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1 **Low-cycle full-field residual strains in cortical bone and their**
2 **influence on tissue fracture evaluated via in situ stepwise and**
3 **continuous X-ray computed tomography**

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22 Abstract

23 As a composite material, the mechanical properties of bone are highly dependent on its
24 hierarchical organisation, thus, macroscopic mechanical properties are dictated by local
25 phenomena, such as microdamage resulting from repetitive cyclic loading of daily activities.
26 Such microdamage is associated with plastic deformation and appears as a gradual
27 accumulation of residual strains. The aim of this study is to investigate local residual strains in
28 cortical bone tissue following compressive cyclic loading, using in situ X-ray computed
29 tomography (XCT) and digital volume correlation (DVC) to provide a deeper insight on the
30 three-dimensional (3D) relationship between residual strain accumulation, cortical bone
31 microstructure and failure patterns. Through a progressive in situ XCT loading-unloading
32 scheme, localisation of local residual strains was observed in highly compressed regions. In
33 addition, a multi-scale in situ XCT cyclic test highlighted the differences on residual strain
34 distribution at the microscale and tissue level, where high strains were observed in regions
35 with the thinnest vascular canals and predicted the failure location following overloading.
36 Finally, through a continuous in situ XCT compression test of cycled specimens, the full-field
37 strain evolution and failure pattern indicated the reduced ability of bone to plastically deform
38 after damage accumulation due to high number of cyclic loads. Altogether, the novel
39 experimental methods employed in this study, combining high-resolution in situ XCT
40 mechanics and DVC, showed a great potential to investigate 3D full-field residual strain
41 development under repetitive loading and its complex interaction with bone microstructure,
42 microdamage and fracture.

43 **Keywords:** Cortical bone, in situ mechanics, cyclic loading, X-ray computed tomography,
44 digital volume correlation, residual strains.

45

46 1. Introduction

47 Cortical bone is a complex composite material whose structure is hierarchically organized from
48 the nano- to the macroscale to withstand physiological loads and resist fracture (Wolfram and
49 Schwiedrzik, 2016). Yet, the mechanical competence of bone is often impaired by the
50 accumulation of microdamage due to isolated overloading events (Gauthier et al., 2019;
51 Morgan et al., 2005) or after suffering fatigue from a large number of loading cycles (Burr et
52 al., 1997; Diab et al., 2006; Schaffler et al., 1995; Zioupos and Currey, 1998). Microdamage
53 in cortical bone tissue is manifested in the form of microcracks or diffuse damage (O'Brien et
54 al., 2007). Microcracks, in particular, are thought to play an important role in bone fracture
55 behavior as well as in bone remodelling, mechanotransduction and the bone toughening
56 mechanism (Voide et al., 2009). The formation of microcracks is dependent on the loading
57 mode, (Mirzaali et al., 2015; Reilly and Currey, 1999) and they are also influenced by the
58 morphological complexity and porosity of cortical bone (Loundagin et al., 2020; Turnbull et al.,
59 2014). Under cyclic loading, microcracks can grow and cause fractures in bone, clinically
60 known as stress fracture (Zioupos et al., 1996), thus there is considerable interest in
61 understanding the failure mechanism of cortical bone following cyclic loading.

62 The mechanics of cortical bone subjected to cyclic loading has been previously investigated
63 with a focus on the fatigue life and microcrack propagation (Fletcher et al., 2014; Kim et al.,
64 2007; Nalla et al., 2005; Zioupos et al., 2008, 2001). Traditionally, the degradation in the
65 mechanical properties due to repetitive loads has been derived from micromechanical tests
66 (i.e. cyclic tensile/compressive loading) using damage indicators such as modulus reduction
67 in relation to the cycle number and residual strains upon unloading (Bajaj et al., 2014; Fleck
68 and Eifler, 2007; K. Winwood et al., 2006; K. L. Winwood et al., 2006). Residual strains are
69 generally assessed as the translation along the strain axis at zero stress from traditional
70 stress-strain curves, thus being a measurement of the plastic deformation of the material (K.
71 Winwood et al., 2006; K. L. Winwood et al., 2006). The influence of residual strain in the fatigue
72 life and strength of cortical bone has been characterized in several studies (Fleck and Eifler,

73 2007; Morgan et al., 2005; K. Winwood et al., 2006; K. L. Winwood et al., 2006); however,
74 how the microarchitecture affects the fatigue life of bone and, in particular, the residual strain
75 accumulation is still lacking, mainly due to the difficulty of relating the macroscopic mechanical
76 behavior to the cortical bone microstructure. Since the structure-mechanics relationship is a
77 key factor in bone damage, a three-dimensional (3D) characterization of the residual strains
78 due to cyclic loading remains essential.

