significantly correlated with  $Q_{44}^{-1}$  ( $r = 0.40$ ). Among the  
 181 collagen variables,  $Q_{44}^{-1}$ , was weakly correlated with DHLNL ( $r = 0.32$ ) and HLNL ( $r = 0.28$ ).

182 The variables that are not significantly correlated with  $Q_{44}^{-1}$ , as shown in Table 3, were not included in the  
 183 subsequent regression analyses.

Table 3: Spearman rank correlation coefficient  $r$  between  $Q_{44}^{-1}$  and microstructural properties. <sup>1</sup> $p < 0.05$ , <sup>2</sup> $p < 0.01$ , <sup>3</sup> $p < 0.001$ , n.s. not significant.

Microstructure variables								
	Ct.Po	PoS/PoV	PoN	PoDm	PoS <sub>p</sub>	PoP <sub>f</sub>	ConnD	SMI
$r$	0.68 <sup>3</sup>	-0.68 <sup>3</sup>	0.51 <sup>3</sup>	0.68 <sup>3</sup>	-0.44 <sup>2</sup>	-0.61 <sup>3</sup>	n.s.	n.s.
Mineral variables								
	DMB	MinOrga	MinMat	Carbon L      M		CryInd		
$r$	-0.68 <sup>3</sup>	n.s.	n.s.	n.s.	0.40 <sup>1</sup>	n.s.		
Collagen variables								
	CollMat	DHLNL	HLNL	PYD	DPD	PEN	Coll	
$r$	n.s.	0.32 <sup>1</sup>	0.28 <sup>1</sup>	n.s.	n.s.	n.s.	n.s.	

### 184 3.3. Multivariate regression model

185 In the multivariate regression models,  $Q_{44}^{-1}$  is the dependent variable and the microstructure, mineral and  
186 collagen variables are the independent variables (Table 4). Overall, Ct.Po and DMB are the most significant  
187 factors contributing to the variability of  $Q_{44}^{-1}$ . As for the collagen variables, the only significant variable is  
188 DHLNL which only accounts for a minor part of the variability of  $Q_{44}^{-1}$  (Adj- $r^2$  is 5.7%). In the multiple  
189 regression models using microstructure variables, Ct.Po explains most of the variations of  $Q_{44}^{-1}$  (Adj- $r^2$  is  
190 53.2%). Among the mineral variables, DMB is the most significant factor (Adj- $r^2$  is 43.2%).

191 The most significant variable of each group, i.e. Ct.Po, DMB and DHLNL, was then retained to derive a  
192 multiple linear regression model. The result is a model with only two variables, Ct.Po and DMB, explaining  
193 59.1% of the variability of  $Q_{44}^{-1}$  (Table 4). The contribution of Ct.Po and DMB to  $Q_{44}^{-1}$  are illustrated in  
194 Figure 6.

Table 4: Multiple linear regression models of  $Q_{44}^{-1}$ . In the two-variable models, only Ct.Po and DMB are included. Note that the explanatory variables have been normalized. <sup>1</sup> $p < 0.05$ , <sup>3</sup> $p < 0.0001$ .

Predicted variable	Explanatory variables	Linear model	Adj- $r^2$ (%)	RMSE
$Q_{44}^{-1}$	microstructure	$0.0396 + 0.0055 \times \overline{\text{Ct.Po}}$	53.2 <sup>3</sup>	0.0021
$Q_{44}^{-1}$	mineral	$0.0379 - 0.0051 \times \overline{\text{DMB}}$	43.2 <sup>3</sup>	0.0023
$Q_{44}^{-1}$	collagen	$0.0377 + 0.0016 \times \overline{\text{DHLNL}}$	5.7 <sup>1</sup>	0.0030
$Q_{44}^{-1}$	Ct.Po + DMB	$0.0392 + 0.0039 \times \overline{\text{Ct.Po}} - 0.0025 \times \overline{\text{DMB}}$	59.1 <sup>3</sup>	0.0020

