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Nonlinear ultrasound monitoring of fatigue microdamage accumulation in cortical bone

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***Abstract*— Accumulation of bone micro-damage is suspected to lead to severe impairment of mechanical properties with an increase in skeletal fragility and fracture risk. The objective of the study was to evaluate the potential of Nonlinear Resonant Ultrasound Spectroscopy (NRUS) for measuring micro-damage accumulation in cortical bone using four-point bending cycling fatigue. Sixteen human cortical bone specimens were machined as parallelepiped beams. Damage progression was controlled by measuring the linear elastic beam theory modulus (E_{LEBT}), known to reflect microdamage accumulation. Before and between each damage step, the nonlinear ultrasonic elastic coefficient was measured by NRUS. At the end of each cycling fatigue, a subset of bone samples was measured by µCT at the European Synchrotron Radiation Facility. Results showing a progressive increase of nonlinear ultrasonic elastic coefficient along fatigue cycling suggest that NRUS measurements are sensitive to micro-damage accumulation. The results mentioned above were validated using synchrotron radiation µCT. The variation of elastic nonlinearity was found to be significantly correlated to the variation of number density of small microcracks which almost doubled in damaged regions.**

Keywords-component; nonlinear; NRUS; microcracks; bone; fatigue

I. INTRODUCTION

Bone microdamage is a natural phenomenon caused by daily loading, such as walking, jumping or carrying heavy load. Microdamage manifests as linear microcracks and diffuse damage. It is typically of little consequence under normal bone self-repair capability through targeted remodeling. Impaired repair capabilities following upon deficient turnover caused by disease or drug absorption result in an accumulation of microdamage which is then suspected to lead to severe impairment of mechanical properties such as decrease of bone toughness, stiffness and ultimate load. Such loss of biomechanical competence may lead ultimately to an increase in skeletal fragility and fracture risk [1].

Histomorphometry is the current gold standard to characterize damage accumulation *in vitro*. This technique is inherently invasive and destructive, is limited to the measurement of a small number of 2-D cross-sections and does not allow investigating the whole volume. These limitations have led to the emergence of alternative techniques. Micro-computed tomography (µCT) with contrast agent has been suggested for quantification and localization of microdamage in the whole specimen volume. However the resolution of about 10µm remains well below that allowed by histomorphometry and is not efficient to detect isolated microcracks and to assess their geometry. This limitation is overpassed by synchrotron radiation micro-computed tomography (SR-µCT) which is the only 3-D imaging technique capable to assess bone microcrack geometry at a micro-scale resolution [2].

At ultrasonic frequencies (approximately 100 kHz—2 MHz), linear characteristics of elastic wave transmission through bone, such as frequency-dependent attenuation and speed of sound, are widely used to assess skeletal status and predict osteoporotic fracture risk [3]. However, it was found that these parameters are insensitive to mechanically induced damage in human bone likely due to the fact that these parameters governing the wave equation fail to be sensitive to discontinuities at the meso or micro scale [4].

More recently, nonlinear acoustics techniques well known to be far more sensitive than linear acoustics to detect microcracks in diverse materials (e.g. concrete, composite), were applied to bone microdamage detection without quantification [5, 6]. A preliminary *in vitro* study by our group suggested that accumulation of damage induced by mechanical fatigue in compression of cortical bone was reflected by hysteretic nonlinear elastic properties measured by nonlinear resonant ultrasound spectroscopy (NRUS) [7] . The results mentioned above have to be validated against histology or high resolution µCT. In the present paper, we

report the results of NRUS measurements in cortical bone specimens that were subjected to four-point bending fatigue. The results are compared to the level of damage measured by SR-µCT measurements.

II. MATERIAL AND METHODS

A. Specimen preparation and measuring protocol

Sixteen human cortical bone samples were prepared from the femoral mid-diaphysis of four female donors (age = 88.5±9.8). Femurs were removed during multi-organ collection. Ethical approval for the collection of samples was granted by the Human Ethics Committee of the Centre du don des Corps at the University Paris Descartes (Paris, France). The tissue donors or their legal guardians provided informed written consent to give their tissue for investigation, in accord with legal clauses stated in the French Code of Public Health. The specimens were wet machined as parallelepiped beams (50*4*2mm), defatted and stored at -20°C until experiments. Initial NRUS measurements were performed for all samples to determine their initial nonlinear properties before any mechanical fatigue. The specimens were then taken through a fatigue protocol in four-point bending as described below, during which mechanical parameters were determined. NRUS measurements were repeated after each cycling session. Four damage steps were achieved. After each damage step, three specimens were removed for 3-D SR-µCT investigations of microdamage.