79 To date, the only experimental technique that allows for 3D full-field strain is digital volume
80 correlation (DVC), which in combination with X-ray computed tomography (XCT) has been
81 extensively used in bone mechanics to investigate the deformation mechanism under different
82 loading conditions (Christen et al., 2012; Peña Fernández et al., 2020, 2019). Particularly,
83 Christen et al. (2012) investigated the role of cortical bone microstructure in the initiation and
84 propagation of microcracks in notched cortical bone specimens under compression by
85 measuring the local strains in the tissue, revealing the complex interaction between microcrack
86 propagation and bone microarchitecture. However, the measured strains resulted from time-
87 lapsed compression testing, while the effect of cyclic loading and residual strains upon
88 unloading were not the object of that study. The potential of DVC in evaluating residuals strains
89 under cyclic compression has been previously explored by Tozzi et al. (2014) in bone-
90 biomaterial composites. The progressive damage accumulation under cyclic loading at the
91 bone-biomaterial interface was shown, as evidenced by the initiation of cracks associated with
92 high residual strains. Nevertheless, the intricacy of such biphasic structure together with the
93 limited resolution of the XCT images (i.e. 20 μm) achieved, could not allow the characterization
94 of local residual strain within bone tissue as well as its spatial correlation with bone
95 microstructure. Therefore, by using high-resolution XCT in combination with cyclic mechanical
96 testing and DVC an in depth understanding of the local residual strain in cortical bone tissue
97 will be enabled.

98 In this work, a series of experiments were carried out in order to assess the ability of DVC
99 based on high-resolution XCT images to evaluate 3D full-field residual strains in cortical bone

100 tissue subjected to **low-cycle compressive loading**. In particular, this study aims at
 101 investigating the residual strain accumulation in relation to the applied level of compression
 102 and number of cycles as well as the spatial correlation of local residual strains, intracortical
 103 porosity and failure patterns following a compressive overload.

104 2. Materials and methods

105 2.1. Specimen preparation

106 Cortical bone specimens were obtained from the diaphysis of a fresh bovine femur. A 20 mm-
 107 thick section was cut from the middle of the femur and a diamond-coated coring tool was used
 108 to extract 4 mm diameter cylindrical plugs. The ends of the specimens were then trimmed with
 109 a bandsaw to achieve a 10 mm length. All cutting occurred under constant water irrigation and
 110 all specimens were wrapped in gauze, soaked in phosphate buffered saline (PBS) and stored
 111 at $-20\text{ }^{\circ}\text{C}$ until testing. Prior to the experiment the ends of the specimen were cleaned, dried
 112 and embedded into brass endcaps using a custom jig to minimize testing uncertainties and
 113 achieving a nominal final length of 8 mm (2:1 aspect ratio).

114 2.2. In situ XCT mechanical testing

115 The specimens were divided in three groups and underwent three different in situ XCT
 116 mechanical tests, as described in Table 1 and Figure 1.

117 Table 1. Description and aims of the in situ XCT mechanical tests performed in this study. n: number of cortical
 118 bone specimens. DLS: Diamond Light Source.

Experiment	XCT system	Loading stage	n	Aim
Progressive compression test	Zeiss Versa 510	Deben CT500	3	To explore the capability of DVC to assess differences between volumetric and residual strains.
Multi-scale cyclic compression test	Zeiss Versa 510	Deben CT5000	3	To examine the spatial correlation between localized strains, intracortical

				porosity and failure patterns after overloading in a multiscale manner.
Continuous compression test	I13-2 DLS	Deben CT5000	3	To investigate differences in volumetric strain progression and failure mechanism in relation to the number of applied cyclic loads.

