On the mechanical characterization of bovine bone tissue under compressive loading

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ABSTRACT

This study reveals a pioneer characterization of bovine trabecular bone tissue under dynamic compressive loading in the range of 20-140 ºC, within physiological loading frequencies (1-20 Hz). The presented results demonstrate that the viscous behaviour in compression mode up to 140 ºC is regulated by the collagen viscoelastic properties, showing three frequency-independent main peaks in tanδ and loss modulus at 75, 100, and 130 ºC. The referred peaks are, respectively, related to crystalline structure breakdown, water structural evaporation, and the glass transition temperature (T_g) of collagen. At these low frequencies, the system is governed by a frequency-dependent elastic response and a frequency-independent energy dissipation. However, an additional experimental test at physiological temperature (37 ºC), in the range of 10-200 Hz, has shown that bone trabecular tissue submitted to compressive loading at higher frequencies is ruled by a frequency-dependent behaviour of the viscous component.

Keywords: Trabecular bone tissue, compression, viscoelasticity, dynamic mechanical analysis

INTRODUCTION

Long bones, such as femur and tibia, have been widely studied in the past few years to evaluate the mechanical response in tensile and compressive viscoelastic loading environments. Despite many investigations, the complex multi-lamellar bone composition left many other topics for studying, since bone mechanical behaviour depends on different imposed loading, preferential bone fibrils direction, harvesting location, mineral and organic relative compositions, and time. Bone comprises a well-organized hierarchical, anisotropic structure [1] that is essentially composed of mineral and organic phases, and water [2]. Different cell groups, responsible for production and maintenance of the extracellular matrix composed by type-I collagen, and hydroxyapatite are the most important elements in the bone structure. Trabecular bone, which is highly porous (50 to 95%), represents 15-20% of the whole bone in body and has lower mineral contents than cortical bone tissue [1]. Despite this lower relative composition, trabecular bone plays an important role in the global mechanical strength, support, and energy absorption, and it is continuously modifying as a function of external stimuli and regeneration/resorption phenomena [1]. The contribution of the mechanical response of trabecular bone in dynamic and long-term responses is thus fundamental. According to [3], poro-viscoelasticity parameters in bone are frequently associated to water and collagen contents, which contribute to the main bone response under loading [4]. The possibility to establish a relation between microstructure and viscoelasticity at a microscopic scale is even more interesting to figure out promising bone tissue substitutes [5] to solve relevant clinical issues (bone fracture and bone-implant systems) [4]. Despite the high interest in the mechanical behaviour of bones, no comprehensive dynamic mechanical analysis (DMA) under compression was found in the literature. In this study, a fundamental compressive mechanical characterization of bovine trabecular-oriented specimens was carried out, in the full range of physiological loading frequencies, by dynamic compressive testing protocols.

MATERIALS AND METHODS

Cylindrical specimens harvested from desiccated bovine femoral heads were used in this study. Bone samples were cut perpendicularly to the intertrochanteric line, from flattened epiphyses regions, using a 10 mm diameter trephine, with 9 mm height (on average). Extremities were then faced against sandpaper (180 and 260 grit size) in a grinding machine, while keeping bone samples in a prismatic slab previously drilled to guarantee the parallelism of specimen ends. The direction of the biggest dimension was chosen according to the pseudo principal loading direction [4]. A physiological saline solution (PBS) was continuously buffered onto bone samples to preserve the hydration condition. Following these processes, bone specimens were fully cleaned, measured (height and cross-area), and immersed in saline solution inside Eppendorf tubes. Then, bone samples were stored in a freezer at -20 °C. Bone thawing occurred at room temperature (22 °C), which has been previously shown not to change the dynamic mechanical properties [6].

Dynamic mechanical analysis (DMA) was performed using a DMA 7100 from Hitachi® (Japan) in programed compression methods. The analyses were carried out in a nitrogen atmosphere (200 ml/min) to ensure an inert environment. The values of compression moduli were registered over a range of frequencies from 1 to 20 Hz, in synthetic oscillation (the first frequency was set to 1 Hz, while the remaining ones were automatically displayed at 2, 4, 10, and 20 Hz). Temperature dependence of tanδ, storage and loss moduli (*E*' and *E*'') were measured in the temperature range of 20-140 °C, at 3 ºC/min.

RESULTS AND DISCUSSION

DMA compression analysis of trabecular bovine bone tissue at different frequencies (1, 2, 4, 10, and 20 Hz) shows that storage modulus (*E*') between 20 and 60 ºC remains unchanged in the range of 160-190 MPa (Figure 1). From 70 ºC to 100 ºC, a large peak in the storage modulus was observed, which increased with the imposed frequency (1-20 Hz). Moreover, after 120 ºC a rapid increase in the storage modulus reaches more than 350 MPa for all the frequencies, suggesting that type-I collagen component of the bone structure is undergoing structural and viscoelastic modifications. This behaviour is attributed to a phase transition of the amorphous regions of collagen at approximately 130 ºC [3, 7]. The values of the loss modulus (*E*'') showed three main peaks at 75, 100, and 130 ºC without a clear relationship between the imposed frequency and the dissipated energy (Figure 1). The first temperature is related to the well-known shrinkage of collagen due to crystalline structure breakdown that increases the viscous component. The second temperature is related to water mobility within the pores of the solid structure and bounded to collagen [8]. The peak at 130 ºC is associated to the glass transition temperature (T_g) of collagen, as confirmed by the peaks shift in tanδ plot (Figure 1). Another test at fixed temperature (37 ºC) up to 200 Hz (data not shown) indicates, as expected, that the system is governed by a viscous behaviour under compression, in which the storage modulus (150 MPa) does not change within the applied frequency. However, the loss modulus increases with the applied frequency from 10 to 20 MPa, indicating growing energy dissipation.

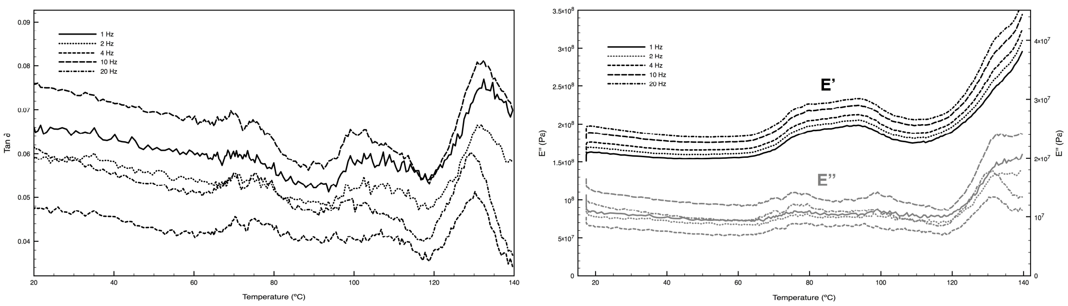

Figure 1. Tanδ, loss, and storage moduli of DMA compressive analysis.

CONCLUSIONS

Bovine trabecular bone is a very complex porous tissue formed by solid and fluid materials that govern its viscoelastic behaviour as a function of the applied frequencies. Above 120 ºC, a phase transition was clearly identified in both storage modulus and tanδ curves. Three peaks were registered through tanδ plots associated to collagen viscous modifications, including a T_g at 130ºC. A frequency-dependent behaviour of the viscous component is only observed at higher frequencies (40 Hz up to 200 Hz).

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