The effects of osteoporosis and osteoarthritis in the mechanical and structural properties of trabecular bone

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Abstract

In the present work, an evaluation of osteoporosis (OP) and osteoarthritis (OA) effects on the mechanical and structural properties of trabecular bone was made. Femoral heads were obtained from patients of both genders, who underwent total hip arthroplasty, due either to a low energy fractured neck of the femur presumably related to OP or to hip osteoarthritis. Using a mechanical drilling machine, cylindrical samples were collected and submitted to compression. Measures of the cortical thickness and diameter of the femoral heads were taken, as well as assessment of the density of trabecular bone. From the biomechanical compression tests, it was clear that the OP bone has, as expected, lower mechanical resistence. These results aren't, however, reflected by the difference in the density, which indicates that the quality of bone is more important that the quantity of bone for its overall mechanical performance. Comparing genders, it´s clear that in OP bone, females have lower mechanical properties than males. The femoral head diameter seems to have a large influence, at least in women, in the susceptibility to develop OP Structural characterization was performed with optical and scanning electron microscopy (OM and SEM). From the SEM´s images, the cellular structure of trabecular bone before and after the compressive tests was observed, which allowed understanding the yielding process of the rod-like and the plate-like structures. A partial relation was found between the structural's measurements from the microscopic images and the biomechanical results, which accentuate the importance of qualitative parameters in the characterization of trabecular bone. The two main trabecular structures, rods and perforated plates, have different degrees of resistance to mechanical solicitation, which suggests that they play different roles in the trabecular organization.

Key words: Osteoporosis; Osteoarthritis; Trabecular bone; Compressive tests; Scanning electron microscopy; Optical Microscopy.

Introduction

Osteoarthritis (OA) and Osteoporosis (OP) are the two most common, age-related, musculoskeletal disorders in the elderly, and are associated with considerable mortality and morbidity. Some studies have reported that these two disorders have a negative association between them [1-3]. In fact, patients with osteoarthritis of the hip (coxarthrosis, cOA), have less intracapsular hip fractures [1]. However, other studies have cast some doubts on these observations [2,3]. OP is a bone disease, characterized by a reduced amount of bone mass, whereas OA is generally considered to be a disease of joint cartilage, with secondary bone changes, such as marginal osteophytes and subchondral sclerosis [2,3]. There is evidence that suggest that the initiation and propagation of OA involves a disruption of the normal mechanical equilibrium between bone and cartilage, but, it is unclear if it is initiated in the cartilage layer, bone layer or both layers simultaneously [3]. Some studies have shown that the femoral heads of patients with cOA have higher bone mineral density, stiffness and ability to absorb energy [1].

Long bones are made up of an outer shell of dense compact bone, enclosing a core of porous trabecular bone [4]. An understanding of the mechanical behavior of trabecular bone is relevant for several biomedical applications, including the design of artificial hips, because most of the bone replaced by artificial hip is trabecular. Moreover, an improved knowledge of the structural relationships in trabecular bone will allow the design of artificial hips with proprieties which would more closely match those of the bone they replace [4]. The cellular structure of trabecular bone is made up of an interconnected network of rods and plates. A network of rods produces low-density, open cells, while high density trabecular bone has a more plate-like structure, with perforations through the plates. The mechanical behavior of trabecular bone is typical of a cellular material. The compressive stress-strain (σ-ε) curve has the three distinct regimes characteristic of all cellular solids: *linear elastic regime*; *stress plateau*, and *densification*. It´s generally accepted that the density of trabecular bone depends on the magnitudes of loads it experiences [5].

The purpose of this study was to compare the mechanical and structural properties in the principal compressive region of femoral heads, from OA and OP patients.

Materials and Methods

The femoral heads used in this work were all retrieved from patients who underwent total hip arthroplasty, for either a fractured neck of the femur attributed to OP, or for cOA, and were matched for sex and disease in 4 groups: female fracture (FF), male fracture (FM), male coxarthrosis (CM) and female coxarthrosis (CF). Some characteristics of the groups are indicated in Tables 1 and 2. Using a driller, two cylinders of trabecular bone were extracted from the region with highest amount of loading *in vivo* in each femoral head (figure 1). One of these cylinders was used in the compressive tests, and the other was cut in several slices to be used in microscopy. Measurements of the femoral heads diameter and the cortex thickness were taken.