119

120 2.2.1. Progressive compression test

121 Cortical bone specimens ($n = 3$) were placed within an environmental chamber filled with PBS
122 in a micromechanical device (CT500, Deben Ltd, UK) that was positioned in the chamber of a
123 high-resolution X-ray microscope (Versa 510, Zeiss, USA) (Figure 1-I). First, a preload of ~ 50
124 N was applied to ensure end contact prior to testing followed by in situ uniaxial compression
125 test at incremental strains of 0.5%, 1% and 2% using a progressive load-unload-reload
126 scheme (Fig. 1-I, right) (Nyman et al., 2009a, 2009b). In each cycle, the specimen was first
127 loaded under displacement control to the target deformation level at a rate of 1 mm/min; then
128 held steady for image acquisition. Thereafter, the specimen was unloaded to zero-strain state
129 (preload configuration) and held there for image acquisition, and then reloaded again to the
130 next strain level. At each loaded-unloaded state XCT images were acquired (80 keV, 7W, 3.5
131 μm voxel size, 2.5 s exposure time, 1800 projections), after two repeated scans in the preload
132 configuration for DVC zero-strain error analysis (Dall'Ara et al., 2017). In total, eight
133 tomographic datasets were acquired for each specimen.

134 2.2.2. Multi-scale cyclic compression test

135 In situ XCT uniaxial cyclic compression testing of cortical bone specimens ($n = 3$) was
136 performed using a loading stage (CT5000, Deben Ltd, UK) placed in the X-ray microscope
137 (Versa 510, Zeiss, USA) (Figure 1-II). Specimens were mounted within a custom-made
138 chamber and immersed in PBS throughout the test. Prior to cyclic testing, XCT images of the
139 intact specimens were acquired at two different resolutions for a multi-scale evaluation. First,

140 an overall XCT scan (110 keV, 10 W, 5 μm voxel size, 5 s exposure time, 1200 projections)
141 was performed to include the entire specimen diameter within the field of view (FOV, 5mm x
142 5mm); then, a high-resolution XCT scan (80 keV, 7 W, 2 μm voxel size, 12 s exposure time,
143 1600 projections) of the centre of the specimen (FOV: 2mm x 2mm) was acquired. Following
144 acquisition of the first pair of XCT images, each of the specimens was subjected to 5, 30 or
145 100 cycles of uniaxial compression at a maximum strain of 0.5% and a frequency of 0.2 Hz,
146 after which the XCT imaging procedure was repeated (unloaded state). Finally, specimens
147 were loaded monotonically up to failure and XCT scans acquired after the load was released.
148 In total, three (i.e. intact, cycled and failed) pairs (i.e. 5 μm and 2 μm voxel size) of images
149 were acquired for each sample. Two additional cortical bone specimens were imaged in the
150 same conditions twice consecutively to allow for DVC zero-strain error analysis (Dall'Ara et
151 al., 2017).

152 2.2.3. Continuous compression test after cyclic loading

153 In situ SR-XCT continuous compression testing ($n = 3$) was performed at the Diamond-
154 Manchester Imaging Branchline I13-2 of Diamond Light Source (UK) (Figure 1-III). A filtered,
155 partially coherent, polychromatic 'pink' beam (5-35 keV) of near-parallel geometry with an
156 undulator gap of 5 mm was used. Projections were recorded by a sCMOS pco.edge 5.5 (PCO
157 AG, Germany) detector, coupled to a 500 μm -thick CdWO_4 scintillator and a visual light
158 microscope with a 2 \times objective lens. Pixel binning (4 \times) was used to achieve better signal and
159 faster framerates, resulting in an effective voxel size of 6.5 μm and a FOV of 4.2 x 3.5 mm.
160 1441 projection images were collected over 180 $^\circ$ of continuous rotation with a exposure time
161 of 15 ms (plus 2 ms read-out), resulting in an estimated radiation dose of ~ 0.53 kGy/tomogram
162 (Marta Peña Fernández et al., 2018). Specimens were placed within the PBS-filled
163 environmental chamber of the loading device (CT5kN, Deben Ltd, UK) and subjected to 5, 30
164 or 100 cycles of uniaxial compression (0.5% maximum strain, 0.2 Hz). Following the cyclic
165 loading scheme, a preload (50 N) was applied to ensure end-contact and continuous loading
166 was performed at a constant crosshead speed of 0.01 mm/min up to failure, with SR-XCT