B. Nonlinear Resonant Ultrasound Spectroscopy (NRUS)

Microcrack accumulation in a material sample is responsible for a softening of the material for increasing excitation amplitudes, leading to a decrease of the resonance frequency when excitation amplitude increases. The NRUS technique exploits the resonant behavior of samples but with progressively increasing excitation levels to retrieve the nonlinear elastic behavior of the material. In resonance, it can be shown that the frequency shift Δf is proportional to the peak strain amplitude $\Delta\varepsilon$ via the nonlinear elastic (α_f) parameter:

$$\frac{f - f_0}{f_0} = \frac{\Delta f}{f_0} = \frac{\alpha_f}{2}\Delta\varepsilon \qquad (1)$$

where f is the resonant frequency at increased strain level, f_0 its corresponding value at the lowest drive amplitude [8]. The parameter α_f, so-called the nonlinear elastic hysteretic parameter, characterizes the hysteretic nonlinearity that occurs for strain levels above approximately 10^{-5} [8] in damage materials and convey information about the amount of hysteretic nonlinearity (damage accumulation) in the material.

The principles of NRUS measurements have been extensively described elsewhere [9]. Briefly, a piezoceramic emitter glued on a backload was bonded at one end of the specimen to ensure free-fix boundary conditions for NRUS measurements. Each sample was probed by a swept-sine encompassing the first resonant modes of the cortical beam (assumed to be pure compression modes under symmetric loading conditions). The peak resonant frequency f is measured as a function of strain applying increasing voltage drive level. The dynamic strain amplitude ε was calculated from the longitudinal particle displacement U at one end of the sample measured by a laser vibrometer (LSV, SIOS, Germany):

$$\varepsilon = \frac{\delta U}{\delta x} = U * k = U * \frac{2\pi}{4L} \qquad (2)$$

where k is the wave number and L is the specimen length. The nonlinear parameter α_f can be calculated from the strain-dependent resonance peak data, using Eq. 1. The usual NRUS measuring protocol was adapted to enhance the sensitivity to subtle variations of bone nonlinearity [10]. Toward this goal, the reference measurement f_0 was repeated before each excitation level and then used to compute α_f. During the NRUS measurements, specimens were kept at fixed temperature (37°C ±0.1°C) and relative humidity (15%±5%) into a climate chamber.

C. Biomechanical testing

The piezoceramic emitter attached to the specimen for NRUS measurements was removed before each mechanical testing. All specimens were progressively damaged by cyclic four-point bending at 2Hz in a saline solution at 37°C (±1°C) using a hydraulic testing machine (INSTRON, 8802, High Wycombe, England) with a 1kN loading cell (accuracy 0.5%) and the internal displacement transducer (accuracy 1%). In this configuration, damage is expected to occur specifically in the mid region of the sample [11], while the ends remaining intact may be used as control. Initial Young's modulus was determined during pre-cycling after 20 cycles (E_{pre}).

From the initial Young's modulus, the load ($Fmax$) corresponding to 5000µε at the mid-span was computed for all specimens [11]. The four-point bending fatigue was then applied between -10N and $-Fmax$. During the cycling session, load and displacement curves were recorded to extract linear elastic beam theory (LEBT) modulus (E_{LEBT}) as defined by Landrigan [12]. E_{LEBT} is a combination of elastic (secant modulus) and plastic (residual strain) biomechanical parameters. After each damage step, the E_{LEBT} modulus is normalized by the initial value measured for the first loading cycle of the first damage step. E_{LEBT} has been shown to decrease as bone microdamage accumulates [11-13]. A progressive damage was performed in four steps (one step=one cycling session), each step corresponding to a reduction of 10% of E_{LEBT}.

D. 3-D synchrotron radiation µCT (SR-µCT)

At the end of each cycling fatigue, a subset of 3 bone samples were measured by SR-µCT at the European Synchrotron Radiation Facility, Grenoble, France on beam-line ID19 [2]. The photon energy was 25 keV and the size of the region of interest (ROI) was 2.8x2.8x1.96mm^3 with a voxel size of 1.4 µm. Two different ROIs were investigated: ROI1 located in the load-free region at one distal end of the sample assumed to be free of damage (except initial pre-fatigue damage) and ROI2 located in the central portion of the

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beam where microdamage is assumed to accumulate. Microdamage was characterized on 12 regularly spaced 2-D transverse cross-sections (Fig. 1) extracted from the 3-D reconstructed bone volumes by measuring the number density of microcracks (Cr.Dn [#.mm²]) and length (Cr.Le [µm]), using the software ImageJ (NIH, USA)) with the plugin NeuronJ (Erik Meijering, The Netherlands).