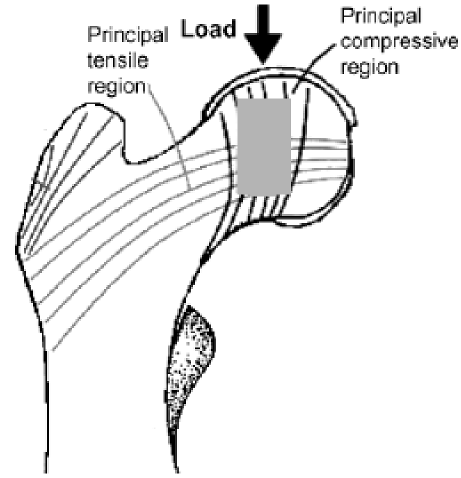

Figure 1. Femoral head schematic diagram with the location where cylindrical samples of trabecular bone were removed.

The tops of the cylinders were cut off in order to remove all the cortical bone and to get a basal surface as parallel as possible. The superficies of the cylinder were polish with a grid paper of 1000µm, at a rotation velocity of 250rpm. Before the compressive tests, the cylinders were de-fatted using a solution 1:1 of chloroform and methanol, and the material density was determined using the Archimedes' principle. All the cylinders had approximately the same diameter (~15mm), but the heights differed considerably (11-31mm), because it was technically very difficult to get samples exactly with the same dimensions. However, compressive tests performed in cylinders of rigid polyurethane foams with similar properties to the trabecular bone, showed that the height of the test cylinder doesn´t have a relevant influence in the results of the tests, since the diameter of the cylinders' doesn´t change.

Biomechanical properties were evaluated in an universal testing machine Instron 5566™. Compression tests were conducted with an elongation rate of 0.1m/s and with a load cell of 10kN. The σ-ε curves were displayed by the Bluehill Software, and the results were treated using MatLab. The tests were performed in the laboratory of mechanical testing of the *Instituto de Ciência e Engenharia de Materiais e Superfícies* (ICEMS) from *Instituto Superior Técnico (IST).*

Scanning electron Microscopy (SEM) images from trabecular samples before and after compressive tests, were acquired with a Hitachi S2400 microscope from the ICEMS/IST facilities, using magnifications from 20x to 100x. In order to increase the number of cOA samples analyzed, a optical microscope Leica DM2500 from the facilities of *Instituto de Medicina Molecular* (IMM), was used to observe non tested trabecular bone slices, with 7µm thickness.

A SPSS Manager Software was used to make a statistical analysis, using either the t-student test or non parametric Mann-Whitney´s test, according to variables distribution. Groups are found to be different if p-value<0.05.

Table 1. Characteristics of the 4 groups of femoral heads.

Group	Number of femoral heads	Age (years)	Femoral head diameter(mm)	Cortical thickness (mm)	Density (g/cm^3)
FF	15	82.5±5.7	43.315±2.070	2.302±0.569	1.297±0.110
FM	8	88.0±5.4	49.553±1.729	2.23±0.362	1.321±0.079
p-value	-	0,397**	0,197**	0,750*	0,333**
CF	8	49.5±12.0	47.735±3.582	1.666±0.563	1.328±0.052
CM	7	45.0±20.7	49.643±3.421	1.834±0.681	1.453±0.300
p-value	-	0,647*	0,313*	0,728**	0,316*

* t-student test

**Mann-Whitney test

Table 2. Details of the femoral heads, considering fractures and coxarthrosis globally.

Grupo	Number of femoral heads	Age (years)	Femoral head diameter(mm)	Cortical thickness (mm)	Density (g/cm^3)
Fractures	23	82.9±16.97	43.66±9.33	2.21±0.587	1.257±0.257
Coxarthrosis	15	45.47±17.16	45.92±11.4	1.671±0.634	1.305±0.378
p-value	-	0*	0.012*	0.05*	0.114*