167 images acquired simultaneously. For all specimens, two repeated scans (preload state) prior
168 to loading were acquired for DVC zero-strain error analysis (Dall'Ara et al., 2017). In total, 18,
169 16 and 13 tomograms were acquired for the specimen cycled 5, 30 and 100 times,
170 respectively; thus, the total radiation dose remained below 15 kGy, minimizing the possibility
171 of radiation-induced damage (Barth et al., 2010; Marta Peña Fernández et al., 2018).

172 2.3. Image postprocessing

173 XCT images acquired in the lab-system were reconstructed using the manufacture's software
174 (TXM Reconstructor, Zeiss, USA). Following image reconstruction, the XCT datasets were
175 rigidly aligned using as reference the first acquired tomogram and denoised using a non-local
176 means filter (Avizo 9.4, ThermoFisher, US). Intracortical porosity was segmented using Otsu's
177 thresholding and the vascular canal network was separated from the osteocyte lacunae by
178 removing the unconnected objects with a volume below $500 \mu\text{m}^3$ (Cardoso et al., 2013). The
179 morphometry of the canal network was determined by the total canal volume (Ca.V), canal
180 volume density (Ca.V/Ct.TV) and mean canal diameter (Ca.D) using BoneJ (Doubé et al.,
181 2010) plugin in FIJI (Schindelin et al., 2012).

182 SR-XCT images were flat-field and dark-field corrected prior to image reconstruction using
183 Savu (Atwood et al., 2015), which incorporated ring artefact suppression. Dedicated Matlab
184 (v2018a, MathWorks, USA) scripts were developed to rigidly aligned the deformed datasets
185 to the reference (unloaded) and to denoise them using an anisotropic diffusion filter.

186 2.4. Digital Volume Correlation

187 DVC (DaVis v10.05, LaVision, Germany) analysis was performed to evaluate the 3D full-field
188 strains in cortical bone specimens subjected to the different mechanical tests. The DaVis
189 software is based on a local approach of deformable registration and further details on the
190 operating principles of the algorithm are detailed elsewhere (M. Peña Fernández et al., 2018).
191 The acquired zero-strain repeated scans were used to evaluate strain uncertainties (i.e. mean
192 absolute strain (MAER) and standard deviation of the error, SDER (Palanca et al., 2016)) with

193 sub-volumes ranging from 8 to 80 voxels. The final DVC-schemes used for each in situ XCT
 194 test and the corresponding strain errors are summarized in Table 2.

195 Table 2. Summary of DVC-schemes used for each experiment and the corresponding strain uncertainties (MAER,
 196 SDER) for the n number of specimens analysed. MAER and SDER are reported as mean (standard deviation).

Experiment	Voxel size (μm)	DVC scheme (voxel)	n	MAER ($\mu\epsilon$)	SDER ($\mu\epsilon$)
Progressive compression test	3.5	64-56-48-40	3	401 (126)	101 (37)
Multi-scale cyclic compression test	5.0	88-80-72-64	2	207 (11)	82 (8)
	2.0	72-64-56-48	2	220 (33)	122 (16)
Continuous compression test	6.5	72-64-56-48	3	467 (24)	112 (11)

197

198 Volumetric strain (Eq. 1) was computed for the evaluation of the strain distribution in
 199 specimens under compressive load (Fig. 1-I, III), whereas Von Mises Equivalent strain (Eq. 2)
 200 was used to assess the residual strain distribution after unloading (Fig. 1-I) or cyclic testing
 201 (Fig. 1-II).

$$\epsilon_{vol} = \epsilon_1 + \epsilon_2 + \epsilon_3 \quad (1)$$

With ϵ_1 , ϵ_2 and ϵ_3 being the principal strains.

