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# **Intrinsic Properties of Osteomalacia Bone evaluated by Nanoindentation and FTIRM Analysis**

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## **Abstract**

Osteomalacia is a pathological bone condition consisting in a deficient primary mineralization of the matrix, leading to an accumulation of osteoid tissue and reduced bone mechanical strength. The amounts, properties and organization of bone constituents at tissue level, are known to influence its mechanical properties. It is then important to investigate the relationship between mechanical behavior and tissue composition at this scale in order to provide a better understanding of bone fragility mechanisms associates with this pathology.

Our purpose was to analyze the links between ultra-structural properties and the mechanical behavior of this pathological bone tissue (osteomalacia) at tissue level (mineral and osteoid separately, or global). Four bone biopsies were taken from patients with osteomalacia, and subsequently embedded, sectioned, and polished. Then nanoindentation tests were performed to determine local elastic modulus  $E$ , contact hardness  $H_c$  and true hardness  $H$  for both mineralized and organic bone phases and for the global bone. The creep of the bone was also studied using a special indentation procedure in order to assess visco-elasto-plastic (creep) bone behavior. This allowed a detailed study of the rheological models adapted to the bone and to calculate the parameters associated to a Burgers model. Ultra-structural parameters were measured by Fourier Transform InfraRed Microspectroscopy (FTIRM) on the same position as the indents.

The use of rheological models confirmed a significant contribution from the organic phase on the viscous character of bone tissue. The elastic  $E$  and the elasto-plastic  $H_c$  deformation were correlated to both collagen maturity and Mineral/Matrix. The pure plastic deformation  $H$  was only correlated to the mineral phase. Our data show that mineral phase greatly affects mechanical variables (moduli and viscosities) and that organic phase (as illustrated in osteoid tissue) may play an important role in the creep behavior of bone. In conclusion, this study brings mechanical and physicochemical values for osteoid and mineral phases.

**Keywords:** Human bone tissue, osteomalacia, nanoindentation, FTIRM.

1

## 2 **I- Introduction**

3 Bone is a multiscale architected composite with a heterogeneous and hierarchical  
4 structure, consisting of mineral deposits composed of crystalline apatite on an organic matrix,  
5 presented mainly by type I collagen network (Bala et al., 2011; Burr, 2002; Oyen, 2019; Rho  
6 et al., 1998). The remodeling process contributes to calcium and phosphorous homeostasis  
7 and leads to the formation of Bone Structural Units (BSUs) (Hadjidakis and Androulakis,  
8 n.d.). The formation starts first with the synthesis of the organic matrix (osteoid) followed by  
9 mineralization of apatite crystals (Bala et al., 2010; Boivin and Meunier, 2003, 2002).

10 Osteomalacia is a bone pathology associated to a deficient primary mineralization of the  
11 matrix, leading to an accumulation of osteoid tissue and reduced bone mechanical strength  
12 (Faibish et al., 2005).

13 The mineral content in bone influences its stiffness and strength (Bala et al., 2011; Boivin  
14 et al., 2008; Follet et al., 2004), while the mechanical function of organic matrix remains  
15 barely understood (Currey, 2003). Patients affected by this osteomalacia suffer from a  
16 decreased mineralized bone leading to an increase in the organic matrix content, through the  
17 increase of the osteoid thickness (Bhan et al., 2018; Bonucci et al., 1969; Faibish et al., 2005;  
18 Turner et al., 1996). [Collagen content and chemistry may also play a role in determining](#)  
19 [viscoelastic behavior of bone](#) (Donnelly et al., 2006; Wu et al., 2012). This enables to  
20 consider this tissue as a good model to access directly to the organic matrix study (Oyen,  
21 2008; Rho et al., 1999).

22 For a better understanding of the biomechanical properties of healthy and diseased bones,  
23 several studies have been done to establish their relationships with the composition at a local  
24 scale (Bala et al., 2011, 2010; Cai et al., 2019; Farlay et al., 2010; Lefèvre et al., 2019).

25 [Indentation techniques have](#) been widely used over the past two decades to study a large  
26 range of viscoelastic materials including biological tissues and in particular bone  
27 viscoelasticity (Bembey et al., 2006; Fan and Rho, 2003; Oyen, 2005; Oyen and Cook, 2003;  
28 Rho and Pharr, 1999; Tang et al., 2007). Nano-indentation technique with a Berkovich tip can  
29 be used to investigate the tissue-level mechanical properties and site-specific variations within  
30 different individual structures (lamellae/osteons) and for different types of tissues (osteoid vs  
31 mineralized) (Bala et al., 2011; Farlay et al., 2019; Rho and Pharr, 1999; Zysset et al., 1999).  
32 Indentation tests are often analyzed assuming an elasto-plastic behaviour of the bone, with

33 time-independent elastic and plastic parameters, such as the Young's modulus  $E$ , contact  
34 hardness  $H_c$  and true hardness  $H$  as determined by (Sakai, 1999).

35 Since bone possesses inherently time-dependent behaviour, the viscous response should also  
36 be taken in consideration when analysing indentation tests (Bembey et al., 2006; Oyen and  
37 Ko, 2007). Specific testing procedures are used to characterize the viscoelastic properties of  
38 mineralized tissues, for example by applying a constant load and subsequently measuring the  
39 creep depth as a function of time. Several analytical approaches have been developed, mostly  
40 based on a combination of viscoelastic constitutive laws to account for load-time responses of  
41 bone and enamel (He and Swain, 2009; Oyen, 2005; Oyen and Ko, 2007). A Visco-Elasto-  
42 Plastic (VEP) creep model for time-dependent indentation (Oyen, 2006) has been investigated  
43 in terms of experimentally-derived variables of load, displacement, and time (Oyen and Cook,  
44 2003). Isaksson et al. have chosen a Burgers model to simulate the VEP creep response of  
45 bone, since it enables to capture the immediate elastic response during indentation (Isaksson  
46 et al., 2010b).

47 Fourier Transform InfraRed Microspectroscopy (FTIRM) presents a powerful micro-  
48 spectroscopic technique which allows the collection of detailed information about bone  
49 composition, mineral and organic properties (such as Mineral/Matrix and collagen maturity  
50 respectively), which are important for the viscous micromechanical properties (Bala et al.,  
51 2011, 2010; Bonucci et al., 1969; Boskey, 2003; Boskey and Imbert, 2017; Carden and  
52 Morris, 2000; Farlay et al., 2010; Farlay and Boivin, 2012).