* t-student test

**Mann-Whitney test

Results

Mechanicals tests

From the compressive tests performed in trabecular bone cylinders, σ-ε curves were obtained using two forms of strain measuring. One by the displacement of the testing machine´s crossbar ($\varepsilon_{crossbar}$), which may result in an overestimation of strain and in an underestimation of the elastic modulus, because of the deformations in the load cell and the test columns [6], and other, using a video-extensometer (ε_{video}), that avoids this problem, measuring the strain directly in the sample, by the distance variation of two points recognized in the sample surface. However, the use of this strain measurement was not always possible, due some problems with the size of the samples and the porous structure of the trabecular bone, with affected the recognition of the points by the system. Trabecular bone deforms as a cellular material in which the collapse propagates in bands. It means one point mark may be in a collapsed region, while the other is in the elastic regime. Another difficult is related to the fluids that are expelled from the bone during compression, which are responsible for the spreading of the ink of the points. So, in several cases, the videoextensometer didn't give good results. Due to all the described problems, it was necessary to find a relation between the elastic modulus calculated using ε_{video}, that was called E_{real}, and the elastic modulus calculated using $\varepsilon_{crosshead}$, $E_{crosshead}$To find this relation, both elastic modulus were determined, in all the samples where it was possible to perform it, and the fowling relation was found:

$$E_{real} = m.E_{crossbar} \tag{1}$$

with, *m* equals to 6.705. This means, that in the cases where it was possible to find out the E_{real} directly from the σ-ε_{video} curves, this was assumed as the elastic modulus of the trabecular structure, E. However, in the cases where this procedure

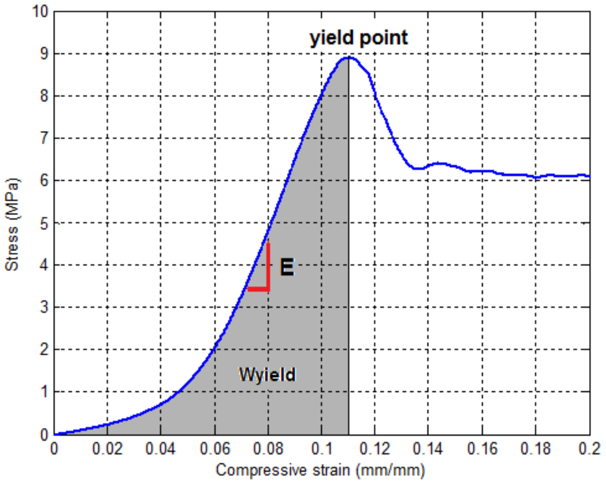

Figure 2. Example of a compressive stress- strain curve, The slope of the straight segment of the curve is the elastic modulus, the maximum value of the curve is the yield point, and the area bellow the curve until the yield point is the absorbed energy.

From those results, it´s clear that, as expected, the OP bone have lower biomechanical properties than the cOA bone. Comparing genders, in the OP groups, the females samples are the ones with less strength and rigidity, but in cOA groups, females seems to have higher biomechanical properties. A constant in every results is the high variability of the results. This is clear for the elastic modulus, where, for instance, the variation in the CM group is from 60 up to 3450 MPa (figure 3).

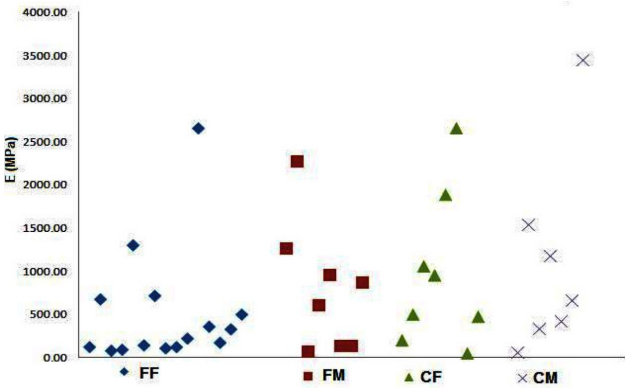

Figure 3. Distribution of E for each sample tested, matched by group.

Table 3. Results of the compressive tests for each group.

Group	σ_{yield} (MPa)	E (MPa)	W_{yield} (N.mm/mm^3)
FF	4.16±2.49	507.77±681.92	0.264±0.175
FM	6.82±3.65	783.45±745.47	0.361±0.195
p-value	0,05*	0.385**	0,243*
CF	10.023±3.904	972.75±896.02	0.533±0.323
CM	8.069±4.518	1091.82±1157.11	0.264±0.156
p-value	0,385*	0.826*	0,049**

* t-student test

**Mann-Whitney test

Table 4. Results of the compressive tests, considering fractures and coxarthrosis globally.

