Assessment of osteoporosis through laser speckle imaging: an *in vitro* model

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Background, Motivation and Objective. The Osteoporosis is a pathological process that decreases the bone mineral density, weakening the bone structure (doi:10.1016/S0140-6736(02)08761-5). It manly affects postmenopausal women (doi:10.1007/s00198-014-2655-z). Its clinical diagnosis is performed by the dual-energy X-ray absorptiometry technique (doi:10.1016/j.mcna.2015.01.010). This technique uses ionizing radiation, limiting the frequency of use and costs, due to radiological protection (doi:10.7326/0003-4819-155-11-201112060-00007). The demineralizing process alters the bone structures at the surface and subsurface levels. Changes on the bone surface and subsurface could be accessed by optical techniques (doi:10.1007/s10103-018-2520-y). However, it is difficult to access the bone structure under on biological tissue using light. This issue could be overcome by accessing the maxilla and mandible bones by the oral cavity. So, the aim of this work was to develop a method to differentiate sound from osteoporotic maxilla and mandible bones in an in vitro model using laser speckle imaging (LSI) technique.

Methods. After Ethical Committee in the Use of Animals of Federal University of ABC (CEUAx N 7604010818) approval, sixteen cubes from mandible and twelve cubes (3 mm) from maxilla of three porcine (*Sus scrofa domesticus*) were obtained. The osteoporosis was simulated following the process described by Lee *et al*. (doi:10.3171/2010.12.SPINE10453). The samples were immersed in Ethylenediaminetetraacetic Acid (EDTA) 0.5 M, by 0 day (no immersion, control group), 7, 15 and 30 days. Ultrasonic bath in distilled water for one hour to applied remove residual EDTA and stops the demineralization process. The samples analysed by an optical profilometry (ZeGage, Zygo, USA), using a 20x (Mirau) interferometric objective, and by custom LSI setup. The LSI was composed by a laser module (Thorlabs CPS635R) emitting 1.3 mW at 638 nm (less than 1 nm of FWHM), expanded by a diverging lens (focal length of -75 mm) to illuminate the sample area (12.5 mm away). The speckle signal was imaged by a CCD camera (200 ms exposure time - Thorlabs - DCC1645-HQ), an adapter (Thorlabs MVLCMC) and objective lens (12x magnification, f-number 2.5 - Thorlabs/Navitar - MVL12X3Z) positioned 95 mm from the sample surface. Ra and Rq surface roughness parameter were obtained from the optical profilometry. The LSI images were processed using a custom software in Matlab R2019b (MathWorks) following the algorithm described by A.M. Hamed *et al*. (doi:10.1016/j.optlastec.2003.09.005) and Rodrigues *et al*. (doi:10.1016/j.promfg.2017.09.077) to obtain the speckle patches ratio (SPR) and the speckle contrast ratio (SCR), respectively. The results obtained from the optical profilometry and the LSI technique were statistically analyzed and compared using Minitab® Statistical Software (Minitab, Inc, State College, PA).

Results. In the optical profilometry measurements the acid attack time increased the roughness amplitude, from -26 µm in the control sample to 146 µm in the 30 days of acid attack sample. For the SCR the control group presented lower values compared to the 7, 14 and 21 days groups. No difference between 7 and 15 days group were observed. The 30 days group presented values

statistically different from all other groups. For the speckle patches ratio analysis, the control group presented significantly higher values when compared to the 7, 15 and 30 days groups. The 7 days group presented statistical difference from 15 and 30 days groups. No difference between 15 and 30 days groups were observed. A negative correlation between SPR, Ra (ρ = -0.895) and Rq (ρ = -0.894) was observed. While a positive correlation between SCR, Ra (ρ = 0.659) and Rq (ρ = 0.651) was observed.

Discussion and Conclusions. It was proposed the LSI technique to assess the demineralization process of osteoporosis using an in vitro model. A custom LSI system and software were implemented to access the SCR and SPR of mandible and maxilla samples. These measurements were compared to the optical profilometry Ra and Rq measurements. Both SCR and SPR differentiates sound from osteoporotic tissue. The authors believe that this technique could be applied for clinical studies to validate it for future human application.

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