$$\epsilon_{eq} = \frac{2}{3} \sqrt{\frac{3(e_{xx}^2 + e_{yy}^2 + e_{zz}^2)}{2} + \frac{3(\gamma_{xy}^2 + \gamma_{xz}^2 + \gamma_{yz}^2)}{4}} \quad (2)$$

$$e_{xx} = \frac{2}{3}\epsilon_{xx} - \frac{1}{3}\epsilon_{yy} - \frac{1}{3}\epsilon_{zz}$$

$$e_{yy} = \frac{2}{3}\epsilon_{yy} - \frac{1}{3}\epsilon_{xx} - \frac{1}{3}\epsilon_{zz}$$

$$e_{zz} = \frac{2}{3}\varepsilon_{zz} - \frac{1}{3}\varepsilon_{xx} - \frac{1}{3}\varepsilon_{yy}$$

$$\gamma_{ij} = 2\varepsilon_{ij}$$

202 3. Results

203 3.1. In situ XCT progressive compression

204 Cortical bone specimens subjected to progressive compression test presented small
 205 differences in their morphology (i.e. Ca.V/Ct.TV = 2.6 ± 0.4 % and Ca.Dm = 55.8 ± 8.5 μm)
 206 and apparent elastic modulus ($E_{\text{app}} = 2.82 \pm 0.11$ GPa). The highest modulus correlated to the
 207 lowest canal volume and the thinnest canals. The internal strain distribution (i.e. ε_{vol} and ε_{eq})
 208 showed a similar pattern for the three specimens. A progressive strain accumulation for both
 209 loaded (ε_{vol}) and unloaded (ε_{eq}) states was observed at increasing applied strain amplitudes,
 210 with the absolute value of ε_{vol} higher than ε_{eq} at all steps (Fig. 2). Maximum local compressive
 211 strains ($\varepsilon_{\text{vol}} < 0$) were higher than tensile ($\varepsilon_{\text{vol}} > 0$) and residual strains (ε_{eq}) (Fig. 2a), with more
 212 than 10% of cortical bone volume showing local compressive values below -4000 μe at 2%
 213 compression for all specimens; whereas tensile strains remained always below 4000 μe and
 214 less than 2% of bone volume experienced residual strains above 4000 μe . All specimens
 215 showed a non-uniform strain distribution, with local strains building up during compression
 216 (Fig. 2b, c). The spatial co-localization of highly strained regions in loaded and unloaded states
 217 is shown in Figure 3 and it highlights the predominance of negative ε_{vol} . Residual strains after
 218 each incremental applied compression accumulated in cortical bone regions that were
 219 previously highly compressed.

220 3.2. In situ multiscale XCT cyclic testing

221 Increasing the resolution from 5 μm to 2 μm for the multiscale XCT imaging allowed to identify
 222 not only the cortical canal network but also the osteocyte lacunae (Fig. 1-II), resulting in
 223 improved DVC spatial resolution from 320 μm to 96 μm , which led to different internal strain
 224 distributions for the overall scan (Fig. 4a) compared to the zoom-in region (Fig. 4b). The overall
 225 residual strains (ε_{eq}) were highly homogeneous for all the specimens and a slight increase of

226 ϵ_{eq} values after 100 cycles was observed compared to the less cycled specimens (Fig. 4a). A
227 more complex and heterogeneous ϵ_{eq} distribution was experienced at tissue level (Fig. 4b),
228 with local strains exceeding those measured at a lower resolution and reaching maximum
229 values over 2000 $\mu\epsilon$ in some areas. Such local strain concentrations were more important
230 after 100 cycles of compression. Highly strained regions were found around thinnest canals
231 for all the specimens (Fig. 5a, b). The specimens subjected to 5 and 30 cycles showed
232 longitudinal cracks after failure (Fig. S3), which were localised in proximity to the previously
233 identified areas with high residual strains (Fig. 5c). No visible damage was identified within the
234 FOV of the highest resolution image of the most cycled specimen (Fig. 5c) and only small
235 cracks were observed when examining the entire FOV (Fig. S3).