Gruop	σ_{ced} (MPa)	E_{real} (MPa)	W_{yield} (N.mm/mm^3)
Fractures	4.944±3.054	602±671.19	0.292±0.178
Coxarthrosis	8.069±4.518	1017.27±926.18	0.410±0.27
p-value	0.002*	0.130*	0.347**

The diameter of the femoral heads seems to have some influence in the biomechanical properties of trabecular bone. Normalizing the femoral head diameter by the patient height, and plotting it against σ_{yield} and E, depicted a linear relation, especially for the osteoporotic samples, where the fitting is excellent (figure 5 (a) and (b)).

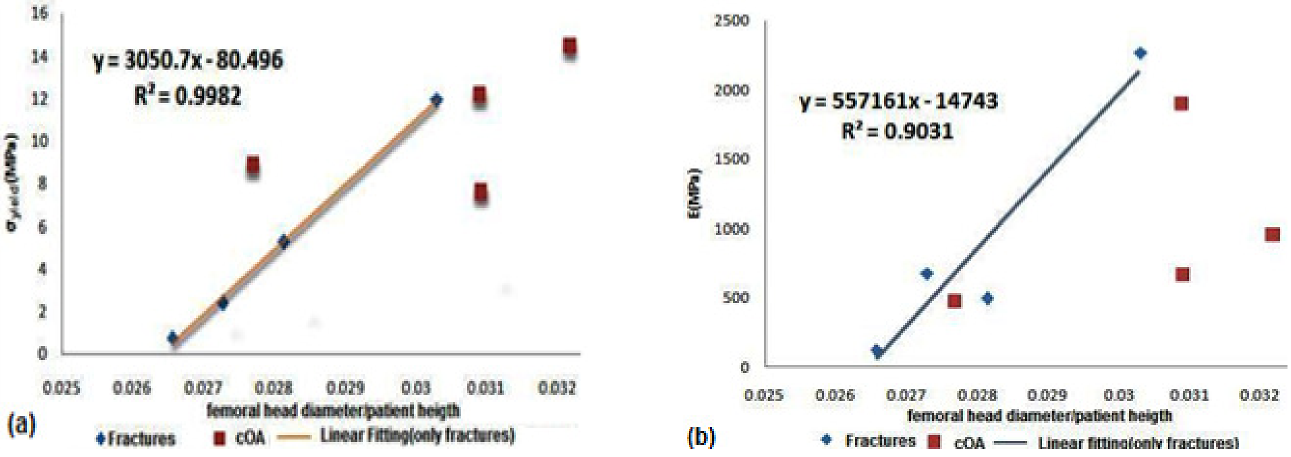

Figure 5. Biomechanical properties plotted against femoral head diameter normalized by the patient height. Linear fitting only for fracture´s samples. (a) yield stress;(b) elastic modulus.