53 Histologic analysis have been done (Bhan et al., 2018, 2010), but only few mechanical  
54 studies have been conducted on osteomalacia bone (Boivin et al., 2008).

55 [Our hypothesis is that specific links exist between micro-structural properties and](#)  
56 [mechanical behavior of osteomalacia bone tissue.](#) The purpose of this study was thus to  
57 characterize the variation in viscous, elastic and plastic properties of bone organic matrix at  
58 tissue level (mineral and osteoid separately, or global). Micromechanical parameters were  
59 assessed by indentation probing, dynamic (Continuous Stiffness Mode, CSM) and quasi-static  
60 (creep) testing protocols. These data were analyzed with Burger rheological model to quantify  
61 the time-dependent visco-elasto-plastic mechanical properties. Furthermore, we evaluated the  
62 relationships between these data with measured Mineral/Matrix and collagen maturity.

63

## 64 **II -Material and Methods**

### 65 **2.1. Sample preparation**

66 Four transiliac bone samples taken from 50±11 year-old patients (2 males, 2 females)  
67 with osteomalacia (one after gastrectomy, one renal osteodystrophy, two unknowns) were  
68 used (approval from Hospital Ethics Committee as an usual care study). Confirmation of the  
69 diagnosis of osteomalacia was defined on bone biopsies as a strong increase in osteoid  
70 thickness with a decrease in the mineral apposition rate. The methods used for the preparation  
71 of samples was described previously (Bala et al., 2011; Boivin et al., 2008). Briefly,  
72 undecalcified samples were fixed in 70% alcohol, dehydrated in absolute alcohol, and then  
73 embedded in poly-methyl methacrylate (PMMA). For instrumented nano-indentation, 150  
74 µm-thick sections were cut in a perpendicular plan to the Haversian canals with a precision  
75 diamond wire saw (Well, Escil, Chassieu, France), then ground progressively (silicium  
76 carbides) to a thickness of 100±1µm and polished with a diamond suspension (0.25µm)  
77 (Boivin and Meunier, 2002). These sections were indented and then re-sectioned into 2µm-  
78 thick sections to perform FTIRM (Polycut E microtome, Leica, Germany), (Figure 1).

### 79 **2.2. Nanoindentation testing**

80 Nano-indentation tests were carried out using a Nano Indenter II machine (Nano  
81 Instruments Inc., USA) equipped with a Berkovich diamond tip. The system was calibrated  
82 with fused silica according to (Oliver and Pharr, 1992)'s protocol. Bone Structural Units  
83 (BSUs) were selected on each bone biopsy, resulting in total amount of 303. Those BSUs  
84 selected were indented. The indent location was chosen at sites distant from visible lacunae or  
85 other discontinuities. Tests were also performed in the impregnation resin PMMA next to the  
86 sample, in order to characterize its properties and its possible influence on the measurement of  
87 bone tissue. Two different procedures were used: a continuous stiffness mode (CSM) test to  
88 estimate elastic and plastic parameters and a creep test to characterize the viscous parameters  
89 of the tissue at the BSU level. In CSM, 260 indents were done on four samples, with 115 in  
90 osteoid tissue and 145 in mineralized tissue, both in cortical and cancellous bone. For Creep  
91 tests, 43 indents were analyzed on three samples, 19 in osteoid tissue and 24 in mineralized  
92 tissue.

93 The CSM test followed the methods used in (Bala et al., 2011) to measure elastic  
94 modulus and hardness versus the displacement of the indenter. This procedure consisted in a  
95 displacement-controlled loading phase until a total penetration depth of 5120 nm. This value

96 enabled to characterize the tissue at the BSU scale, averaging the heterogeneity of the  
97 structure at the lamellar scale and limiting the influence of samples roughness on the  
98 measurements. A  $0.05 \text{ s}^{-1}$  constant strain rate loading stage was followed by a 10 s dwell at  
99 the maximum load, and by a 45 s withdrawal to 10% of maximum displacement, a 50 s hold  
100 period for thermal drift calculation and final withdrawal to zero displacement. The testing  
101 procedure is synthetized in Figure 2.

102 The Young's modulus ( $E$ ) and the contact hardness ( $H_c$ ) were determined following  
103 Oliver & Pharr method for the estimation of the contact area (Oliver and Pharr, 1992), using a  
104 custom Matlab code (Math Works Inc., Natick MA, USA) to process the individual load  
105 displacement curves as detailed in (Bala et al., 2011). For each indent, true hardness ( $H$ ),  
106 which reflects only the plastic character of the material was calculated based on the previous  
107 parameters ( $E$ ) and ( $H_c$ ) (Sakai, 1999). The true hardness ( $H$ ) was then deduced as described  
108 previously by (Bala et al., 2011). The total, elastic, and plastic works were also computed  
109 from the load-displacement curves, and, for analyses, only the ratio  $W_{\text{plast}}/W_{\text{tot}}$  was used since  
110 it represents surrogate measurements of irreversible mechanisms occurring during indentation  
111 (Mirzaali et al., 2016).

112 The creep experiments were conducted at a constant load of 150 mN for 240 s (4 min)  
113 to measure changes in the indentation depth versus time (Figure 2). Both loading and  
114 unloading were performed at a constant loading speed of  $15 \text{ mN} \cdot \text{s}^{-1}$ . The penetration depth  
115 after loading was typically of  $3.5 \text{ }\mu\text{m}$  and  $5 \text{ }\mu\text{m}$  for mineralized and osteoid tissues  
116 respectively, to avoid the influence of surface roughness after polishing as well as to  
117 overcome the influence of heterogeneity at the lamellar scale. The penetration depth during  
118 the dwell at constant load was recorded and was further post-processed to characterize the  
119 non-linear characteristics of the tissues, as well as the one of the PMMA as a benchmark and  
120 further analysis.