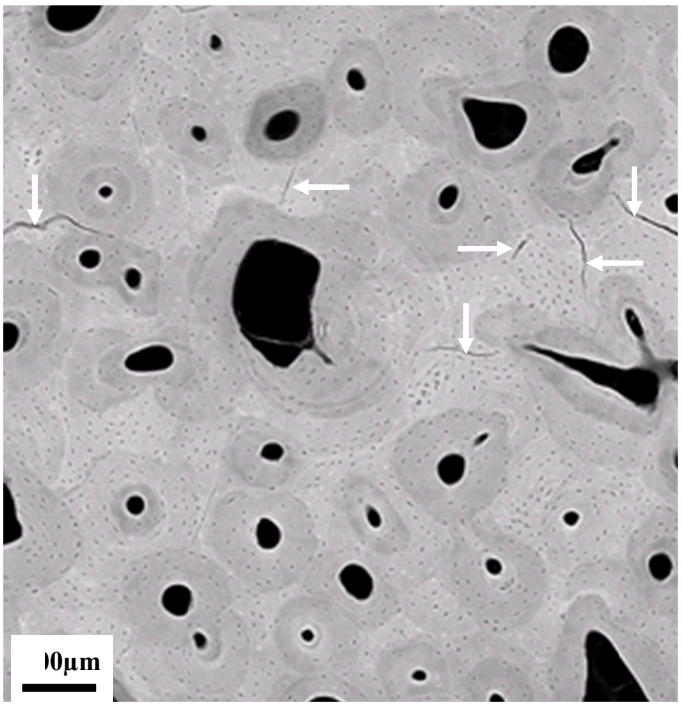

Figure 1. Transverse 2-D high resolution µCT cross-section with microcracks (white arrows)

E. Data analysis

Matlab 7.8 with statistics toolbox 7.6 (Mathworks, USA) was used for statistical analyses. One-way analysis of variance (ANOVA) was used to test for differences among different levels of nonlinear elasticity achieved at each steps of the fatigue protocol. The effect of fatigue loading on microdamage characteristics in the control region and the damaged region was investigated with a non parametric Wilcoxon signed rank test. The relationship of the nonlinear elastic parameter α_f with microdamage characteristics was obtained using regression analyses and Spearman correlation test. The significance level is measured using a p-value $p<0.05$.

III. Results

A. Biomechanical testing

The measurement precision of biomechanical parameters was assessed on five dedicated specimens not included into the final protocol. The initial Young's modulus was estimated for each sample. Then they went through 20 cycles (after system stabilization) of four-point bending test. The process was repeated 6 times with repositioning. The coefficient of variation was found to be 2.4% for E_{LEBT}. The number of cycles required to achieve the desired E_{LEBT} reduction at each damage step was found to vary between the specimens (e.g., 1182±966 cycles for the first cycling session) despite homogeneous initial biomechanical properties (apparent dry density=1792±155g.mm^{-3} and E_{LEBT}=15.1±3.0GPa).

B. Ultrasonic (NRUS) measurements

The measurement precision of NRUS, assessed by the coefficient of variation of three measurements with intermediate debonding and repositioning, was found to be 8.5% for α_f. The initial nonlinear values α_f= -5.6±2.7 for the first compression mode were consistent with values previously reported for undamaged cortical bovine bone (α_f=-5.0±2.5) [10]. $\Delta\alpha_f/\alpha_f$ representing the relative change after each damage step of α_f with respect to its initial value is represented against damage step in Fig. 2 using the Box and Whiskers Plot. Note that the number of specimens included in each cycling session decreased as a function of the damage step, because three specimens were removed for SR-µCT acquisition after each session. In particular, three outliers showing a dramatic relative increase of α_f higher than 150% after the 2nd and 3rd fatigue steps are not represented in Fig. 2 for the sake of clarity and were removed for the ANOVA analysis. One-way ANOVA of $\Delta\alpha_f/\alpha_f$ showed a significant effect of the damage steps (F=7, p=0.001)