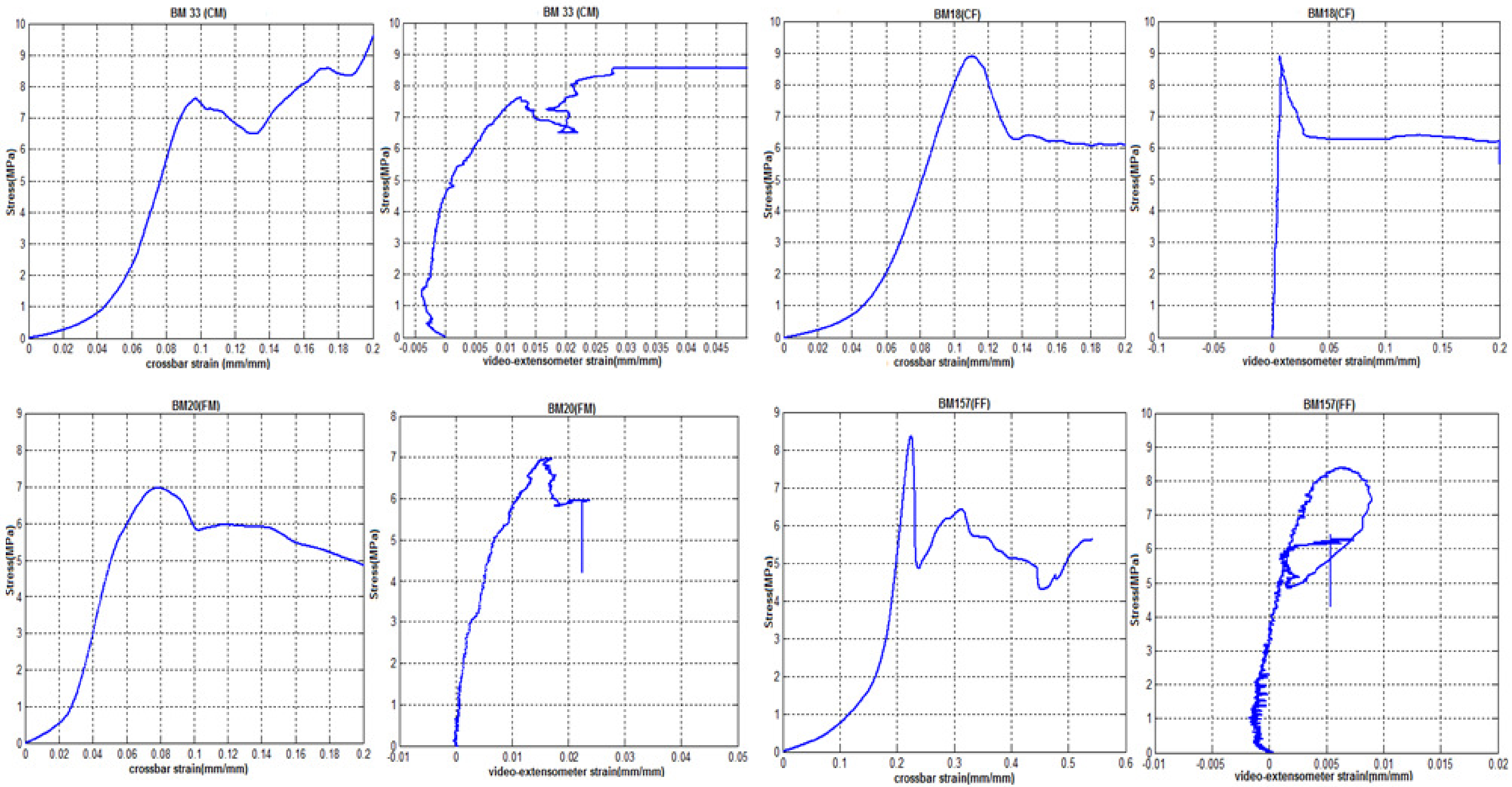

Figure 4. Examples of σ-ε_{video} and σ-$\varepsilon_{crossbar}$ curves from each of the considered groups.

Structural analysis

In this work, both tested and non tested trabecular samples were observed in microcopy, all from female patients. The observation of non tested samples was performed both in SEM and OM, from 5 OP and 5 cOA samples, and from those images, structural measures were taken using the ImageJ software: trabecular thickness, trabecular distance, and fraction of occupied area. The trabecular structure shows a great complexity, being composed by an interconnected network of rods and perforated plates (figure 6 (a) and (b)). No evident distinction was noted in the trabecular structure, between the two groups observed.

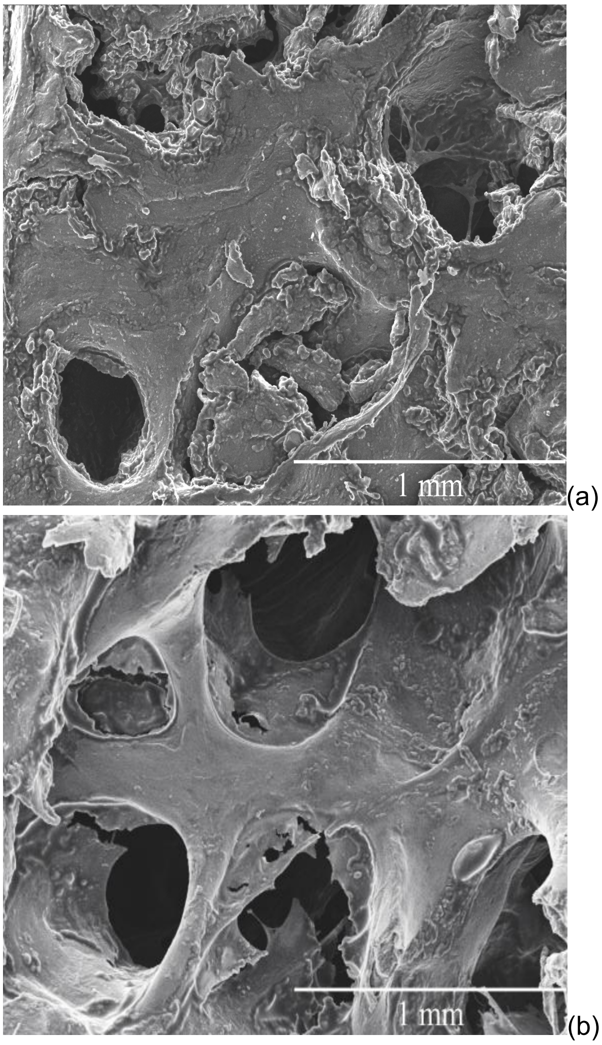

Figure 6. SEM images with 50x magnification. (a) Perforated plate in OP sample; (b) network of rods and plates in OP sample.