121 The analysis procedure was performed following (Vandamme and Ulm, 2006)  
122 approach on both loading and holding segments curves. Burgers model (see Figure 2C)  
123 implies four parameters: two elastic modulus  $E_1$ ,  $E_2$  and two viscosities  $\eta_1$ ,  $\eta_2$ ; parameters  
124 reflecting the local VEP behavior for each indent (Figure 2). The signification of different  
125 parameters should be understood as follows:  $E_1$  (in GPa) governs the "instantaneous" elastic  
126 behavior of the material,  $\eta_1$  (in GPa.s) characterize the permanent deformation, i.e. the plastic  
127 properties, and  $E_2$  (in GPa) and  $\eta_2$  (in GPa.s) the viscoelastic properties.  $L$  is the total length  
128 of creep during the load hold segment ( $\mu\text{m}$ ).

129 The calculation of the parameters was done by fitting experimental curves with the  
130 rheological equations of Burgers model (Fig 2C) for conical indentation as derived by  
131 (Vandamme and Ulm, 2006), using a Levenberg-Marquardt algorithm with a custom Matlab  
132 code. The partial derivatives needed for the analysis procedure have been calculated using  
133 Maple (Maplesoft, Waterloo, Canada) for verification purposes. The Berkovich tip used for  
134 experiments was considered as modeled by an equivalent conical indenter of apex  $70.3^\circ$ . The  
135 Poisson's ratio for all tests is set to 0.3, and the hypothesis of an isotropic behavior is  
136 considered.

### 137 **2.3. Fourier Transform InfraRed Microspectroscopy (FTIRM)**

138 Due to the process (re-cut section of  $2\mu\text{m}$  from the previous one), only three samples  
139 were available. FTIRM was performed on  $30\times 55\ \mu\text{m}^2$  area with 30 positions per sample,  
140 which were tested previously with nanoindentation. The spectra were collected in  
141 transmission mode with a Spectrum 100 spectrometer equipped with an Auto-IMAGE  
142 microscope (Perkin-Elmer, Shelton, Connecticut, USA). Each spectrum corresponded to 100  
143 cumulated scans. The contributions of air and PMMA were subtracted from the individual  
144 spectra, and a baseline at absorbance 2 was corrected and normalized on the  $\nu_3\text{PO}_4$ . Following  
145 the same procedure used by (Bala et al., 2011), the spectra were curve-fitted using  
146 GRAMS/AI software (Thermo galactic, Salem, New Hampshire, USA) to analyze the peaks  
147  $\nu_4\ \text{PO}_4$  ( $500\text{--}650\ \text{cm}^{-1}$ ) and amides I ( $1600\text{--}1700\ \text{cm}^{-1}$ ). A total amount of 160 spectra were  
148 analyzed.

149 The mineral to organic ratio (Mineral/Matrix) was measured as the areas ratio at the  
150 peak of  $1030\ \text{cm}^{-1}$  ( $\nu_1\nu_3\text{PO}_4$ ) and the peak of  $1660\ \text{cm}^{-1}$  which is the main peak of amides I.  
151 Collagen maturity (Col Mat) was evaluated by the ratio of the peaks area ( $1660\ \text{cm}^{-1}$  /  $1690$   
152  $\text{cm}^{-1}$ ) (Farlay et al., 2011; Paschalis et al., 2001). Crystallinity index was calculated as the  
153 inverse of the full width at half max of the  $604\ \text{cm}^{-1}$  peak (Farlay et al., 2010).

### 154 **2.4. Statistical analysis**

155 Statistical analysis was performed under SPSS v16.0 (SPSS inc., Chicago Illinois,  
156 USA) using an alpha risk set at 5%. Results were reported as mean $\pm$  standard deviation (SD).  
157 The distribution of the variables was tested with Kruskal-Wallis & Mann-Whitney  
158 nonparametric procedure. The influence of the microstructure on the micromechanical



159 behavior was studied using linear regression analyses ( $\rho$ : Spearman's Rho correlation  
160 coefficient).

161 For the paired comparison, only indents which have undergone both mechanical and  
162 physicochemical characterizations were used (Figure 1, Physicochemical parameters and  
163 CSM: 116; and creep: 43).

### 164 **III- Results**

165 Significant differences were observed in both mechanical ( $p<0.0001$ ) and  
166 physicochemical parameters ( $p<0.05$ ) according to bone tissue-type (osteoid / mineralized),  
167 (Table 1). We chose to present results for the global tissue, and for the mineral and osteoid  
168 part.

#### 169 **Physicochemical parameters (Table 1, Figure 3 A, Table 2)**

170 The Mineral/Matrix was significantly lower in the osteoid tissue than in the  
171 mineralized one (resp.  $0.20\pm 0.18$ ,  $3.62\pm 1.28$ ,  $p<0.0001$ ). Identically, collagen maturity (Col  
172 Mat) varies from  $3.39\pm 1.34$  in osteoid to  $4.23\pm 1.46$ , ( $p<0.0001$ ) in mineralized tissue,  
173 whereas crystallinity was only available in mineralized tissue  $0.04\pm 0.003$  (Table 1). Col Mat  
174 and Mineral/Matrix were positively correlated in mineralized tissue ( $\rho=0.560$ ;  $p<0.001$ ),  
175 while they were weakly and negatively correlated in osteoid tissue ( $\rho=-0.284$ ;  $p<0.05$ ),  
176 (Figure 3A, Table 2). Mineral/Matrix is significantly correlated with crystallinity in the  
177 mineral compartment ( $0.316$ ,  $p<0.01$ ). Crystallinity is also linked to Col Mat ( $0.238$ ,  $p<0.05$ ).

#### 178 **Mechanical parameters (Table 1&2, Figure 3 B&C, Figure 4)**

179 Figure 3b shows **representative** load - displacement curves for mineral, osteoid and  
180 PMMA resin. A large difference is noted in the maximum load for a similar penetration depth  
181 in each zone, revealing a higher hardness and stiffness of mineral tissue as compared to  
182 osteoid tissue and impregnation resin. Micromechanical CSM variables ( $E$ ,  $H_c$ , and  $H$ )  
183 reflecting the elastic, elasto-plastic and pure **plastic** response respectively were deduced from  
184 load-displacement curves. Their values decreased respectively from  $12.2\pm 2.7$ ,  $0.52\pm 0.12$  and  
185  $1.85\pm 0.5$ GPa in mineralized tissue to  $5.04\pm 0.8$ ,  $0.22\pm 0.05$  and  $0.81\pm 0.3$ GPa in osteoid tissue.  
186 The ratio of irreversible work to the total work represented by  $W_{\text{plast}}/W_{\text{tot}}$  is also slightly  
187 higher in mineral ( $0.71\pm 0.03$ ) than in osteoid tissue ( $0.67\pm 0.03$ ) and is significantly correlated  
188 to  $H/E$  as already noted for structural materials (data not shown, (Cheng and Cheng, 1998)).

