EFFECT OF THE MESOSTRUCTURE ON THE MECHANICAL DYNAMICAL BEHAVIOUR IN CANCELLOUS BONES

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Recibido: Abril 2012. Aprobado: Julio 2012.
Publicado: Enero 2013.

ABSTRACT

The correlation between the mesostructure of cancellous bones, revealed by scanning electron microscopy (SEM), and the mechanical dynamical behaviour as a function of temperature, was studied. The study of the physical and chemical changes in cancellous bones at temperatures over the room temperature through mechanical dynamical tests requires mandatorily the examination of the mesostructure by means of SEM. SEM studies reveal that the contraction of the woven part of bone occurs by heating over 350K. This change in the morphology of the sample at mesoscopic level influences strongly the dynamic response of bones and consequently masks the physical and chemical processes occurring in the collagen during the heating of samples.

Keywords: SEM, cancellous bones, mesostructure, DMA

INTRODUCTION

Bone is a complex material essentially composed by mineral and organic parts and water. The mineral part is composed by carbonated apatite ($Ca_{10}(PO_4)_6(OH)_2$), which is crystalline, and the organic part contains principally type-I collagen, with phosphoproteins, glycoproteins and other proteins. The stoichiometry of apatite in living organisms is not fulfilled because it results deficient in calcium content, being calcium replaced by carbon; which is incorporated from the breathing.

The outstanding features of bones are not only related to their main components. The mesostructure and/or nanostructure play an important role in both the superior stiffness and the strength properties that are found in bones. Indeed, the composition alone does not fully explain the bulk mechanical properties. Mechanical properties can vary significantly in bones as a result of differences in the arrangement of their structure [1-3].

There exist several works reported about mechanical properties and dynamical behaviour of bones, owing to the interest for improving the life quality in old persons and persons suffering some disease as for instance osteoporoses, see references [1-3] and cited papers therein. Most of the works have been done in cortical (compact) bones, taken from either persons, caws, chickens, etc. In contrast, there exist few works studying the mechanical properties of cancellous (porous) bones, e.g. see [4].

The mechanical properties are frequently explained considering changes which are occurring in the collagen and in the mineral concentration [1–3, 5], but without a coupled study with scanning electron microscopy (SEM)

in order to reveal the possible changes in the woven at level of the mesostructure.

In this work, we have studied the correlation between the mesostructure of cancellous bones, revealed by SEM, and the mechanical dynamical behaviour as a function of temperature. We will show that SEM examinations are a requisite for studying the mechanical dynamical response of bones, owing to SEM reveals the appearance of changes in the woven part at mesoscopic level which mask the physical and chemical processes occurring either in the collagen and/or mineral parts.

MATERIALS AND METHODS

Samples

Samples of cancellous bones were taken from ribs of fresh cow meat, which were purchased from three different local supermarkets. Three samples were prepared from the bone of each provider. Samples were taken from the bones by cutting with a jeweller saw, the final size being reached by mechanical polishing. Parallelepiped shaped samples of around 3mm x 4mm x 40mm were used.

Measurements

A SEM *Jeol (JSM-5610LV)* was employed to characterise, at room temperature, the morphology and structure of the samples. Secondary electron images were obtained working at 20 kV. The composition was analysed by means of energy-dispersive X-ray spectroscopy (EDS). The specimens were coated with 5 nm of Silver.

Dynamic mechanical analysis (DMA) studies usually involve the simultaneous measurement of damping, tan(ϕ), and natural oscillating frequency, f (f^2 α dynamical modulus), as a function of temperature [6, 7]. DMA measurements were performed at frequencies close to 1 Hz, as a function of temperature, T, in torsion, under pure argon atmosphere at standard pressure. The heating and cooling rates were of 1K/min. A heating and its corresponding cooling run will be called hereafter a thermal cycle. The maximum shear strain on the sample was 2 x 10^{-4}. The error for tan(ϕ) and dynamic modulus was less than 2%.

RESULTS AND DISCUSSION

Results from DMA exhibit differences between different samples, however showed results in this work represent the general trends of the whole set (nine) of studied samples. The measured values of tan(ϕ) and dynamic modulus, between different samples, exhibited usually a bandwidth of discrepancy around 10%, but in some cases we have also found a bandwidth up to around 20%. Nevertheless, the general trends of the curves during the thermal cycles are completely similar and reproducible.

Figure 1 shows the tan(ϕ) behaviour as a function of temperature for a fresh bone sample during two subsequent thermal cycles. The spectrum of tan(ϕ) exhibits a wide peak between around 280 K and 400 K during the heating run in the first thermal cycle, see circles in Figure 1. During the cooling part of the first thermal cycle, the tan(ϕ) values decrease strongly (triangles). A new heating run (in thermal cycle #2, squares), leads to tan(ϕ) values slightly higher than for the cooling part of the first thermal cycle, up to around 360 K. The cooling in the second thermal cycle gives rise to even smaller tan(ϕ) values than in the previous runs (inverted triangles).