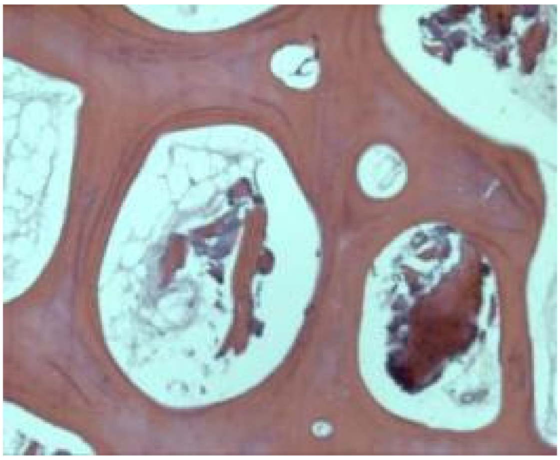

Figure 7. Detail of OM image with 10x magnification from cOA sample.

The structural's measurements are summarized in table 5. The group of patients with femoral neck fracture shows as expected a higher trabecular distance, however the trabecular thickness is also high.

Transversal cuts in the trabecular cylinders, after the compressive tests, were made, and observed in SEM. Fractured trabecular rods are easily find (figure 8), and the irregular surface that formed are evident. However, the location of these ruptures was randomly distributed. Finding damage rods was more difficult, because the majority were completely crashed. In the top of figure 9(a) a perforated rod is visible, broken exactly in the local of the perforation. Some tiny fissures were also detected (figure 9(b)). In some images, it was possible to observe the existence of fractured rods near rods apparently undamaged (figure 10).

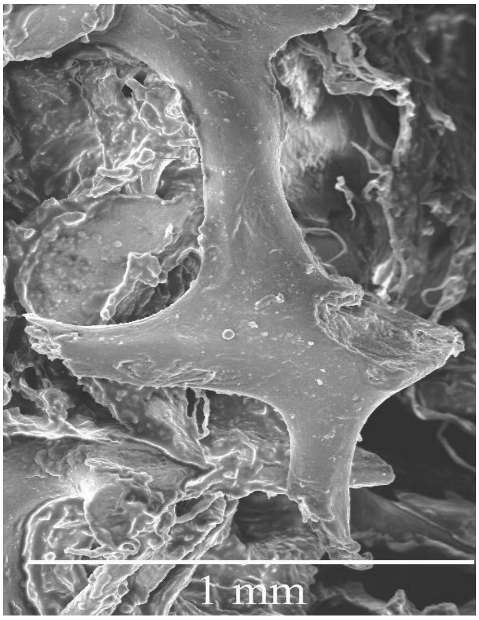

Figure 8. Detail of a SEM image, showing two broken trabecular rods.

Table 5. Age and structural properties measured for each sample, and average values.

Group	Sample ID (type of microscopy)	Age (years)	Trabecular thickness (mm)	Trabecular distance (mm)	Fraction of occupied area (%)
Fracture (OP)	BM11 (SEM)	83	0.306 ±0.155	0.771±0.363	71.03±5.07
	BM13 (SEM)	73	0.143±0.04	0.438±0.159	79.92±5.39
	BM14 (SEM)	85	0.154±0.047	0.497±0.11	67.16±8.2
	BM32 (SEM)	90	0.191±0.093	0.588±0.133	71.34±9.51
	BM131 (SEM)	83	0.148±0.047	0.512±0.118	83.54±3.32
cOA	BM 18 (MO)	66	0.171±0.059	0.426±0.171	-
	BM34 (SEM)	41	0.154±0.052	0.526±0.280	73.86±6.46
	BM73 (MO)	40	0.181±0.056	0.487±0.259	-
	BM74 (MO)	59	0.161±0.065	0.268±0.164	-
	BM106 (SEM)	60	0.173±0.048	0.571±0.270	73.6±7.52
Average values by group					
	Fracture	82.8±6.2	0.2177±0.128	0.631±0.292	74.61±8.92
	cOA	53.2±11.9	0.158±0.051	0.536±0.271	73.73±6.74
	p-value	0.03*	0.004**	0 **	0.650*