189 Linear regression revealed strong and positive correlations ( $\rho=0.946$ ,  $p<0.001$ )  
190 between the elastic modulus ( $E$ ) and the contact hardness ( $H_c$ ), both in osteoid ( $\rho=0.730$ ;  
191  $p<0.001$ ) and  
192 in mineralized tissue ( $\rho=0.848$ ;  $p<0.001$ ) (Figure 3B, Table 2). The true hardness ( $H$ )  
193 reflecting the plastic behavior was also strongly correlated with  $E$  ( $\rho=0.856$ ,  $p<0.001$ ) for both

194 osteoid ( $\rho=0.451$ ;  $p<0.001$ ) and mineralized tissues ( $\rho=0.610$ ;  $p<0.001$ ). Except for  $H_c$  in the  
195 mineral compartment,  $W_{plast}/W_{tot}$  is significantly correlated with other mechanical parameters.  
196 Characteristic time - displacement curves in creep mode for mineral, osteoid tissues and for  
197 PMMA resin are shown in Figure 4. These curves revealed a strong difference between  
198 mechanical behavior between the different zones tested, with a larger creep displacement  $L$   
199 for PMMA resin and for osteoid tissue as compared to mineralized tissue. PMMA exhibits the  
200 largest viscous component. The values of different Creep modulus  $E_1$  and  $E_2$  and viscosities  
201  $\eta_1$  and  $\eta_2$  are larger in mineralized tissues (Table 1) than in osteoid tissue, by a factor ranging  
202 from 1.9 (for  $\eta_2$ ) to 2.9 (for  $\eta_1$ ). From a global interpretation, parameters are significantly and  
203 positively correlated (Table 2). However, the length of creep was negatively correlated with  
204 both elastic modulus of Burgers model.

205 Comparing the CSM and Creep modes using the mean within each compartment  
206 (mineral and osteoid), for the three separate samples,  $E$  and  $E_1$ , reflecting the similar elastic  
207 properties, are significantly correlated ( $\rho=0.943$ ,  $p<0.01$ ). In a similar manner, plastic  
208 parameters are linked ( $H$  and  $\eta_1$ ,  $\rho=0.943$ ;  $p<0.01$ ).

### 209 **Relationships between Physicochemical and Mechanical parameters (Table 2, Figure 5)**

210 Mineral/Matrix was significantly correlated with all micromechanical parameters ( $E$ ;  
211  $H_c$ ;  $H$ ) in the global osteomalacia bone tissue ( $\rho=0.867$ ;  $0.831$ ;  $0.761$  *resp.*,  $p<0.001$ ). These  
212 correlations, even significant, were more moderate within the mineralized tissue ( $\rho=0.647$ ;  
213  $0.558$ ;  $0.458$  *resp.*,  $p<0.001$ ). While in osteoid tissue, only the elastic modulus was  
214 significantly correlated with Mineral/Matrix ( $\rho=0.285$ ,  $p<0.05$ ) (Figure 4, Table 2). Similar  
215 results are observed for the creep mode (Table 2). Linear regression demonstrated a moderate  
216 positive correlation between collagen maturity and respectively  $E$  ( $\rho=0.272$ ,  $p<0.01$ ) and  $H_c$   
217 ( $\rho=0.220$ ,  $p<0.05$ ) for the global tissue. This relationship remains valuable only between the  
218 Col Mat and the elastic modulus  $E$  within the mineralized tissue ( $\rho=0.410$ ,  $p<0.01$ ) and  $H_c$   
219 ( $\rho=0.269$ ,  $p<0.05$ ), and no relationships has been found in osteoid tissue. Relationships are  
220 plotted in Figures 5. Crystallinity does not show relationship except with  $E$ .

221 For all bone samples, the parameters of Burgers model were significantly correlated  
222 with the Mineral/Matrix parameters (Table 2). All these correlations were positive, except for  
223 the creep length  $L$ . The viscosity  $\eta_1$  presents the highest correlation coefficient with the  
224 Mineral/Matrix, ( $\rho=0.828$ ,  $p<0.001$ ). A similar relationship was observed with the collagen

225 maturity ( $\rho = 0.554, p < 0.001$ ). Time-displacement creep curves were plotted for several  
226 indents within the same sample (Figure 4B). Each indent showed similar results with the same  
227 loading condition. More is the creep length, less is the collagen maturity.

#### 228 **IV-Discussion**

229 The present study on human subjects with osteomalacia investigated the relationships  
230 between bone visco-elasto-plastic parameters and ultrastructural components, examined at the  
231 tissue level.

232 For the physicochemical parameters, values obtained in our study confirm what previous  
233 authors (Faibish et al., 2005; Farlay et al., 2019) found with low Mineral/Matrix ratio in  
234 osteomalacia. On the other side, we found a significant difference in Col Mat in osteoid and  
235 mineral phase. There is still a positive correlation between Col Mat and Mineral/Matrix, as in  
236 control bone, showing Col Mat is dependent of the quantity of deposited mineral. In the osteoid  
237 phase, there is a high heterogeneity of Col Mat, but values remain close to those obtained in  
238 the mineral phase. This suggests that **if** the organic matrix is relatively normal, the  
239 mineralization is however disturbed **by calcium deficiency**.

240 The micromechanical properties in both dynamic (CSM mode) and quasi-static (creep  
241 mode) and also those reflecting the ultrastructural variables (Mineral/Matrix, Col Mat) were  
242 consistent with previous studies. (Bala et al., 2011, 2010; Boivin et al., 2008; Farlay et al.,  
243 2010; Isaksson et al., 2010a; Lefèvre et al., 2019). **In particular, the degree of mineralization**  
244 **is strongly correlated with the elastic modulus and hardness** (Bala et al., 2011). **This work also**  
245 **shows its correlation with the viscosity of mineralized tissue**. It is usually claimed that elastic  
246 and plastic properties are related respectively to the mineral and collagen (Viguet-Carrin et  
247 al., 2006). **A link was also recently** found between collagen fibril scale and macroscale for  
248 elastic behavior **in children's** bone (Dépalle et al., 2018). But, to our knowledge, this study is  
249 the first to focus on the link between viscous response and ultrastructural variables.