Figure 2 shows the dynamic shear moduli curves corresponding to thermal cycles plotted in Figure 1.

Moduli curves exhibit more scatter than tan(ϕ) measurements. Despite of the scatter in the measured modulus values, it is clear that after the first heating in thermal cycle # 1, the moduli values have increased for all the next heating and cooling runs.

We are interested in the present study in the temperature range above room temperature. The mechanism controlling the damping and modulus below room temperature are discussed involving the interaction of ice particles with the woven of bones [2], but it is not discussed here.

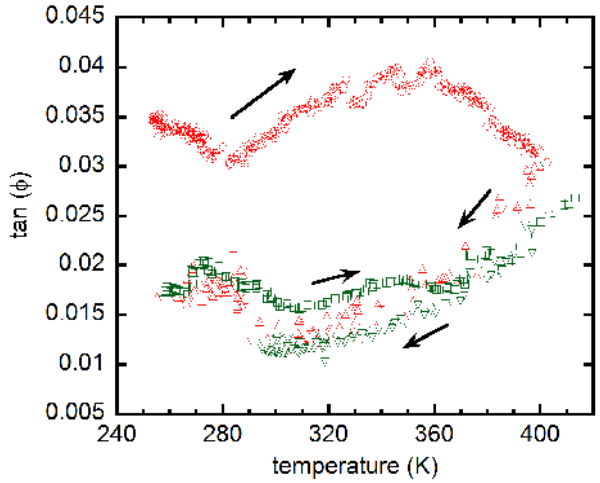

Fig. 1. tan(ϕ) vs. T during thermal cycles: First thermal cycle: circles and triangles. Circles: first heating. Triangles: first cooling. Squares and inverted triangles: second thermal cycle. Squares: second heating. Inverted triangles: second cooling. Arrows indicate the warming and cooling runs.

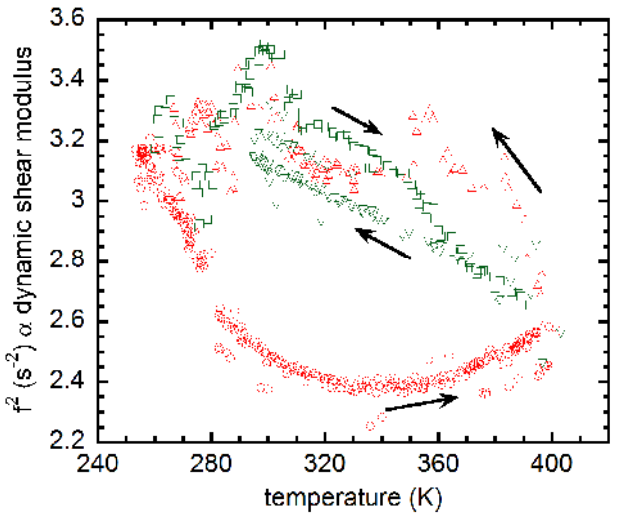

Fig. 2. Dynamic shear moduli vs. T during thermal cycles. Symbols mean as in Figure 1.

The mineral phase of bone is crystalline hydroxyapatite, which is elastic and responsible for the stiffness of bone. As a consequence of its elastic nature, the mineral phase cannot be a source of damping, within these temperature and strain ranges. Due to the complex structure of bone, the mechanical loss can be due to a variety of mechanisms. As the structure contains holes and canals of different sizes, damping due to liquid flow in porous media can be important in fresh bones [3].

The wide tan(ϕ) peak between around 280 K and 400 K, has been already related in the literature for cortical bones, to the viscous motion of the collagen and the water into the bone channels and cavities. The decrease in tan(ϕ) values, after the first heating up to around 400 K, can be related to the deterioration of the collagen (at molecular level) due to the heat treatment. Besides this, the values of damping slightly higher during the warming of the second thermal cycle can be promoted by a partial recovery of the collagen. While, the subsequent decrease of damping during the second cooling run can be the result of a further deterioration of the collagen [2, 3].

The increase in the modulus during the heating part in the first thermal cycle can be related to the loss of water during warming [2].