236 3.3. In situ SR-XCT continuous compression

237 The evolution of ϵ_{vol} during continuous compression for the specimens subjected to 5, 30 and
238 100 cycles is shown in Figure 6 in terms of average and standard deviation of strain in the
239 analysed volumes. All samples presented an almost linear increasing trend on the average
240 ϵ_{vol} values prior to failure. Strain heterogeneity increased after yielding and built-up on the
241 onset of crack formation (Supplementary Video 1). Failure occurred earlier for the most cycled
242 specimen at a lower average ϵ_{vol} ($\sim -1600 \mu\epsilon$) compared to the specimens cycled 5 or 30
243 times, which experienced minimum ϵ_{vol} of $\sim -4000 \mu\epsilon$ before failure (Fig. 6). The strain
244 distribution was highly homogeneous for the all specimens prior to failure and heterogeneity
245 in the strain field only increased when cracks were visible (Fig. 7), with positive ϵ_{vol} in regions
246 of crack opening and negative ϵ_{vol} in regions highly compacted. The damage initiation and
247 progression between the specimens was different, with the less cycled specimens displaying
248 a structural collapse due to the presence of a main longitudinal crack running through the
249 entire cortical volume, while cracks in the more cycled specimen did not pierce the volume
250 longitudinally (Supplementary Video).

251 4. Discussion

252 The accumulation of microdamage during cyclic loading plays a key role in weakening cortical
253 bone and leading to complete fracture as a result of the degradation in its mechanical
254 properties, which is evidenced by the development of residual strains upon unloading. The
255 main goal of this study was to explore the capability of DVC to assess local residual strains in
256 cortical bone tissue following in situ XCT cyclic loading in order to gain a greater understanding
257 of the 3D relationships between residual strain accumulation, cortical bone microstructure and
258 failure pattern.

259 The first experiment herein presented aimed at investigating the spatial correlation between
260 volumetric strains in loaded cortical bone specimens and residual strains upon unloading
261 following a progressive loading scheme (Fig. 1-I) similar to that introduced by Wang and
262 Nyman (2007). Maximum ϵ_{vol} and ϵ_{eq} local strains in the tissue increased in a nearly linear
263 relationship with the applied deformation (Fig. 2), consistent with the observations of Nyman
264 et al. (2009a, 2009b). DVC-computed ϵ_{eq} were used as a non-directional measurement of the
265 residual (plastic) local strains (Morgeneyer et al., 2014); this enabled a good representation of
266 the strain localization with respect to bone microstructure and allowed a direct comparison
267 with ϵ_{vol} in the loaded specimens (Fig. 2, 3). In this study, the DVC computation successfully
268 showed the coupling of residual strain accumulation and highly compressed regions at low
269 levels of global strains (Fig. 3). Similar findings were observed by Tozzi et al. (2014) in bone-
270 biomaterial composites, where high residual strains after cyclic loading were found in the most
271 strained regions during uniaxial compression. However, the XCT spatial resolution achieved
272 in that study (i.e. 20 μm) did not allow for an in-depth characterization of localized residual
273 strain within the bone tissue.

274 The multi-scale in situ XCT cyclic test evidenced differences on the residual strain distributions
275 after cyclic loading at different dimensional scales (Fig. 4). Despite the ϵ_{eq} was found to slightly
276 increase with higher number of cycles at the microscale (Fig. 4a), the presence of high local
277 residual strains as an indicator of microdamage could only be appreciated at the tissue level

278 (Fig. 4b). The inhomogeneity of local strains in the bone matrix was previously described by
279 Nicolella et al. (2005) and Hoc et al. (2006) at a higher resolution (up to 0.4 μm) using digital
280 image correlation based on optical microscopy images. In agreement with those studies, local
281 residual tissue strains here were mostly above 2000 $\mu\epsilon$, whereas microscopic average strains
282 only accounted for 500 $\mu\epsilon$. These results highlight the need for a multi-scale mechanical
283 characterization of bone, as the macroscopic properties (i.e. modulus reduction, global
284 residual strains) are not sufficient to accurately predict the source and potential incidence of
285 damage due to local residual strains build-up in the tissue.