250

251 In CSM mode, compared to (Bala et al., 2011), we found that both collagen maturity and  
252 in mineral tissue were  $4.23 \pm 1.46$  , and  $0.040 \pm 0.003$  respectively, compared to  $4.69 \pm 0.76$   
253 and  $0.040 \pm 0.002$  in (Bala et al., 2011). Comparing with Vickers micro-hardness, (Boivin et  
254 al., 2008) concluded from their observations in human iliac bone that osteoid tissue  
255 represented about one third of the Hc of mineralized matrix, as in our study. As (Bala et al.,  
256 2011), we found that both collagen maturity and Mineral/Matrix play a role in elastic

257 deformation. Col Mat was also found correlated with the elasto-plastic  $H_c$  deformations, but  
258 not with the pure plastic H. But in this previous study, osteoporotic bones were used.

259 In creep mode, the osteoid tissue presents a larger value of creep length L than  
260 mineralized tissue, which reflects the primary role of organic phase in the viscous behavior of  
261 bone. For the entire tissue, L, was found also inversely correlated with the collagen maturity.  
262 Nano-indentation on demineralized tissue with acidic treatments, shows plastic deformations  
263 close to the tip of indenter correlated to a loss of the supramolecular bending structure in  
264 collagen fibrils (Tai et al., 2005). There is also evidence showing the role of collagen in the  
265 time-dependent deformation of bone under loading during indentation testing (Isaksson et al.,  
266 2010b).

267 We have shown that both collagen maturity & Mineral/Matrix were significantly  
268 correlated with most parameters of creep model, when considering the entire bone (both  
269 mineralized and osteoid tissues), confirming our hypothesis. There is also a trend to have a  
270 higher length L linked to collagen maturity in the osteoid tissue, but it is only significant when  
271 considering the global bone. Nevertheless, the major influence of Mineral/Matrix on the  
272 mechanical parameters of osteomalacia bone is clearly evidenced in this work.

273 Limitations: We investigated exclusively osteoid from diseased cases and for a quite small  
274 number of samples due to the difficulty in obtaining such specific cases. Moreover, the  
275 impregnation of bone samples by PMMA certainly has an influence on the mechanical  
276 properties of the tissues as compared to fresh samples. The qualitative comparison of  
277 properties between mineralized and osteoid tissues is however not influenced by the  
278 impregnation of PMMA resin, at the latter shows the lowest mechanical properties (Figures 3,  
279 4 and 5). The impregnation of the porous phase by PMMA may explain the low correlation  
280 between mechanical parameters with structural parameters apart from the strong influence of  
281 mineral fraction confirmed in this study. The comparison of osteoid tissue with collagen is  
282 also certainly affected by the conservation protocol of the tested samples. The use of fresh  
283 sample seems however impossible, at least, using those technics. Following the study of  
284 (Diez-Perez et al., 2010), an *in vivo* measurement of parameters reflecting bone quality could  
285 be a possible option (Crespo, 2020).

286 **In conclusion**, this study brings mechanical and physicochemical values for osteoid and  
287 mineral phases. Only few studies were able to establish those relationships. However, our data  
288 show that mineral phase greatly affects mechanical variables (moduli and viscosities) and that

289 organic phase (as illustrated in osteoid tissue) may play an important role in the creep  
290 behavior of bone. The ratio osteoid/mineral in osteomalacia bone is such higher than in a  
291 control bone, that osteoid tissue influences the behavior of the entire bone, and lead to a more  
292 ductile bone. This could cause an increase of fractures risk as the remaining mineral has to  
293 sustain the entire daily activities load.

294

#### 295 **Conflict of interest statement**

296 None of the authors have any conflicts of interest.

297

#### 298 **Ethics**

299 The work was performed in compliance with ethical standards.

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## Figure List

**Figure 1.** Different steps of identifying the type of bone tissue for each indent using the reflection and the transmission electron microscopy. **A)** Identification of indents with the image obtained by reflection microscopy, **B)** Accurate positioning of indents and their types using a color code: -Triangle: "CSM" procedure, -Round: "Creep" procedure, -Orange: Osteoid tissue, -Green: Mineralized tissue, -Blue: Resin, **C)** Image obtained by transmission electron microscopy

**Figure 2. A)** Load-time (left) and load-displacement (right) curves obtained by instrumented indentation with CSM procedure, **B)** Load-time curves (right) and load-displacement (left) obtained by instrumented indentation with Creep procedure. **C)** Equations of Burgers Rheological model applied on loading and holding segments where:  $E_1$ ,  $E_2$  correspond to elastic modules and  $\eta_1$ ,  $\eta_2$  to viscosities.  $E_1 = E_0$ ,  $E_2 = G_V * 2 * (1 + \nu_0)$ . In the case of bone tissue,  $\eta_1 \gg \eta_2$ . (with *resp*  $\eta_1 = \eta_M$  and  $\eta_2 = \eta_V$  from (Vandamme and Ulm, 2006) ).

**Figure 3. A)** Relationship between collagen maturity and Mineral/Matrix ratio in both osteoid and mineralized tissue. **B)** Representative indentation in CSM mode into the two types of tissues (mineralized, Osteoid) and for Methyl methacrylate PMMA, **C)** Correlations between contact hardness ( $H_c$ ), true hardness ( $H$ ) and elastic modulus ( $E$ ). Those plots show the strong linear dependence of  $H_c$  with elastic properties whereas  $H$ , reflecting plastic deformation is moderately linked to  $E$ .

**Figure 4 A)** Representative creep curve, **B)** Example of a displacement-time curves obtained during instrumented indentation method with the "creep" mode for six indents from the same sample, located in global tissue. An indicator of the maturity of collagen is placed to see the variation of this parameter according to the creep length.

**Figure 5** Correlations obtained between parameters measured by FTIRM (left) Mineral/Matrix ratio, (Right) Collagen maturity and the micromechanical properties: **A)**  $E$ , **B)**  $H$ , and **C)**  $H_c$ , for the global bone tissue measurement and with separation of mineralized and osteoid phases. Mineral: dark black circle, Osteoid: open orange circle. Values for the measured parameter in the resin (PMMA) are in square green. Corresponding Rho Spearman correlation coefficient are indicated for each group.