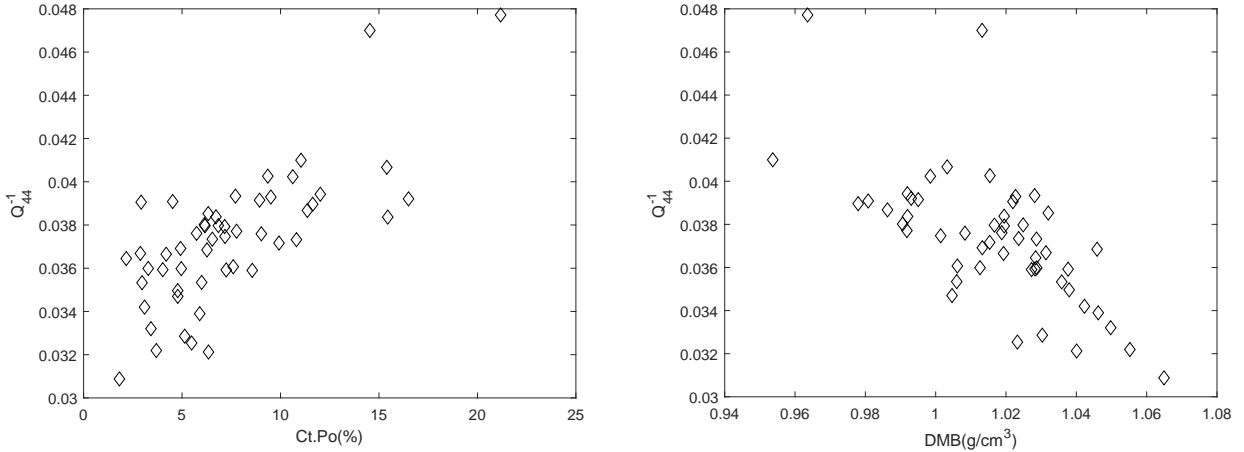


Figure 6:  $Q_{44}^{-1}$  as a function of Ct.Po (left) and DMB (right)

#### 195 4. Discussion

196 We measured with RUS the shear damping coefficient  $Q_{44}^{-1}$ , equivalent to a torsion loss tangent (usually  
 197 denoted  $\tan \delta$ ), in 52 specimens of human cortical bone from 26 elderly donors. We then investigated the  
 198 relationships between  $Q_{44}^{-1}$  and some compositional and microstructural characteristics measured with FTIRM,  
 199 biochemical analysis, and SR- $\mu$ CT.

200 The shear damping values in the present study ( $0.0371 \pm 0.0031$ ) fall in the range of values usually reported  
 201 [18]. The variations of  $Q_{44}^{-1}$  were essentially determined by the variations of vascular porosity and mineral  
 202 content: a multiple linear regression model with these two variables explained 59.1% of the variability of  $Q_{44}^{-1}$ .  
 203 Damping increased with specimen's porosity and decreased with mineral content. Adding collagen variables  
 204 did not improve this model. These relationships between damping, vascular porosity and mineral content  
 205 have not been reported before as far as we know. These results are consistent with the finding previously  
 206 reported [23] that  $Q_{44}$  (also measured with RUS) is correlated to mass density ( $r^2 = 0.72$ ); indeed, density  
 207 increases as porosity decreases and mineral content increases. Interestingly, this behavior for  $Q_{44}^{-1}$  is similar  
 208 to that observed for stiffness coefficients [25] which decrease with porosity and increase with mineral but are  
 209 also weakly dependent on collagen variables.

210 Viscoelasticity in bone may arise from a variety of mechanisms, including fluid motion inside pores, ther-  
 211 moelastic coupling, motions at interfaces such as the cement line and between lamellae of mineralized collagen,  
 212 and molecular deformation of collagen [3]. The relative importance of these mechanisms depend on the time  
 213 scale of the experiment (or excitation frequency in a dynamic experiment). Garner et al. [18] have investigated  
 214 the shear damping with an excitation frequency between  $10^{-2}$  and  $10^5$  Hz, showing a minimum of damping  
 215 around 10 Hz and a range of  $\tan \delta$  of approximately 0.01-0.08. With RUS, the measurement frequency cor-  
 216 responds to the natural resonance of the specimen; in this study, this frequency varied in a narrow range  
 217 centered at  $146.1 (\pm 8.4)$  kHz. Measurement of damping around 150 kHz have seldom been reported as most  
 218 of the viscoelastic data was obtained with dynamic mechanical analyzers (DMA) below 20 Hz or creep tests.  
 219 Accordingly, our results can only be compared with that of others with caution.