Nevertheless, in the above referenced works a correlation with the mesostructure of the samples was not made. In the next paragraphs we will show that for studying the physical and chemical transformations which develop in bones as a function of temperature, up to around 400 K, it is critical to perform SEM studies for revealing the changes in the woven at mesoscopic scale; which appear during heating. In fact, it is not possible to understand from the reported works, that the wide peak in damping between 280 K and 400 K, which has its maximum at around 340 K, is related with some deterioration of the collagen, given that the elastic modulus increases markedly in the second thermal cycle. If a deterioration, either in the collagen fibrils or in the tropocollagen appears, as an isolated phenomenon, it would lead to a decrease in the dynamic modulus. For this reason is mandatory to reveal the evolution of the mesostructure of the sample as the temperature increases by means of SEM.

Figures 3 and 4 show the morphology of the woven part of cancellous bones for samples prior and after the DMA tests, respectively. As it can be seen comparing these Figures, the sample after the thermal cycles (up to 400 K)

exhibits an arrangement more compact than the fresh bone sample. This compactness is promoted by the contraction of the woven part assisted by the loss of water and fluids during warming. From several SEM examinations, samples after the warming runs exhibit a mesostructure more compact than the fresh samples.

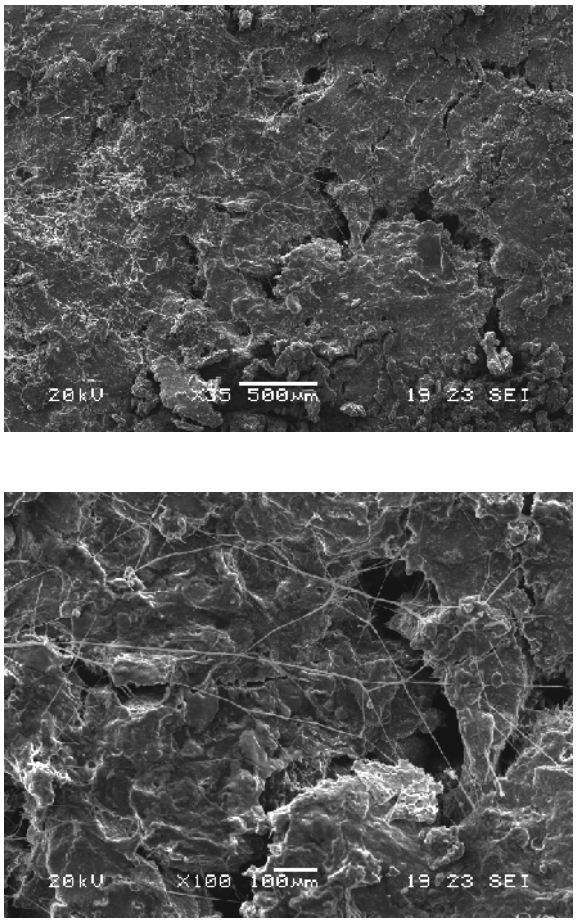

Fig. 3. SEM micrographs for a fresh bone (previously to DMA tests).

By comparing the hierarchical arrangement of cancellous bones at level of the collagen fibrils with the arrangement of muscles, a reasonable correspondence from the point of view of the mechanical properties can be obtained. In meat it has been reported that over 330 K the contraction of connective tissue begins. It gives rise to a large extracellular space when shortened and there is more room for the connective tissue to contract without is restricted by the myofibrilar mass. Therefore, a higher number of fibbers per unit of cross-sectional area lead to a larger elastic modulus [8]. This mechanism results in agreement both with the reorganisation of the mesostructure shown in Figures 4, and with the increase in the modulus during the thermal cycles (see Figure 2).

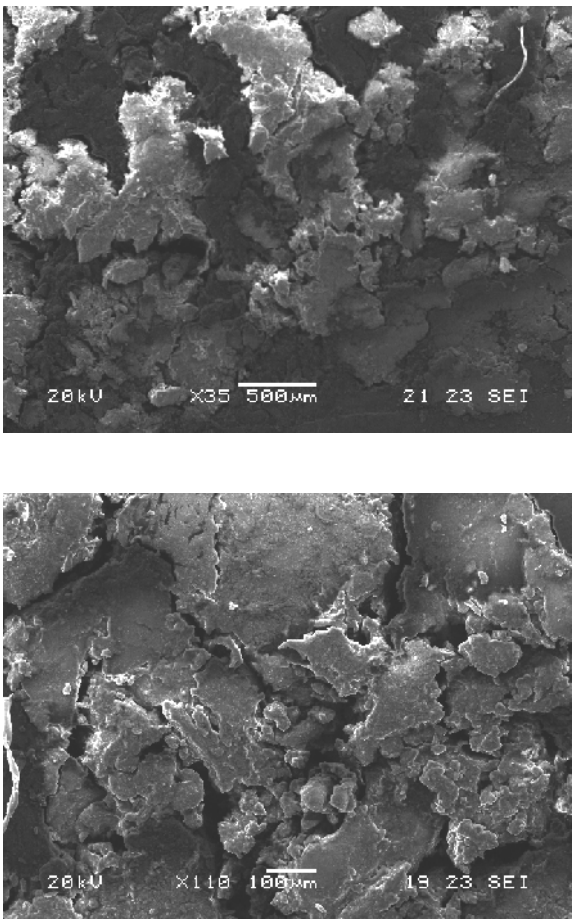

Fig. 4. SEM micrographs for a bone sample after the DMA thermal cycles up to 400 K.