## Table List

**Table 1.** Descriptive statistics+ p-value for Mann-Whitney differences between mineral and osteoid phases

**Table 2.** Spearman coefficient of correlation between CSM, Burgers model mechanical properties and physicochemical properties. (-): NS: Non significant, \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$

**Table 1**

	Parameters	unit	Tissue Type	n	Mean $\pm$ SD	Median	Min	Max	P-value	
<b>FTIRM</b>	Mineral/Matrix		Global	156	2.15 $\pm$ 1.96	1.86	0.065	5.83	<0.0001	
			Osteoid	67	0.20 $\pm$ 0.18	0.13	0.065	0.78		
			Mineral	93	3.62 $\pm$ 1.28	3.93	0.18	5.83		
	Crystallinity	cm								
				Osteoid		N/A				
				Mineral	86	0.040 $\pm$ 0.003	0.040	0.030	0.048	N/A
<b>CSM</b>	Collagen maturity		Global	141	3.90 $\pm$ 1.47	3.96	1.04	7.79	<0.0001	
			Osteoid	55	3.39 $\pm$ 1.34	3.49	1.31	6.39		
			Mineral	86	4.23 $\pm$ 1.46	4.19	1.04	7.79		
	E	GPa	Global	259	9.03 $\pm$ 4.11	8.12	3.36	16.84	<0.0001	
			Osteoid	115	5.04 $\pm$ 0.77	4.90	3.36	7.58		
			Mineral	144	12.22 $\pm$ 2.66	12.92	5.01	16.84		
			PMMA	20	3.59 $\pm$ 0.46	3.70	2.72	4.20		
Hc	GPa	Global	259	0.39 $\pm$ 0.18	0.38	0.11	0.76	<0.0001		
		Osteoid	115	0.22 $\pm$ 0.05	0.22	0.11	0.39			
		Mineral	144	0.52 $\pm$ 0.12	0.53	0.23	0.76			
			PMMA	20	0.15 $\pm$ 0.03	0.16	0.09	0.20		
<b>CREEP</b>	H	GPa	Global	259	1.39 $\pm$ 0.67	1.27	0.28	3.02	<0.0001	
			Osteoid	115	0.81 $\pm$ 0.28	0.77	0.28	1.99		
			Mineral	144	1.85 $\pm$ 0.51	1.89	0.62	3.02		
				PMMA	20	0.49 $\pm$ 0.16	0.54	0.25	0.73	
	W <sub>plast</sub> /W <sub>tot</sub>		Global	259	0.70 $\pm$ 0.03	0.70	0.61	0.77	<0.0001	
			Osteoid	115	0.67 $\pm$ 0.03	0.67	0.61	0.74		
Mineral			144	0.71 $\pm$ 0.03	0.72	0.61	0.77			
			PMMA	20	0.68 $\pm$ 0.02	0.68	0.66	0.72		
<b>FTIRM</b>	E <sub>1</sub>	GPa	Global	41	31.62 $\pm$ 19.71	27.42	7.18	78.65	0.0002	
			Osteoid	18	17.70 $\pm$ 10.05	14.39	10.62	51.34		
			Mineral	23	42.52 $\pm$ 10.05	38.18	14.9	78.65		
	E <sub>2</sub>	GPa	Global	43	5.65 $\pm$ 2.15	6.34	2.58	9.08	<0.0001	
			Osteoid	19	3.50 $\pm$ 0.39	3.42	2.58	4.41		
			Mineral	24	7.35 $\pm$ 1.19	7.44	4.26	9.08		
	$\eta_1$	GPa.s	Global	43	2491 $\pm$ 1326	2647	955	4763	<0.0001	
			Osteoid	19	1213 $\pm$ 259	1097	955	1933		
			Mineral	24	3504 $\pm$ 861	3521	1517	4763		
	$\eta_2$	GPa.s	Global	43	4.86 $\pm$ 1.85	4.31	1.49	9.76	<0.0001	
			Osteoid	19	3.23 $\pm$ 0.71	3.49	1.49	4.28		
			Mineral	24	6.15 $\pm$ 1.39	5.94	3.70	9.76		
L	$\mu$ m	Global	43	0.61 $\pm$ 0.20	0.55	0.36	0.94	<0.0001		
		Osteoid	19	0.81 $\pm$ 0.11	0.84	0.60	0.94			
		Mineral	24	0.46 $\pm$ 0.08	0.44	0.36	0.70			

**Table 2**

			Mineral/Matrix	Crystallinity	Collagen maturity	E	Hc	H	W <sub>Plast</sub> /W <sub>tot</sub>	E <sub>1</sub>	E <sub>2</sub>	η <sub>1</sub>	η <sub>2</sub>
<b>IRM</b>	Crystallinity	Mineral	0.316**										
	Collagen maturity	Global	0.395***										
		Osteoid	-0.284*	N/A									
		Mineral	0.560***	0.238*									
<b>CSM</b>	E	Global	0.867***		0.272**								
		Osteoid	0.285*	N/A	-0.08NS								
		Mineral	0.647***	0.269*	0.410**								
	Hc	Global	0.831***		0.220*	0.946***							
		Osteoid	0.163 NS	N/A	-0.07 NS	0.730***							
		Mineral	0.558***	0.250 NS	0.269*	0.848***							
	H	Global	0.761***		0.183 NS	0.856***	0.970***						
		Osteoid	0.04 NS	N/A	-0.04 NS	0.451***	0.914***						
		Mineral	0.458***	0.207 NS	0.166 NS	0.610***	0.924***						
	W <sub>Plast</sub> /W <sub>tot</sub>	Global	0.575***	-0.01NS	0.106NS	0.681***	0.481***	0.322***					
		Osteoid	0.15NS	N/A	-0.06NS	0.233*	-0.383***	-0.693***					
		Mineral	0.111NS	-0.01NS	0.08NS	0.415***	0.02NS	-0.252**					
<b>CREEP</b>	E <sub>1</sub>	Global	0.718***		0.401*	0.943**	1*	1*	0.600 NS				
		Osteoid	0.474*	N/A	-0.286 NS								
		Mineral	0.133 NS	0.304 NS	0.068 NS								
	E <sub>2</sub>	Global	0.797***		0.539***	0.971***	0.971***	0.971***	0.618 NS	0.648***			
		Osteoid	0.339 NS	N/A	0.132 NS					-0.255 NS			
		Mineral	0.172 NS	0.157 NS	0.116 NS					0.322 NS			
	η <sub>1</sub>	Global	0.828***		0.554***	0.829*	0.943**	0.943**	0.714 NS	0.807**	0.896***		
		Osteoid	0.261 NS	N/A	0.42 NS					0.136 NS	0.616***		
		Mineral	0.406*	0.388 NS	0.018 NS					0.756**	0.61***		
	η <sub>2</sub>	Global	0.714***		0.465***	0.878*	0.878*	0.878*	0.878*	0.515**	0.909***	0.777***	
		Osteoid	-0.08 NS	N/A	-0.06 NS					-0.625**	0.504*	0.694NS	
		Mineral	0.003 NS	0.170 NS	0.025 NS					-0.015NS	0.769***	0.24NS	
	L	Global	-0.831***		-0.605***	-0.899*	-0.812*	-0.812*	-0.67NS	-0.700***	-0.890***	-0.971***	-0.890*
		Osteoid	-0.25 NS	N/A	-0.47 <sup>BL</sup>					-0.122NS	-0.660***	-0.886***	0.132NS
		Mineral	-0.445*	-0.222NS	-0.134 NS					-0.685***	-0.565***	-0.910***	-0.06NS