220 The relative importance of damping mechanisms also depends on the length scale of the measurement.  
221 Shepherd et al. [32] reported concurrent measurement of shear damping with a creep test on dogbone-shape  
222 specimens (several millimeters) and nanoindentation measurement of creep and found millimeter scale damping  
223 (relaxation time) about an order of magnitude larger than microscopic damping; furthermore the damping  
224 values at the two scales did not correlate. Accordingly, it is hypothesized that damping mechanisms, not  
225 captured by nanoindentation, dominate at the scale of a few millimeters: e.g., viscous damping related to  
226 fluid flow in pores, motion at mesoscale interfaces in the Haversian microstructure, or thermoelastic coupling  
227 at the mesoscale in the heterogeneous mineralized matrix. The correlation we found in the present study  
228 between porosity and damping is consistent with this hypothesis: with increased porosity the heterogeneity of  
229 the microstructure increases (leading to inhomogeneous thermoelastic damping) as well as the contact surface  
230 between fluid in pores and bone matrix (viscous damping due to fluid flow).

231 We built a multilinear model suggesting that when porosity is controlled, the variation of mineral content  
232 explains a small additional part of the variability of damping (Adj- $r^2$  of 59.1 vs. 53.2). Inter-specimen variation  
233 of mineral content may reflect different degree of homogeneity of the bone matrix (e.g., proportions of osteonal  
234 vs. interstitial tissue, age of osteons) which could affect damping through inhomogeneous thermoelastic effect  
235 and affect the viscous loss in nanoscale motion within the mineralized collagen fibrils [3]. At the scale of the  
236 mineralized collagen molecule, it was evidenced with molecular dynamics simulations that the mineral content  
237 contributes to the attenuation of stress waves [33]. The mineral characteristics other than DMB were weakly or  
238 non-significantly correlated to damping. Using nanoindentation and assessing mineral properties with FTIRM,  
239 Ojanen et al. [17] also found that mineral variables, except crystallinity, were not correlated to creep viscosity.

240 The role of matrix proteins in damping is well established [34, 35]. However, authors who have investigated  
241 the relationships between collagen and viscoelastic properties of native specimens (not chemically altered)  
242 reported weak or non significant correlations [16, 17]. In line with these works, we found that collagen  
243 variables, including cross-links properties were weakly or non significantly correlated to  $Q_{44}^{-1}$ . One possible  
244 explanation is that the variations of collagen properties in the population of donors considered are too small  
245 and that at the macroscale, the variations of damping due to changes in microstructure are dominant.

246 Our data fills a gap of knowledge as we have measured cortical bone shear damping at frequencies around  
247 150 kHz which have seldom been considered [18]. This frequency regime is not accessible with most widespread  
248 measurement techniques: it is intermediate between the lower frequencies typically accessible with commercial  
249 DMA devices to measure the phase shift  $\delta$  between a forced excitation and the oscillatory response, and  
250 higher frequencies (MHz range) of conventional ultrasonic transducers to measure wave attenuation. The  
251 measurement frequency region considered is a priori not relevant for the study of the physiological behavior of  
252 bone as physiological loading hardly contains frequencies above 100 Hz. However, some engineering applications  
253 could benefit from a better quantification of damping around 150 kHz. Low intensity pulsed ultrasound  
254 (LIPUS) devices used to stimulate bone healing work in a frequency range between 45 kHz and 3 MHz [36].  
255 Ultrasonic bone drilling in orthopedic surgery uses frequencies in the range 20-50kHz [37, 38]. Finally, some  
256 devices for the assessment of cortical bone properties for the monitoring of bone health with guided waves use  
257 frequencies around 100 kHz [39, 40].

258 It has been suggested that viscoelasticity could serve as a biomarker of skeletal fragility and bone disease  
259 [13, 14, 16]. Most often, this idea is related to the assumption that interindividual variations of viscoelasticity  
260 reflect the variability in the quality of the bone matrix which should prevent crack propagation. Our results  
261 suggest that collagen variations only have a minor effect at the mesoscale (i.e., the scale of a few millimeters),  
262 if any, on damping variability. In contrast, we found that damping is correlated to porosity, which is a well  
263 documented risk factor for fragility fracture [41] and which is related to bone strength *ex vivo* [42]. This means  
264 that damping, as it reflects porosity, is related to bone fragility, supporting the consideration of viscoelasticity  
265 measurement as a potential biomarker of fragility.