286 Cortical bone mechanical properties are strongly governed by its microstructure and
287 intracortical porosity has previously been reported to explain a significant amount of variance
288 in bone strength and fatigue life (Carter et al., 1976; Loundagin et al., 2020; Turnbull et al.,
289 2014; Zioupos et al., 2008). Particular attention has also been given to the contribution of
290 canal diameter as a predictor of the overall fatigue behaviour of cortical bone (Loundagin et
291 al., 2020; Yeni et al., 1997), with vascular canals as stress concentrators. In the current study,
292 highest local residual strains (i.e. above 1500 $\mu\epsilon$) following cyclic loading were observed in
293 regions with thinnest canals (Fig. 5). Despite the presence of a high number of smaller canals
294 in cortical bone may increase its fracture toughness and fatigue life (Loundagin et al., 2020;
295 Yeni et al., 1997), the DVC-computed ϵ_{eq} suggested that localized areas with the thinnest
296 canals may accumulate more microdamage due to larger amount of stress concentration in
297 which microcracks typically initiate. Interestingly, such highly strained regions after 5 and 10
298 compressive cycles could predict the location where fracture occurred following overloading
299 (Fig. 5). Conversely, fracture in the more cycled specimen (i.e. 100 cycles) could not be
300 identified within the imaged FOV despite an evident force drop was observed in the
301 mechanical curve (Fig. S4), suggesting the main failure may have occurred outside the FOV.
302 To better understand the effect that damage accumulation may have had on the overall
303 fracture outcome, the final in situ SR-XCT test investigated the full-field ϵ_{vol} distribution during
304 continuous compression, showing a major fracture throughout the specimens subjected to 5

305 and 10 cycles and several cracks progressively propagating through the most cycled
306 specimen (100 cycles) (Supplementary Video 1). Following cyclic loading, some damage had
307 already occurred and the ability of bone to progress further damage would decrease, leading
308 to a decrease in fracture initiation toughness (Fletcher et al., 2014). This in line with the lower
309 ϵ_{vol} magnitudes (i.e. below 3000 $\mu\epsilon$ in compression) that such specimen (100 cycles)
310 accumulated during continuous compression prior to failure, which eventually led to fracture
311 in a more brittle way due to the reduced bone plasticity when compared to the less cycled
312 specimens. Additionally, following failure, DVC-computed strains based on real-time
313 compression indicated tensile strain in regions where cracks opened and compressive strain
314 in highly compacted areas (Fig. 7b, Supplementary video 1).

315 This study has some limitations. A small number of specimens were used, making the study
316 unable to support statistical analysis; therefore, providing a more qualitative than quantitative
317 evaluation as typically achieved in high-resolution XCT-based DVC studies. Additionally, all
318 experiments presented were performed on different specimens, hence a correlation between
319 the observations could not be determined. Finally, the accumulation of residual strains at
320 increasing compressive cycles was not conducted, due to the long acquisition time needed for
321 high-resolution imaging; extending the duration of the test could impact the mechanical
322 properties of bone due to the effect of X-ray irradiation (Barth et al., 2010; Marta Peña
323 Fernández et al., 2018). Nonetheless, the combination of in situ high-resolution XCT imaging
324 and DVC employed in this study allowed for a deeper understanding on the mechanical
325 behaviour and failure mechanisms of cortical bone following cyclic loading, showing for the
326 first time 3D full-field residual strain accumulation at low cycles as a potential predictor of
327 tissue failure due to overloading. The results reported in this study have the potential to
328 produce a significant impact in the understanding of fracture mechanism in pathological
329 conditions (i.e. osteoporosis), by further investigating the complex interplay of local residual
330 strain accumulation, increased porosity and fatigue microcracks initiation/propagation pattern.

331 Conflict of interest

332 The authors declare no conflict of interest.

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339 CRediT author statement

340 **Marta Peña Fernández:** Conceptualization, Methodology, Validation, Formal Analysis,
341 Investigation, Data Curation, Writing – Original Draft, Writing – Review and Editing,
342 Visualization, Project administration, Funding Acquisition. **Alexander P. Kao:** Investigation,
343 Writing – Review and Editing. **Frank Witte:** Resources, Writing – Review and Editing, Funding
344 Acquisition. **Hari Arora:** Investigation, Writing – Review and Editing, Funding
345 Acquisition. **Gianluca Tozzi:** Conceptualization, Resources, Writing – Reviewing and Editing,
346 Supervision, Project administration, Funding Acquisition.

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