\* : p<0.05, \*\* : p<0.01 ; \*\*\* : p<0.001, BL : Borderline 0.05<p<0.06, NS : non significant

Figure 1

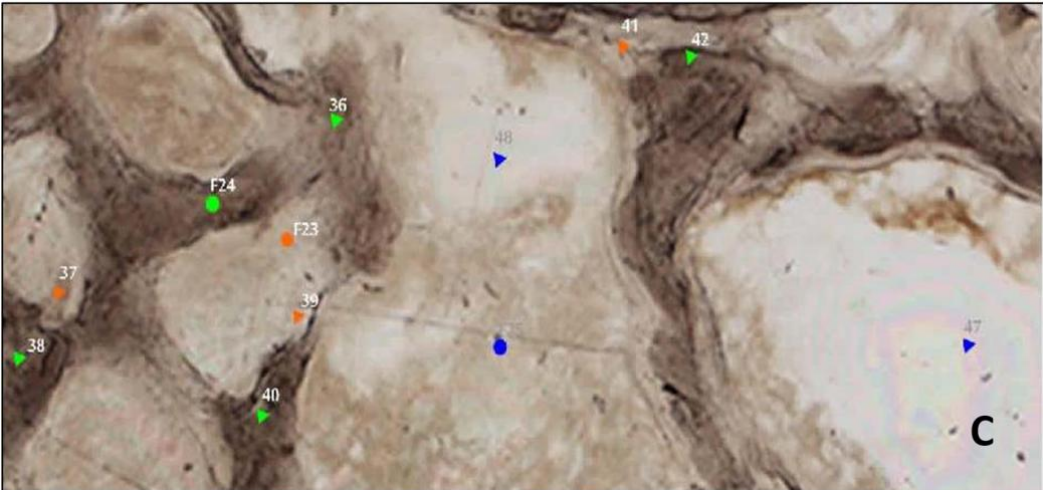


Figure 2

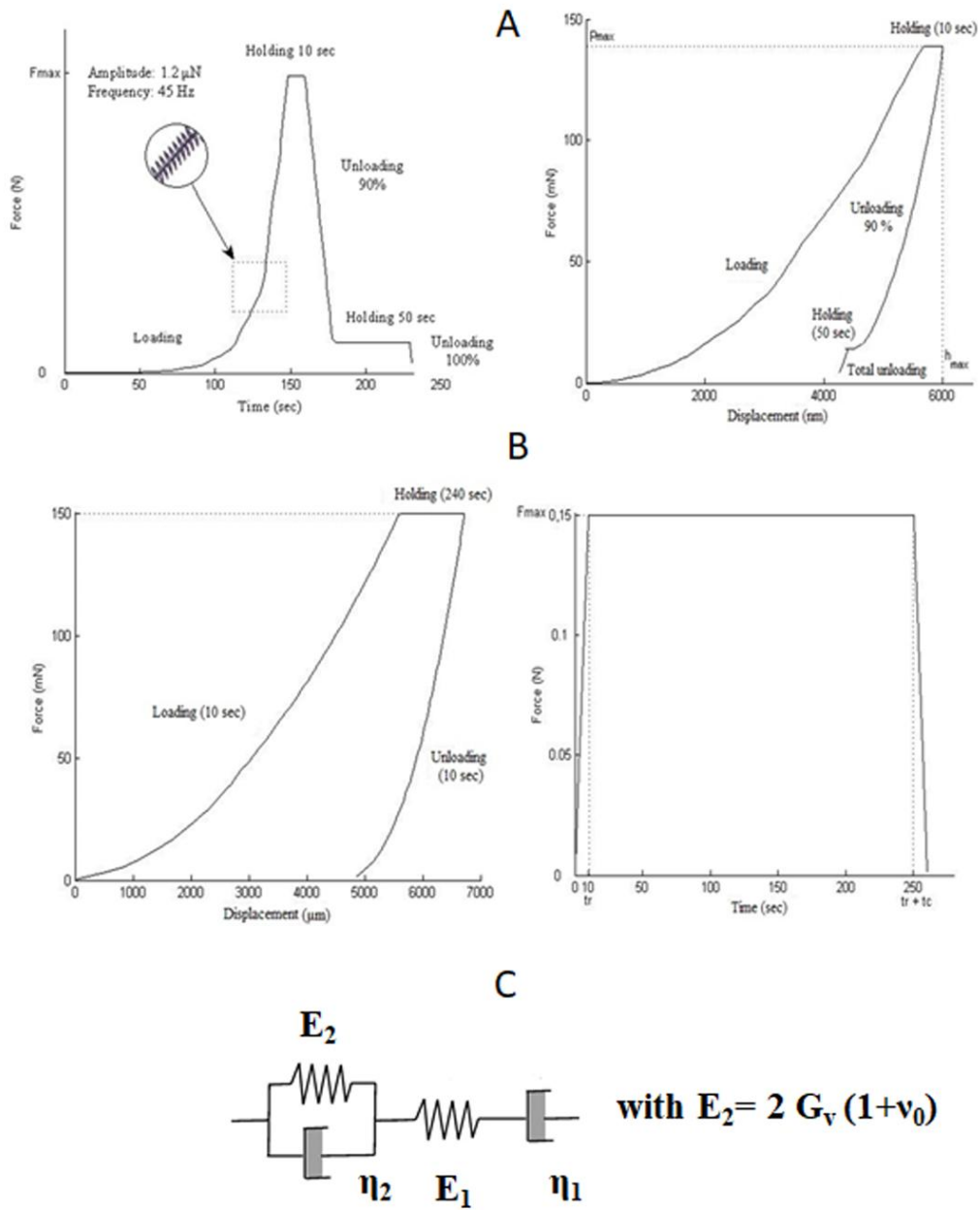


Figure 3.