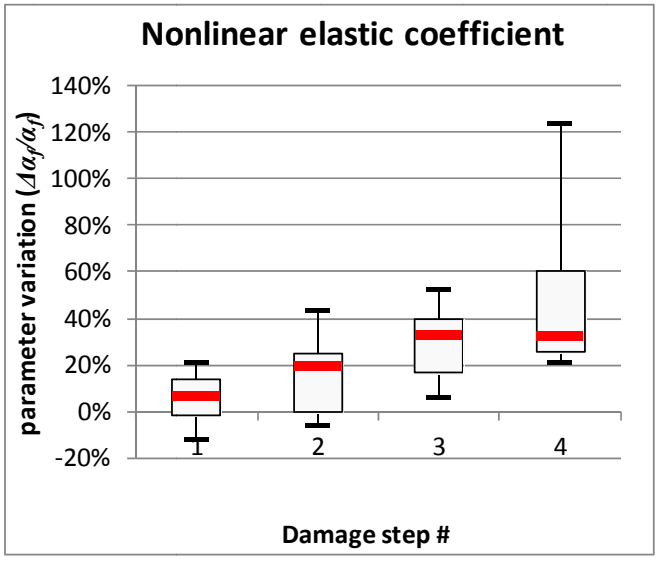

Figure 2. Nonlinear elastic coefficient variation ($\Delta\alpha_f/\alpha_f$) after each damage step

C. Damage characteristics

Taking into account all detected microcracks on µCT images, the measured microcracks characteristics were not statistically different between ROI1 (Cr.Le=71.8±13.7µm; Cr.Dn=1.92±1.19#/mm²) and ROI2 (Cr.Le=65.3±10.3µm; Cr.Dn =2.20 ±1.25#/mm²). In contrast, fatigue cycling resulted in a significant increase of the density of small microcracks. Indeed, by taking into account only small microcracks with a length falling in the first quartile (Cr.Le<31.93±8.05µm), crack density almost doubled between the control region ROI1 (Cr.Dn= 0.37±0.26#/mm²) and the damage region ROI2

(Cr.Dn= 0.63±0.45#/mm²) (p=0.01). Moreover, the relative variation of density of small microcracks between ROI2 and ROI1 was found to be significantly correlated to the relative variation $\Delta\alpha_f/\alpha_f$ (r^2=0.6, p<0.05) (Fig.3).

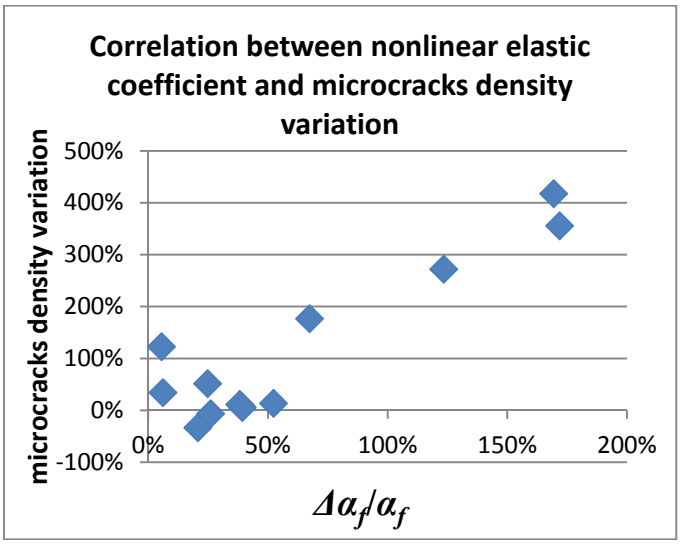

Figure 3. Correlation between nonlinear elastic coefficient variation ($\Delta\alpha_f/\alpha_f$) and tiny microcracks density variation

IV. Discussion and Conclusion

This is the first study reporting the modulus E_{LEBT}, the nonlinear elastic hysteretic parameter α_f and microdamage characteristics derived from SR-µCT that were concurrently assessed in human cortical bone specimens during a four point-bending fatigue cycling protocol. The progressive 10% decrease of E_{LEBT} after each fatigue cycling session is strongly suggestive of microdamage accumulation [11, 13-15]. Our results showed a progressive decrease of the normalized hysteretic parameter α_f, suggesting that NRUS measurements are indeed sensitive to damage accumulation.

High resolution µCT investigations evidenced a significant increase in small microcracks number which almost doubled in damaged regions compared to their number in load-free (control) regions. These small microcracks, with length falling in the lowest quartile, are suspected to be newly formed microcracks as a result of fatigue cycling. The relative variation of nonlinear elasticity was significantly related to the relative variation of the number density of these small cracks.

Altogether, our results evidence, for the first time, a relationship between the nonlinear elastic parameter α_f measured by NRUS and bone microdamage characteristics reflected by the density of supposedly newly formed microcracks during fatigue cycling. To conclude, our experimental results are indicative of the potential of NRUS measurements for monitoring non-invasively microdamage accumulation in cortical bone.

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