266 This study has some limitations. The accuracy of shear damping measurement is limited by the fact that  
267 it was evaluated from the measurement of the quality factor of a resonance peak by using an approximate  
268 formulae (Eq. 5). We evaluated that only about 90% of the damping value was correctly captured. The  
269 resonance peak of the specimens ranged from 115.4 to 160.3 kHz depending on the specimens dimensions,  
270 stiffness, and mass. We have disregarded a possible effect of frequency on shear damping independent of the  
271 effect of the microstructural and compositional variables. We believe this is reasonable as we did not find  
272 any correlation between the frequency of the peak and damping. Besides, bone specimens were collected at a  
273 single skeletal site (lateral and medial quadrants of the femoral diaphysis) of bones from elderly donors without  
274 documentation on the existence of bone pathologies. Therefore, the findings in this work are limited to the  
275 femoral mid-diaphysis of an aged group of donors. Further studies are warranted to investigate whether these  
276 conclusions can be extended to other skeletal sites of bone. Finally, studies with bone material representative  
277 of that of patients (e.g., osteopenic and osteoporotic patients) should be conducted in order to assess the extent  
278 to which viscoelasticity could reflect fragility for specific bone diseases.

## 279 **Declaration of interests**

280 The authors declare that they have no known competing financial interests or personal relationships that  
281 could have appeared to influence the work reported in this paper.

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292 **Appendix A. Effect of the selected bandwidth for signal analysis on the peak frequency and**  
 293 **quality factor.**

294 The quality factor of the first peak  $Q_1$  in the RUS spectrum (Fig. 4) was determined by fitting the Lorentzian  
 295 model, as explained in Methods, to a portion (bandwidth) of the spectrum containing one (Case 1) or two  
 296 (Case 2) peaks. To test the effect of the choice of bandwidth we also fitted the spectrum with a bandwidth  
 297 limited to the first peak for the 40 specimens initially in Case 2 (first two peaks relative close). Results are  
 298 given in Table A.5. There was no significant difference for  $Q_1$  and  $f_1$  tested by Wilcoxon test. There was a  
 299 slightly larger standard deviation of  $Q_1$  calculated from the several repetitions of the measurements for each  
 300 specimen due to the uptrend of the low frequency part of the second peak that was also taken into account.  
 301 Overall, these results indicate that the bandwidth selection method has a negligible effect on the values of  $f_1$   
 302 and  $Q_1$  in this study.

Table A.5: Comparison of the results (mean $\pm$ SD) of  $f_1$  and  $Q_1$  obtained by fitting the first peak or alternatively the first two peaks. The last line summarizes the standard deviation (mean (SD)) of  $Q_1$  calculated from the several repetitions of the measurements for each specimen.

$f_1$ — Two peaks	$f_1$ — One peak
$145.9 \pm 6.5$	$145.9 \pm 6.5$
$Q_1$ — Two peaks	$Q_1$ — One peak
$29.98 \pm 1.81$	$30.04 \pm 1.73$
SD of $Q_1$ — Two peaks	SD of $Q_1$ — One peak
$1.04 \pm 0.70$	$1.18 \pm 1.01$

303 **Appendix B. Effect of defatting and X-ray radiation on viscoelastic damping**

304 In order to clarify whether  $Q_{44}^{-1}$  would be affected by defatting and irradiation, RUS measurements were  
 305 conducted three times on each specimen from a subset of 24 specimens: i) on the native specimen right after  
 306 preparation, ii) after being defatted for 18 h in a chemical bath of diethyl ether and methanol (1:1), iii) after  
 307 SR- $\mu$ CT imaging which delivers a moderate radiation dose of 2.5 kGy. More details concerning defatting  
 308 and imaging protocols were given in [25]. Table B.6 summarizes the  $Q_{44}^{-1}$  values determined from the three  
 309 measurements.

Table B.6: A summary of the results (Mean $\pm$ SD) of  $Q_{44}^{-1}$  in native, defatted and irradiated specimens.

$Q_{44}^{-1}$ — Native	$Q_{44}^{-1}$ — Defatted	$Q_{44}^{-1}$ — Irradiated
$0.0375 \pm 0.0034$	$0.0385 \pm 0.0031$	$0.0378 \pm 0.0028$

310 Multiple comparison of means did not show significant difference between the different states. There was  
 311 no bias and the mean and standard deviation of differences appeared to be constant throughout the range  
 312 of  $Q_{44}^{-1}$  values. This analysis complements the analysis, conducted on the same specimens, of the effect of  
 313 defatting and irradiation on elastic properties which was reported in [26].

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