* t-student test

** Mann-whitney test

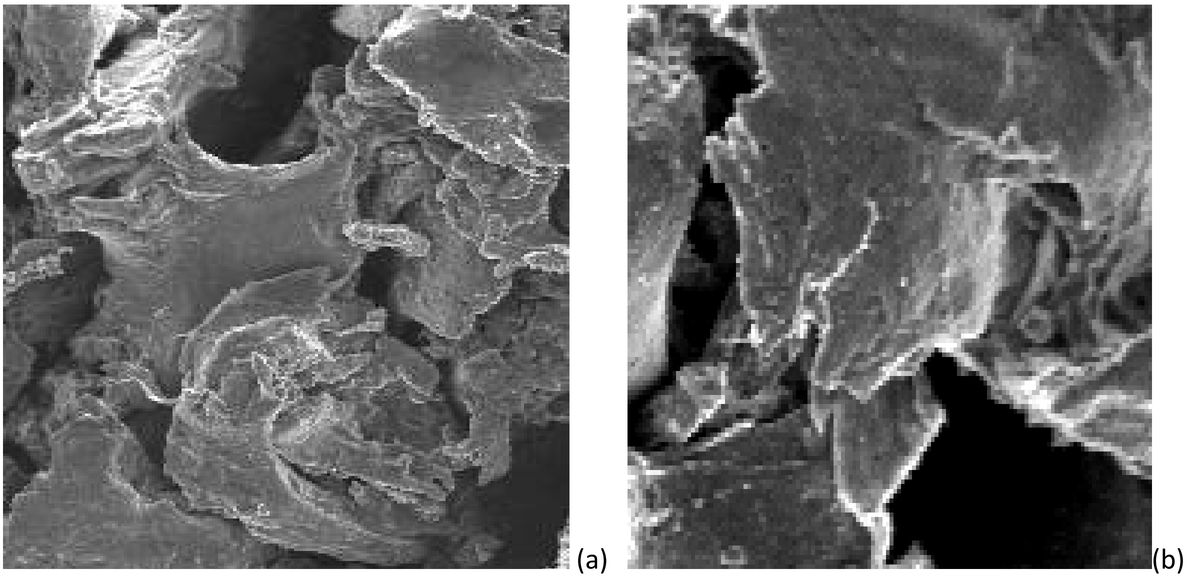

Figure 9. Damaged trabecular plate-like structures. (a) Broken plate structure in the perforation location (b) Detail of a fissure in a trabecular plate.