Moreover, this modification in the mesostructure of the sample during warming influences also to the damping behaviour. The contraction of the woven in the bone should lead to a decrease in the tan(ϕ) values, since the mobility of the collagen fibrils decreases. Indeed, in meat a strong increase in the dynamic modulus appears at around 330 K which is accompanied by a decrease in the tan(ϕ) response [9].

It should be pointed out that, the temperature at the maximum in tan(ϕ) (around 340 K) corresponds with the minimum in the dynamic modulus (see Figures 1 and 2),

there of the decrease in the tan(ϕ) values for temperature higher than around 340 K can be related to the compaction in the mesostructure. Consequently, for studying the physical and chemical processes which develop in the collagen as a function of temperature, the contribution of the change in the mesostructure must be subtracted firstly, since the contraction appears as the dominant process in the bulk of the bone samples (see Figures 4).

In the other hand, a change in the chemical composition of bones after DMA thermal cycles has been also revealed by EDS studies. EDS spectra for a fresh bone and for a bone after the thermal cycles are shown in Figures 5.a and 5.b, the results being summarised in Table 1. The error bandwidth in contents of each element, determined from EDS studies is around 0.5%.

The elimination of water and fluids from the bone could originate the composition change revealed by means of EDS.

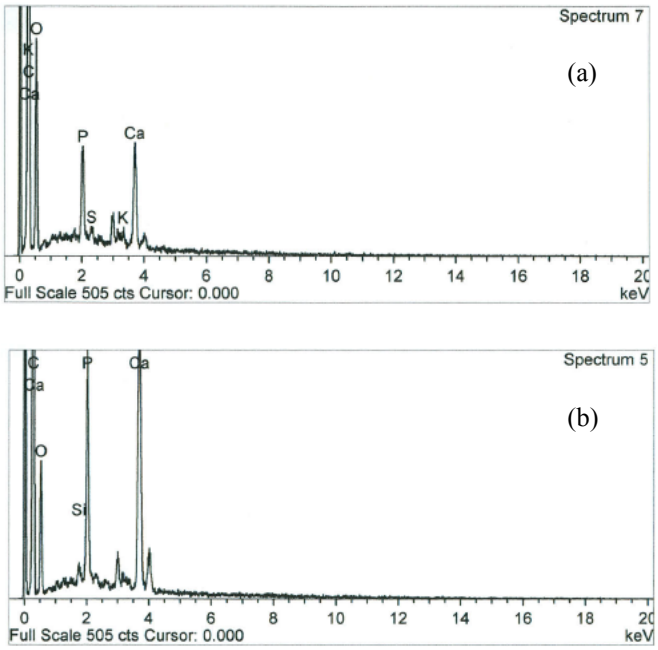

Fig. 5. EDS for bones with different thermal treatments. (a) fresh bone. (b) EDS bone after the thermal cycles up to 400 K.

This change in the mineral part could influence also the DMA response. In fact, the mineral parts are contained between the tropocollagen in a regularly separated fashion [4, 10]. However, the contraction effect of the woven part which appears as an overlaid relaxation process, masks all the other mechanisms which are occurring; due to the larger volume involved in this relaxation. Therefore, it must be suppressed firstly, before of analysing the physical and chemical changes that develop within the bone structure from DMA studies as a function of temperature.

Table 1. EDS results for a fresh bone and for a bone after the thermal cycles up to 400K. Data are from Figure 5.

Element (K)	Fresh bone (wt. %)	After thermal cycles (wt. %)
C	76.3	66.5
O	20.8	21.9
Si	--	0.2
P	1.0	4.0
S	0.1	--
K	0.1	<0.1
Ca	1.6	7.4

CONCLUSIONS

The study of the physical and chemical changes in cancellous bones at temperatures over 290 K, which could be revealed by means of DMA, requires mandatorily the examination of the mesostructure by means of SEM. SEM studies reveal that the contraction of the woven part of bone occurs by heating over 340K. This change in the morphology of the sample at mesoscopic level influences strongly the dynamic response of bones and consequently masks the physical and chemical processes occurring in the collagen during the heating of samples.

ACKNOWLEDGMENT

This work was partially supported by the CONICET PIP-2098.

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