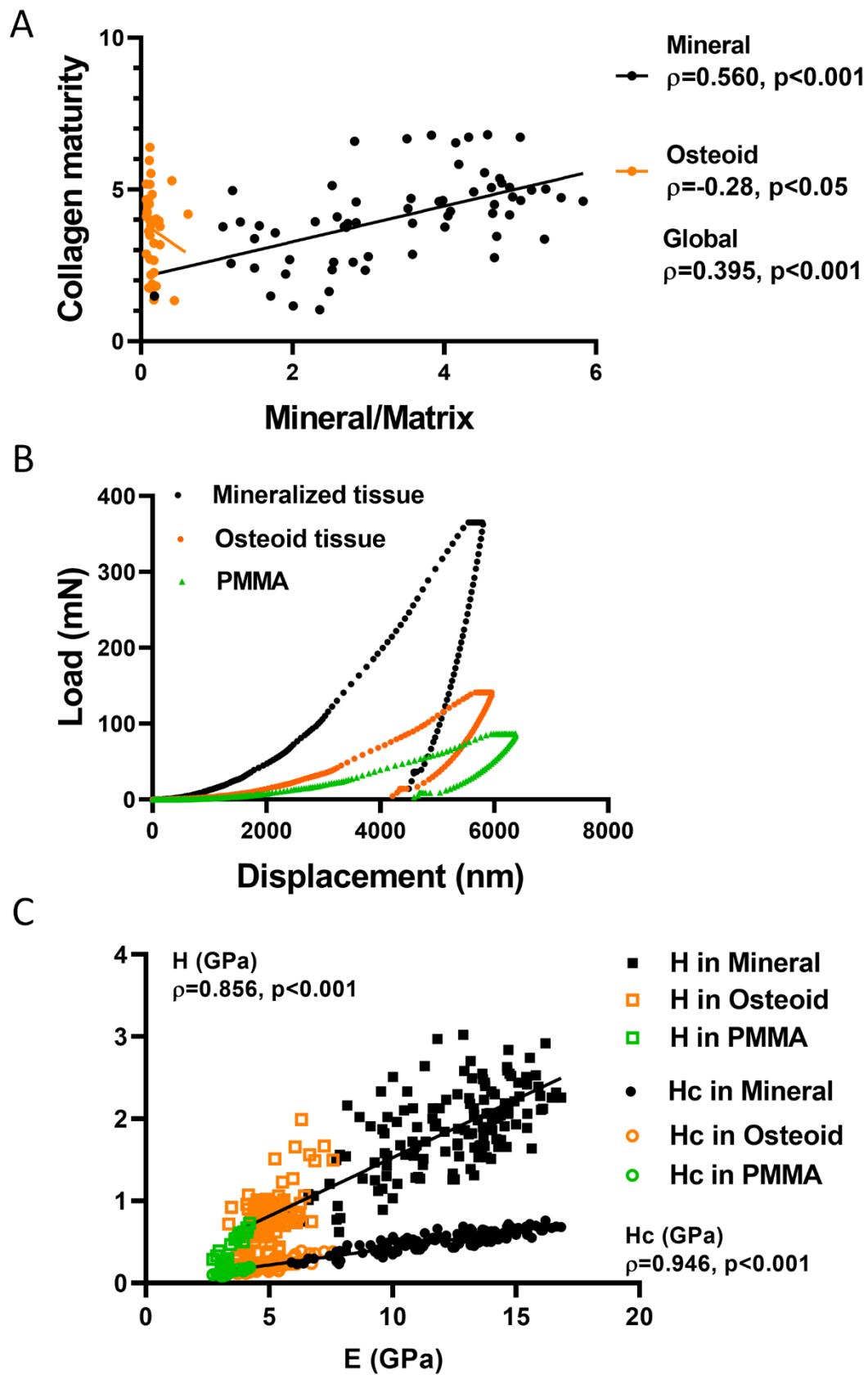


Figure 4

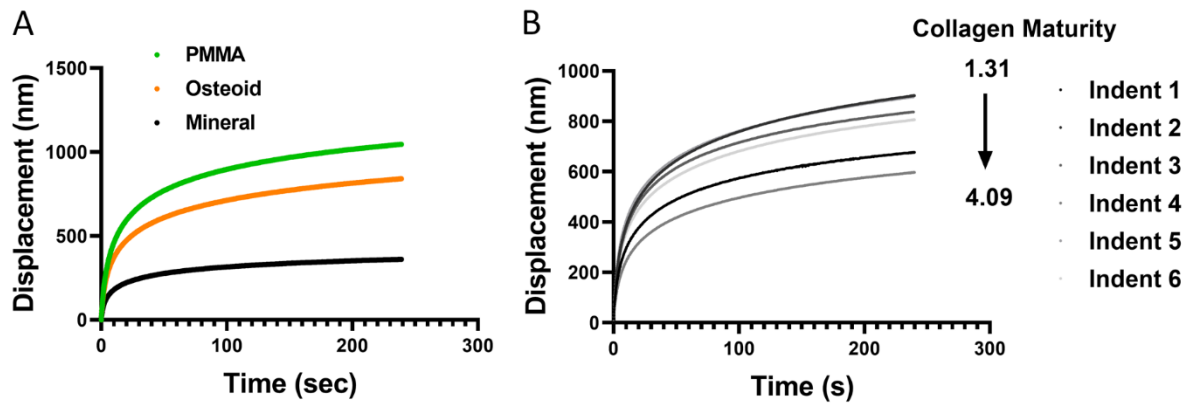




Figure 5