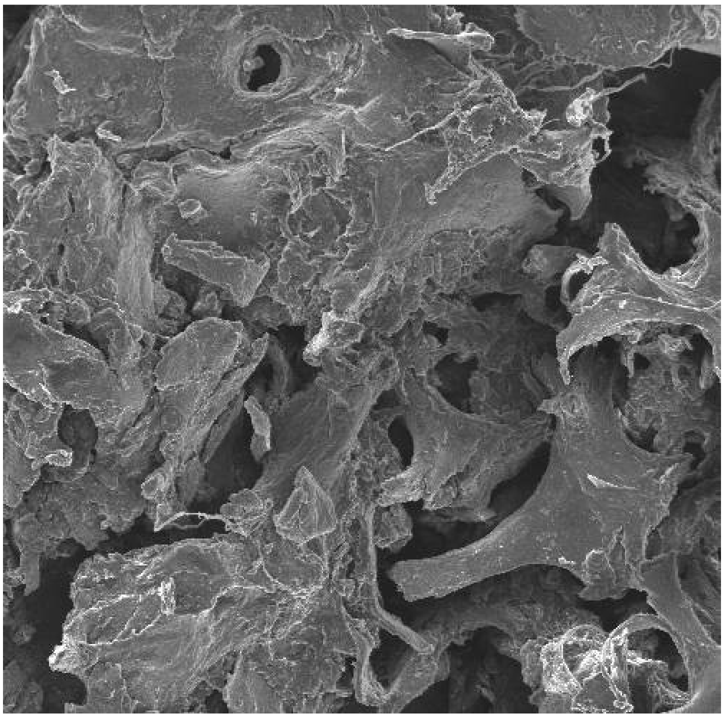

Figure 10. Damaged rods near an apparently intact perforated plate

Discussion of results

The evaluation of the cylinders extracted from the femoral heads shows that the average femoral head diameter of the CF group is much higher than the average femoral head diameter of the FF group, while the diameter of the femoral diameter of the two males groups seems to be very similar. This may indicate that women with smaller femoral heads can be more prone to have an osteoporotic fracture. However, this comparison was performed with cOA patients, who were associated to larger femoral heads in a published study [7]. On the other hand, the femoral head diameter hasn´t be often study as a fracture related parameter, however, in at least two different works, no relation was found between the fracture probability and the radiologic diameter of the femoral head [8,9]. Curiously, the two OP groups have a thicker cortex than the OA groups, and, comparing OP gender groups, the cortical thickness of the female group is the higher. This is unexpected, because males have much larger femoral heads. These results are hard to compare with the literature because the evaluation of the cortical thickness has only be performed in the femoral neck region, and in this location this parameter is higher in patients with cOA. Hypothetically, a thicker cortex could create a "shield effect" for the *in vivo* stresses, and, by the Wolff law, it could settle a reduced trabecular development. As expected, because OP is characterized by bone loss, the samples of these groups are less dense than the cOA sample, however the differences are not significant. The results from the biomechanical tests are all characterized by great standard deviations, which reflect the huge variability of biologic tissues. However, and in spite of this variation, it´s clear that in general both OP groups have the worst biomechanical properties, especially the female group, which shows lower strength and rigidity, which reflects the higher probability of women to suffer fractures. Comparing the results from the cOA groups, the females have higher yield stress and ability to absorb energy, but have a slightly lower rigidity. These results could support the hypothesis that OA acts like a protector, or, at least, a delaying factor for the occurrence of fragility fractures. It also should be noted that, because the densities of OP and cOA bone were not significantly different, the differences of the mechanical properties between these groups can't be explained by discrepancies in the quantity of bone, but by qualitative disparities. The direct correlation found between the femoral head dimensions normalized by the patient height and the biomechanical properties is very interesting. In fact, if confirmed in a larger cohort, it can constitute a useful tool to estimate the resistance and the rigidity of trabecular bone, and therefore the risk of fracture in OP patients. Two types of interconnected structures were mainly found in the microscopic observation of trabecular samples: rods and perforated plates. The disposition of these structures is very heterogeneous, and no evident difference was found between samples of different groups. Structural measures show that OP bone has a higher average trabecular thickness and distance. This can be explain by a redistribution of bone in less but more thicker and more spaced trabeculae, which may be compatible with a diminution of the mechanical resistance. However, differences in the age between the two groups might have also contributed for this effect. Comparing these results with the results from mechanical tests, it is interesting to highlight that the sample BM11, which is the one with the higher trabecular thickness and distance, has the second lower σ_{yield} (0.98MPa). In accordance, the sample BM74, which is the one with lower trabecular distance, has the higher rigidity (2660MPa) and the second higher σ_{yield} (11.6 MPa). However, there are samples that do not perform in accordance with this observation, which means that structural properties, per se, do not fully explain the mechanicals properties of trabecular bone. The microscopic analysis of trabecular samples after the compressive tests, offers some additional data on the way trabecular bone reacts to compressive stresses and how the yield of the different structures occurs. The rod-like structures seem to yield in a very irregular way, with no obvious standard. There are some evidences that the yielding of the trabecular plates occurs by fissure propagation and that the perforation site can be a more vulnerable location. Broken rods were observed near to undamaged plates, which indicate that rods are much more fragile than plates and are responsible for the first yield observed in the compressive test. In other words, the breaking of those rods separate the elastic from the plastic regime. No obvious difference in the way the yield occurs were discernible, between the two groups observed.

Conclusions

From the biomechanical compression tests, it was clear that the OP bone has, as expected, lower mechanical resistance. These results aren’t, however, reflected by the difference in the density, which indicates that the quality of bone is more important that the quantity of bone for its overall mechanical performance. The femoral head diameter seems to have a large influence, at least in women, in the susceptibility to develop OP. A partial relation was found between the structural’s measurements from the microscopic images and the biomechanical results, which accentuate the importance of qualitative parameters in the characterization of trabecular bone. The two main trabecular structures, rods and perforated plates, have different degrees of resistance to mechanical solicitation, which suggests that they play different roles in the trabecular organization.

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